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Research Article

THE COLLECTION OF AZALEAS AND CAMELLIAS OF THE BOTANICAL GARDEN IN IAȘI – BOTANICAL, HORTICULTURAL, HISTORICAL, AESTHETIC AND EDUCATIONAL VALUES

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Abstract: The azalea and camellia collection of the "Anastasie Fătu" Botanical Garden of the "Alexandru Ioan Cuza" University of Iași holds an important significance for the horticultural value of the plant inventory cultivated in greenhouses. It includes 45 valuable taxa, mainly due to their size and age. The specimens in the collection are showcased through exhibitions organized annually since 1975, so in 2025 the 50th edition took place. In the Romanian botanical space, this collection has represented a constant, even in cases where specialized information regarding these taxa was rare or confusing.

Keywords: *Camellia* genus, exotic plants exhibition, informal education, phytonyms, *Rhododendron* genus.

Introduction

The Greenhouse Complex of the "Anastasie Fătu" Botanical Garden of the "Alexandru Ioan Cuza" University of Iași has a botanical inventory that includes about 2,200 taxonomic units (almost 2/3 of the spontaneous taxa in Romania). The taxa of the *Camellia* and *Rhododendron* genera are 45 in number, representing 2% of the total inventory of the Greenhouses, but constitute a representative collection, valuable due to the age and size of the specimens, but especially due to their rarity and history. The joining of these two groups of plants is justified by their belonging to phylogenetically related families (they belong to the same botanical order – Ericales), by the similar requirements regarding the cultivation method and by some popular names that refer to roses.

The specimens from this collection are accessible to the visiting public only during the Exotic Plants Exhibitions, events organized annually within the Greenhouse Complex section of the "Anastasie Fătu" Botanical Garden.

Little information is known about the cultivation of these types of plants in the Romanian space (especially Moldova), although their presence in the assortment of plants sold for horticultural purposes is reported before the 1870s.

Material and methods

The botanical material mainly referred to in this work consists of the hundreds of specimens that are part of the *Camellia* and *Rhododendron* genera collection cultivated within the Greenhouse Complex section of the "Anastasie Fătu" Botanical Garden of the "Alexandru Ioan Cuza" University of Iași.

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The current collection of azaleas and camellias represents a continuation of the initiative carried out in the greenhouse on the previous site of the garden (until 1962-1963), located in the immediate vicinity of the "Alexandru Ioan Cuza" University building on Carol I Boulevard, called at that time 23 August street. The plant material that makes up this collection was purchased over time from various sources represented by private collections (Sibiu) or horticultural research centers (Codlea, Cluj-Napoca) that cultivated this type of plants [TEODORESCU & MITITIUC, 2002].

Aspects regarding the taxonomy, cultivation conditions, educational and decorative value of these plants were analyzed. For this, internal documents (registers, inventories, personal observations, etc.), catalogs and specialized literature, as well as other relevant articles or publications were analyzed.

The characteristics of the cultivars mentioned in the paper are consistent with the information in the specialized literature [GALLE, 1987; ROGER & RIX, 1999; LESLIE, 2004; LOGAN, 1991; BUNDESSORTENAMT; RHS].

The leaf micromorphological aspects were observed using the Optika SZM-1 stereo microscope and were captured using the Levenhuk M1 200 Plus Digital Microscope Camera.

Results and discussions

Botanical values

Azalea collection / *Rhododendron* genus

The terms azalea and rhododendron designate taxa belonging to the genus *Rhododendron* L., which includes over 1,000 species [GIBBS & al. 2011] organized in different systematic units, depending on morphological characteristics.

The species are distributed in nature from the arctic regions to the tropics, in areas with very different climates. The greatest diversity of species of the genus *Rhododendron* is found in the eastern area of the Himalayas, but their natural distribution area extends from southeast Asia (Indo-China, Korea, Japan and Taiwan) to northern Australia. A much smaller number of species are distributed in North America and Europe (*R. ferrugineum* L., *R. luteum* Sweet, *R. ponticum* L.). Approximately one third of the known species are united in a group of tropical rhododendrons called "vireyas". They develop mainly in warm climate areas of Borneo, New Guinea, Sulawesi, Sumatra, Philippines [CĂPRAR & al. 2014].

In Romania, only one representative of this genus grows spontaneously – *R. myrtifolium* Schott & Kotschy (syn. *R. kotschyi* Simonk., Figure 4), frequently called [BORZA, 1968] **smârdar** or **bujor de munte** (mountain peony). It is a protected plant that grows in the subalpine zone of the Eastern and Southern Carpathians. The flowers have therapeutic properties and are also used for culinary purposes.

As the name suggests, rhododendrons are trees or shrubs remarkable for the presence of bell-shaped (campanulate), trumpet/chalice (hypocrateriform) or funnel-shaped (infundibuliform) flowers. The rough leaves have dimensions that can vary from 1(2) to approximately 70 cm in length.

Despite the undeniable beauty of the flowers, azaleas are toxic, due to their bitter, astringent and narcotic properties. Over time, cases of poisoning have been reported, either due to honey or nectar, and this fact has been explained by pharmacognosy studies. The toxicity of the species is due to the presence of grayanotoxin, a diterpene that in high concentrations affects the gastrointestinal, cardiac and central nervous systems. Most cases of poisoning are known as mad honey disease, as it is due to the ingestion of honey contaminated with grayanotoxin [JANSEN & al. 2012].

Most of the taxa in the greenhouse collection are indoor azaleas, sometimes called Indian azaleas by horticulturists. The name alludes to the area of the islands in the Indian Ocean (especially Java), from where the first azaleas were brought to Europe, but that has often created confusion regarding their origin [TEODORESCU, 2003]. Amateur horticulturists still use the name *Azalea indica* for both *R. indicum* (L.) Sweet and *R. simsii* Planch., because the first specimens of *R. simsii* were exported to England after 1800 (probably 1808, WILSON, 1921) under the name *indicum*. These are the best known of the parental species of most indoor azaleas, to which are added the lesser-known R. *scabrum* G. Don and *R. mucronatum* (Blume) G. Don [HEURSEL, 1994], and recent genetic studies also support the contribution of the species *R. ripense* Makino [KOBAYASHI & al. 2021].

Rhododendron simsii is probably the most studied of the few azalea species suitable for indoor cultivation and is accepted as the main ancestor of modern varieties [CHRISTIAENS, 2014]. The species is native to East Asia, where it grows in the hilly areas of China, Thailand, Laos and Myanmar at altitudes of 1000-2600 meters. Once it reached Europe, in the early 19th century, the species was subjected to hybridization, over time obtaining a large number of horticultural varieties. Belgium and Germany are the European countries suitable for the title of leader in the production of hybrids.

There are over 25,000 horticultural varieties in the world [LESLIE, 2004], but of these only a few hundred are involved in the indoor azalea trade.

The qualities of cultivars that are constantly being improved in the horticultural field refer to the shape and color of the flowers, the shape of the leaves, the vigor of growth, the general appearance of the plant, the precocity of flowering, as well as the resistance to diseases and pests [CHRISTIAENS, 2014].

In order to be able to describe and identify the varieties of azalea, artificial classifications of flower types have been developed, the varieties obtained through horticultural improvement having intermediate or combined characters. One of the most frequently used classifications [LEE & al. 1952] presents 6 types of flowers (Figure 2):

- Single – the most frequent form, with five petals and stamens in multiples of five;
- Semi-double – some stamens are “transformed in petal-like structures, so that the true petals and the transformed stamens look substantially alike”;
- Double – some stamens are transformed in petal-like structures;
- Single hose-in-hose – combination of the two types;
- Semi-double hose-in-hose – combination of the two types;
- Double hose-in-hose – combination of the two types.

The term hose-in-hose refers to a mutation affecting flower appearance, present in cultivars of the Kurume group. In this case, the sepals are converted into petaloid sepals, so the first rosette (usually of the calyx) has an appearance much more similar to the petals [CHEON & al. 2016]; this shape is genetically determined.

The difficulties in completing the database of *R. simsii* cultivars are illustrated by the situation of the cultivar 'Violacea'. It was commercialized [VERKADE, 1948] and described in the literature as having burgundy flowers [SHEN, 2004], but is not listed in the international register [LESLIE, 2004].

Table 1. Peculiarities of some azalea varieties from the Botanical Garden Iași collection

Cultivar	Synonyms	Year of registration	Parentage	Form / horticultural group
'Aquarell(e)'		1992	Sport of 'Helmut Vogel'	Semidouble / Indian
'Concinnum'		1848/1849	unknown	Single / Indian
'De Waele's Favorite'		1958	Radiation-induced mutation of 'Knut Erwén'	Semidouble / Indian
'Eri Schäme'	'Eri', 'Eric(h) Schäme', 'Pink Ruffles'	1928	Sport from 'Paul Schäme'	Semidouble / Indian
'Henny'		1955	unknown	Semidouble
'Hexe'	'Firefly'	1878 (?)	'Duc de Nassau' × 'Amoenum'	Single hose-in-hose / Indian
'Hino-Crimson'		1944	'Amoenum' × 'Hinodegiri'	Single hose-in-hose / Kurume
'Pax'		1923	'Président Comte Oswald de Kerchove' × 'Madame Petrick'	Double / Indian
'Petrick Alba'	'Schepen's Kerstperel'	1915	Sport of 'Madame Auguste van Damme'	Semidouble / Indian
'Robert van Oost'		1941	'Theodor Findeisen' × 'Paul Schäme'	Double / semi-double / Indian
'Rolko'	Enzett Rolko	1968	Artificially induced mutation of 'Ernst Thiers'	Single / Indian
'Sachsenstern'		1996	unknown	Single / Indian
'Terra Nova'		1994	Sport of 'Helmut Vogel'	Double/ Indian

The chromatic palette of the corollas of the cultivars in the collection covers a narrow group of shades. This is due to the parental species that generally has a large share in the genetic heritage – *R. simsii*. In nature, specimens with flowers with shades from vermillion to red have been observed [HANG & al. 2010]. The specimens of this species used in horticultural breeding have colors determined by anthocyanins and flavonols that influence the decorative qualities of the obtained cultivars. In order to better study the different characteristics related to the pigments that give color, the cultivars of *R. simsii* have been divided into 6 categories: white, red, carmine, pink, violet (purple) and lilac [HEURSEL, 1981]. Other characteristics of this species valued in breeding practice are early flowering and the ability to produce natural variations (sports).

The cultivars in the greenhouse collection have white ('Pax', 'Petrick alba'), pink ('Terra Nova'), cinnabar red ('Robert van Oost'), carmine red ('Hino-Crimson'), purple ('Concinnum') or bicolor ('Aquarelle', 'De Waele's Favorite', 'Eric Schäme', 'Sachsenstern') corollas.

Most varieties have as their main ancestor the species *R. simsii*, so they retain many morphological peculiarities, including those related to the indument. *R. simsii* belongs to the Tsutsusi section, characterized by the presence of simple hairs and stipitate glands [CHAMBERLAIN & RAE, 1990]. These are responsible for the irritating sensation it causes to people who come into contact with the leaves of these plants (Figure 1).

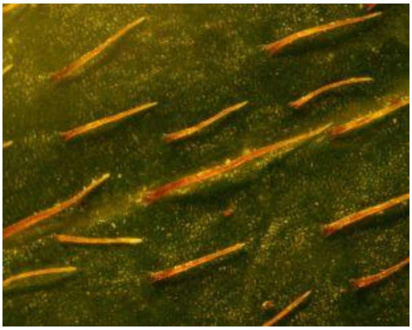

Figure 1. *Rhododendron* 'Hexe'
Leaf: trichomes on dorsal face
135 x (original)

From the genus *Rhododendron*, the species *R. decorum* Franch. and *R. luteum* Sweet are also cultivated in the greenhouses of the Botanical Garden Iași, both obtained through international exchange of botanical material. Since 1998, some taxa of the genus are available for exchange of vegetal material through the Seed Catalogue (1998).

Camelias collection / *Camellia* genus

The genus *Camellia* L. groups about 230 species originating from forested areas of tropical and subtropical Asia, from sea level to altitudes of over 2000 m.

The Latin name *Camellia* is due to the great botanist Carl Linnaeus who wanted to honor the work of the Jesuit missionary Georg Kamel (1661-1706). He spent a large part of his life in the Philippines, where he collected, described and studied numerous plants from the spontaneous flora. He maintained an extensive correspondence with the botanists of the time that he signed Pater Camellus. Georg Kamel never met Carl Linnaeus (who was born in 1707) and apparently never saw a camellia, not even a herbarium. It was not until 1712 that Engelbert Kaempfer described a camellia for the first time, following his trip to Japan, where the plants are designated by the term tsubaki [LOGAN, 1991].

Camellias are small shrubs or trees with evergreen, leathery, glossy leaves; the variously colored flowers are solitary or frequently grouped in groups of 3.

The history of the introduction of camellias into Europe is unclear. Some unproven claims show that the first specimens arrived from Japan in Portugal in the 16^{th} century [TREHANE, 2007]. *Camellia japonica* was introduced as an ornamental plant in Europe in 1793 [BERLESE, 1840], however it seems that before 1745 a camellia specimen was cultivated in Essex (England) obtained from seeds [TREHANE, 2007].

The greenhouse collection includes 15 cultivars from four parental species: *C. japonica* L. (12), *C. cuspidata* (Kochs) Bean, *C. reticulata* Lindl. and *C. sinensis* (L.) Kuntze.

The classification of the thousands of cultivars of this genus is difficult. In his famous monograph (among the first of its kind in Europe), BERLÈSE (1840) proposed their classification according to "general" color into two main categories ("classes"): unicolor and bicolor. Currently, an artificial classification is frequently used that proposes 6 types of flower, which are differentiated according to the characteristics of the petals and stamens. It is usually used for *C. japonica* [camelliasaustralia] and allows the description and identification of cultivars:

- *Single*: a maximum of eight petals in a single row, with an uninterrupted cluster of stamens.
- *Semi-double*: two or more rows of petals, with an uninterrupted cluster of stamens.
- *Irregular semi-double*: a semi-double with an interrupted cluster of stamens. Sometime this type is replaced by rose form double [LOGAN, 1991].
- *Formal double*: any number of petals, regularly disposed, tiered or imbricated, but no visible stamens.
- *Informal double* (previously peony form): a double with any number of petals and petaloids. Stamens may or may not be visible.
- *Elegans form* (previously anemone form): an informal double with one or more rows of large outer petals lying flat or undulating; the centre a convex mass of intermingled petaloids and stamens.

The color palette within the collection is relatively narrow (Figure 3); white (cv. of *C. sinensis*, *C. japonica* 'Alba simplex', 'Snow Ball'), pink (cv. of *C. reticulata*, *C. japonica* 'Elegans'), carmine-red (*C. japonica* 'Alexander Hunter', 'Robert Casamajor'), red ('Dr. King', 'Evelyn'). Three cultivars have flowers with bicolored petals: 'Brusfield's Yellow' (white marginal petals and central petaloid stamens with light yellow shades), 'Elegans Variegated' (pink with irregular white spots) and 'Flashlight' (white petals with irregular deep pink striations).

It is worth noting that some varieties are quite old: 'Alba Simplex' – 1816-1817, 'Elegans' – 1831, 'Adolphe Audusson' – 1910.

Since 2013, some camellia specimens have been grown in soil, thus providing their roots with a much more generous space than in a regular pot. Under the new conditions, these specimens have had much more vigorous and rapid development, reaching heights of over 3.5 m. Since 1991, some taxa of the genus are available for botanical material exchange through the Seed Catalog of Iași Botanical Garden (1991).

WANG & al. (2021) have documented the difficulties in identifying camellia cultivars, mainly due to the large number of synonyms in the specialized literature. According to the authors, the largest number of synonyms (121!) belongs to a variety obtained in 1788 ('Masayoshi'). The variety 'Elegans', present in the Botanical Garden of Iași collection, apparently has 68 synonyms.

Other problems affecting precise identification are represented by morphological variability that can manifest itself "among specimens grouped in the same cultivar" or "within a single plant" [COUSELO & al. 2010]. TREHANE (2007) notes that the shape of the corolla itself can vary within the same cultivar, depending on the characteristics of the climate in which it is grown ("formal double" flower in "cool, wet climates", but "peony-form" in "warmer, drier conditions").

Horticultural values

In the inventory of the Greenhouse Complex, azalea and camellia taxa have always been considered to belong to a "specialized collection" of great horticultural importance. That is why LAZĂR (1982) grouped them in the theme "Horticulture", considered one of the four themes that must be developed in a botanical garden (alongside "Botanics", "Ethnobotany" and "Relaxation and rest").

RHS specialists state that it is difficult to cultivate indoor azaleas, as does TOMA (2009), contrary to what SADOFSKY & MILIȚIU (1966) recommended. However, it seems that their optimism was not justified, because after about 60 years, the cultivation of potted azaleas is quite rare among amateur horticulturists in Romania.

The fact that these plants have been successfully grown in Botanical Garden of Iași greenhouses for over 50 years is nevertheless proof that they are worth introducing into the assortment of plants cultivated and used for interior decoration, but certain rules must be observed.

Successful cultivation of indoor azaleas requires compliance with three main rules: the use of an acidic substrate, ensuring high atmospheric humidity (over 70%) and avoiding high temperatures (heat waves). Compliance with these ecological requirements is mandatory not only for the survival of the specimens, but especially for the development and flowering of the plants, a phenomenon that occurs in the winter months [TEODORESCU & MITITIUC, 2002].

The conditions in the greenhouse are constantly influenced by the natural and climatic conditions of the city of Iași, especially when the microclimate is little artificially controlled, in the period late spring - summer - early autumn (June-September), in the absence of heating. The relatively strong temperature fluctuations throughout the year must be compensated by different methods: increasing the temperature – in cold periods, decreasing the temperature – in hot periods. A very important aspect, but difficult to control, is represented by atmospheric humidity. In the climatic conditions of the city of Iași-Romania, this aspect becomes a real challenge, because in July and August the atmospheric humidity drops a lot, in accordance with the excessive continental climate that manifests itself in this area. In order to prevent damage to the plants and especially the formation of flower buds, it is necessary to intervene with additional watering or ensure artificial fog conditions that ensure the necessary humidity of 70-80%. In addition, these measures have the secondary effect of lowering the temperature by a few degrees.

Among the numerous specimens (over 500) of the collection are some that are over 45 years old and impressive in size: azaleas with a crown of about 3 m in diameter or camellias with a trunk over 2 m high. Their true value is expressed in the flowering period, which is not uniform. Some varieties of azaleas and camellias have early flowering, others late, so that the flowering period is not synchronous, but extends over a period of 3-4 months (December-March).

Several specimens of azaleas and camellias have been selected to be cultivated in bonsai style, thus becoming part of the collection that holds over 200 plants from 95 taxa.

Historical values

The Exotic Plants Exhibition is an annual event organized within the Greenhouse Complex section of the "Anastasie Fătu" Botanical Garden of the "Alexandru Ioan Cuza" University of Iași. The initial nucleus is represented by the collection of remarkable horticultural value of azaleas and camellias. The maximum flowering period of these plants is between January and March, this being the only time interval in which the collection is accessible to the visiting public [LAZĂR, 1988; TEODORESCU, 2003].

It is very likely that the azaleas and camellias exhibition organized during the winter period, starting with 1975 [MITITIUC & TONIUC, 2006], represents the first exhibition event organized annually, dedicated to scientific collections in a botanical garden in Romania.

Currently, this event is part of the cycle of thematic exhibitions with flowers, fruits and seeds, and contributes essentially to the uniqueness of the "Anastasie Fătu" Botanical Garden of the "Alexandru Ioan Cuza" University of Iași, and thus individualizes it in relation to similar institutions in the country and abroad. Through these traditional events, the team of the Botanical Garden of Iași offers scientific and cultural support, both to the community of Iași, as well as to visitors from the country or abroad [PETRE & IFRIM, 2016].

The beginning of these events was initially marked by the introduction, for didactic and research purposes, of a small number of azaleas and camellias, in the spaces of the greenhouse complex that were intended for their cultivation and maintenance, rather than for presentation to visitors in the form of "micro-exhibitions" [LAZĂR, 1988]. Over time, the exposure for visiting acquired the proportions of an exhibition event, especially after 2000.

Between 1975-2008 (Figures 5, 6), the azaleas and camellias were exhibited to the public in small spaces, specifically intended for the maintenance of these collections of plants with special ecological requirements. The small dimensions and narrow access paths had the advantage of offering visitors a direct approach to the plants, but they had the disadvantage of a lack of perspective and fragmentation of the overall image of the collection. During the flowering period, the azaleas and camellias were given special care, which highlighted their special decorative character. Often this aspect was enhanced with the help of conifers that harmoniously completed the aesthetic image. Throughout this period, the events were held under the generic name of the *Azaleas and Camellias Exhibition*, except for 2008 when it was called *The Magic of Flowers*.

Starting with 2009, these events took place in the much more generous space of the exhibition greenhouse. Along with the main collections on display (azaleas and camellia cultivars), visitors could admire specimens belonging to numerous groups of exotic plants: orchids, peperomias, begonias, phytonias, arrowroots, crotons, euphorbias, crotons, dracaenas, ferns, citrus, cacti and succulents.

A prefiguration of the holding of exhibitions in a broader concept can be considered the manner in which, within the framework of the autumn exhibitions, until 2005, there was permanently a space intended for the exhibition of plants cultivated in the Greenhouse Complex. There, year after year, attractive specimens were exhibited to visitors, selected according to ornamental, but also ecological, biogeographical or taxonomic criteria.

Between 2009-2013, the event called *Messages with Flowers*, organized in the rehabilitated space and with a palette enriched with plant material, allowed for the diversification of the themes proposed to visitors.

During the 2011 edition (Figure 7) of the exhibition called *Messages with Flowers*, a message and photo contest dedicated to flowers was organized with the aim of promoting interest in the diversity of plant species in the collections of the Botanical Garden of Iași and inviting visitors to an exercise in imagination through which to express their own vision of the plant elements in the exhibition.

Under the same generous name – *Messages with Flowers* – subsequent editions were with the theme of the symbols of spring (2012), of the symbols of the Far East, inextricably linked to the plant collections through their Asian origin (2013). In 2015, the event was held with the theme of Dragobete (Romanian traditional feast in February), Mărțișor (Romanian traditional feast in March) and Chinese New Year.

The 2014 edition marked the inauguration of 2 new compartments intended for the cultivation of azaleas and camellias, spaces where the 2016 edition was organized, an event that opened the cycle of anniversary events dedicated to the 200th anniversary of the birth of naturalist Anastasie Fătu, founder of the first botanical garden in Romania, a garden that celebrated 160 years of existence.

In the exhibitions from 2015-2020, visual artists from Iași exhibited paintings or artistic installations under the theme Heart on the Symes. The parallel holding of these cultural events stimulated the artistic expression initiatives of the garden's landscapers, who used plant remains, represented by dry roots, stem bark, leaves, flowers or dried fruits, but also rocks or natural fibers, with which they created very refined decorative elements. The sources of inspiration were diverse: love (2016 edition), birds in the culture of peoples (2018 edition) or Japanese gardens (2019 edition, Figure 8).

In 2020, plants from the flora of Japan were selected for the landscape arrangements, as well as special specimens from the collection of tropical ferns. On this occasion, a permanent exhibition was inaugurated with species and hybrids of carnivorous plants, originating from different areas of the world. Also at that time, a new element was represented by the creation of vegetable walls made of perennial grass plants with contrasting foliage (species from the genera *Begonia*, *Fittonia*, *Peperomia*, *Pilea*, *Saxifraga*).

In 2021, due to restrictions during the pandemic, the exhibition could not be visited, as the greenhouses were closed, therefore the landscaping inspired by Asian gardens, made with exotic plants or, in the case of dry ones, made of gravel, stone blocks and sand, as well as artistic installations made of plant waste, inspired by the world of insects, were viewed online.

The exhibitions in the following years brought new elements from Asian culture to the attention of visitors, for example, plant and animal symbols in 2022, elements from Asian philosophy in 2023, iconic images of the Sino-Japanese space (2024). They were also exhibited in a diorama where habitats for species that show impressive adaptations to the environment are suggested, representative specimens from the Bromeliaceae family, which also includes the pineapple.

The year 2025 marked the 50th edition, held under the theme *Flowers in Celebration*, in which *Camellia* varieties were highlighted, as well as the ecological requirements of some plant groups.

Educational values

For those who know the history of the Botanical Garden of Iași, the phrase "giunimea studioasă" / "studying youth" is well known [BURDUJA & TOMA, 1979] and we consider it appropriate to point out a few aspects that highlight the instructive-educational value of the collection in different periods of time in an academic context.

The Botanical Garden is permanently a place of instruction, but the exhibition event mentioned is a special opportunity to educate the public through informal means. On this occasion, the educational act is carried out through a harmonious combination of information and affect. The design of the exhibition, each time different, has a double function: to ensure a special emotional state, but also to provide a special framework for mnemonic performances in the process of acquiring scientific information that accompanies the exhibits or that is transmitted in the exhibition context.

This information comes from very diverse fields: taxonomic, utilitarian, phytogeographic, horticultural, ecological, ethnobotanical, artistic, etc. Those from the taxonomic and utilitarian fields, for example, are presented in the form of written texts, those from the phytogeographic, horticultural or artistic fields are deduced from the location of the plants, their appearance or the accompanying decorative elements. It becomes obvious that the exhibition event can be considered important for the activity of ecological education, by capitalizing on informal learning techniques. Thus, the exhibition, as a specific manifestation within the botanical garden, is not "just an attraction" [WILSON & GREENE, 1994], but becomes a social and permanent learning space [WOLLENTZ & al. 2022].

The scientific value of the collection with an age of over 50 years is relevant especially in relation to the Romanian botanical context of the equivalent time period.

Camellia flowers (species *C. japonica*) were known in the Romanian space quite quickly after their entry into Europe (circa 1780). As we will see, the flowers became a real fashion around 1865, after which in a few decades they entered a shadow cone. Interestingly, they seem unknown for a long time in the field of Romanian taxonomy, the Theaceae family to which they belong not even being mentioned in the monumental work Flora RPR-RSR [ȚOPA, 1960], although *Thea sinensis* (syn. *Camellia sinensis*) is a species of economic importance worldwide. Unfortunately, the situation remains unchanged in the valuable work of CIOCÂRLAN (2000), although here we find much less known species such as: *Anthurium scherzerianum* or *Cycas circinalis*. It is likely that this fact is related to the lack of interest in the Romanian space towards tea consumption, almost non-existent until a few decades ago; in any case, far behind coffee consumption (*Coffea* sp.).

As for azaleas, their presence in specialized works reflects the pace at which they have become increasingly well-known. The Ericaceae family (to which they belong) has 13 representatives from 6 genera in the spontaneous flora, including the genus *Rhododendron*. The species *R. simsii* is mentioned only in the 2000 [CIOCÂRLAN, 2000] volume, not in Flora RPR [ȚOPA, 1960]. CIOCÂRLAN (2000) mentions that *R. indicum* and *R. simsii* (both synonyms of *Azalea indica*) are grown in cold greenhouses, but does not specify that these are actually horticultural varieties, in no way spontaneous species.

We discover inaccuracies regarding azaleas, especially in specialized works in the horticultural field. Thus SADOFSKY & MILIȚIU (1966) show regarding azaleas that "*some* of them also grow in our mountains ...", although it is a single species. TOMA (2009) mentions varieties of *R. simsii* with yellow and orange flowers, but these probably belong to other species of the genus, since we have shown above that these colors are not characteristic of indoor azaleas. In addition, in the protocols established for the characterization of the color of the corolla of varieties of this species, only 9 groups are mentioned: white, three shades of pink, three shades of red, purple and violet [CPVO-TP, 2007], a diversification from previous classifications which only proposed 4 categories: purple, carmine red, red and white [HEURSEL, 1994]. Confusion regarding the taxonomy of the genus *Rhododendron* as well as the differentiation of horticultural groups are also found in works in the field of botany / plant anatomy [ANDRICI & al. 2005].

This lack of clarity of information is unexpected, if we consider that as early as 1864 a list of plants offered for sale was published in Iași, among which we find the species *Camellia japonica* "(in Sorten)", *Rhododendron* (!) *arboreum* and *R. hibrida* in the category "Plantes de serre froide / Kalthauspflanzen".

Phytonymy aspects

The popular names "azaleas" and "camellias" seem familiar nowadays, this type of plant being relatively frequently encountered in horticultural supply or in amateur horticulturist collections. Most likely, the increasingly easy access to information has contributed to this, but this aspect has evolved over time.

It is worth noting that the Romanian phytonyms for species of the genus *Camellia* are much more widespread and well-known compared to those for the genus *Rhododendron*. And this despite the fact that in the spontaneous flora there is a representative of the genus *Rhododendron* – *R. myrtifolium* (syn. *R. kotschyi*), which grows in mountainous areas.

There are probably at least two explanations for this situation.

1. *Rhododendron myrtifolium* is mentioned in the specialized literature with the popular names of smirdar or bujor de munte (mountain peony). Less well-known are the names: bujor (peony), coacăză, cocăzar (gooseberry), merișor (cranberry), trandafir de munte (mountain rose). We note that none of the phytonyms mention the terms rhododendron or azalea. Probably the popular names smîrdar or bujor de munte (mountain peony) did not facilitate the establishment of a botanical connection.

2. The name "camellia" became very well-known due to the numerous editions of the translations of the novel "The Lady of the Camellias".

From the analysis of the available information, it seems that there is no awareness among the uninformed public that the spontaneous species *R. myrtifolium* is from the same family as the azalea varieties found in gardens and flower shops. It is worth noting that the illustrious romanian folklorist Simion Florea MARIAN (2008) presents the phytonym "bujor" (peony) mainly as describing the species *R. myrtifolium* and only mentions that it can also be used to designate other plants, such as *Paeonia officinalis*.

The term azalea appears for a long time mainly in literary works, especially poems [ZAMFIRESCU, 1899; IOSIF, 1939], but the authors probably had information about the plant either from their travels in western Europe (Italy, Austria) where the plants were much better known, or from foreign literature.

In the current period, DRĂGULESCU (2014) mentions the phytonym "azalea" collected from the Sibiu area for the species *R. indicum* or an unknown species of the genus. In addition he mentioned *Azalea indica* as a different taxon, despite he is a synonym of *R. indicum*. It is very likely that actually it is about varieties that have multiple parental species, so once again the difficulty of correctly identifying taxa is evident.

Camellias have a similar status, although they are known at least as the name of a flower due to the numerous editions of the translations of the novel "The Lady of the Camellias".

The image of Elena Cuza - in the memoirs of Lucia BORȘ (1936) - who appears as a great lover of camellias is very valuable.

"In her white dress ..., caught in bouquets of white **camellias**, her figure ... seemed to melt into the shiny black of her rich hair, gathered in the fashion of the Empress of France, Joséphine, wearing those flowers, which were also dear to her, and which represented at that time an entire era of romanticism."

In fact, camellia flowers were for a long time the preserve of the Romanian elite, following the model of the Western aristocracy.

"The fashion of the time gave the shows varied and curious decorations: dresses ..., vests ..., and everywhere natural flowers, worn in women's hair or at the waist, in men's buttonholes or in children's arms, and among them the most precious being the red or white **camellia** according to the taste or the significance given to it." [BORȘ, 1936].

In the same context, it is worth mentioning a composition by Ciprian Porumbescu, originally called "Florile dalbe" and which was dedicated to Mrs. Teresa Kanitz, honorary president of the Romanian students' society in Vienna "România Jună". This piano waltz was performed by Maestro Eduard Strauss with his orchestra at the "România Jună" ball in 1880. Since the conductor found the title incomprehensible, he requested that it be changed to one "that the Viennese would understand" [BORZA, 2012]; thus, the waltz was renamed "The Camellias". This little story illustrates the importance of phytonyms that confer a sense of knowledge and familiarity, as well as their importance in the tradition and culture of a people.

Conclusions

The azaleas and camellias collection represents an important link between generations, on the one hand, but also between specialists and visitors; it largely gives continuity to the existence of the botanical garden, especially in the context in which its turbulent history did not ensure the permanence of its location.

The horticultural and educational value of this collection has been manifested over time and has benefited both specialists and the uninitiated public. The exhibition events held around these collections have represented opportunities for ecological education, by capitalizing on the plant heritage preserved and enriched within the botanical garden. Information from very diverse fields is disseminated through informal education means: taxonomic, utilitarian, phytogeographic, horticultural, ecological, ethnobotanical, artistic, etc. The exhibition venue represents a social space and longlife learning.

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a. Single
('Concinnum')

b. Single hose-in-hose
('Hexe')

c. Double
('Terra Nova')

d. Double hose-in-hose
(not in collection)

e. Semi-double
('De Waele's Favorite')

f. Semi-double hose-in-hose ('Varvaeniana')

Figure 2. Types of *Rhododendron* flowers (a, b, c, e, f - original, d – source www.rhs.org.uk)

Figure 3. *Camellia* flowers from collection: left - *C. cuspidata* cultivar, middle - *C. japonica* 'Snow Ball', right - *C. japonica* 'Elegans Variegated' (original)

Figure 4. *Rhododendron myrtifolium* on Rodna Mountains (photo C. M. Ifrim)

Figure 5. View from 2008 *Azaleas and Camellias* Exhibition (photo V. Delinschi)

Figure 6. View from 1990 *Azaleas and Camellias* Exhibition (photo A. Toniuc)

Figure 7. View from 2011 *Messages with Flowers* Exhibition (photo C. M. Ifrim)

Figure 8. View from 2019 *Exotic Plants* Exhibition (photo C. M. Ifrim)

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Research Article

ANATOMICAL STRUCTURE OF THE PERENNIAL STEM IN DIFFERENT *ROSA* L. SPECIES

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Abstract: The observations comprise a comparative study of the anatomical structure of the perennial stem in the species *Rosa canina* L., *R. damascena* Mill., *R. rubiginosa* L., and R. *rugosa* Thunb., cultivated in the Botanical Garden of Iași.

Cambial activity gives rise to a relatively thin outer ring of secondary phloem and to several rings of secondary xylem, which indicate the age of the examined branch. Within the phloem ring, conducting elements (sieve tubes and companion cells), phloem parenchyma cells (some containing idioblasts), and irregularly dispersed libriform fibres with thickened, lignified walls can be distinguished, these fibres being more abundant in *R. rugosa*. In *R. rubiginosa*, the phloem fibres are arranged in two layers separated by phloem parenchyma. At the periphery of the phloem ring, in contact with the inner layers of the primary cortex, thick bands of sclerenchymatous fibres with very thick, heavily lignified walls (with a punctiform lumen) are visible, especially in *R. canina* and *R. damascena*.

Analysis of the secondary xylem shows its ring-porous aspect in all observed species. Each growth ring displays on its inner side vessels of larger diameter (representing the earlywood), separated by libriform fibres with strongly thickened, intensely lignified walls. On the outer side, each ring contains fewer vessels of smaller diameter, with libriform fibres predominating – a feature common to all examined species. In contact with the pith, remnants of the primary xylem can still be observed, completely lignified and protruding as small bundles into the pith.

All annual rings of secondary xylem are traversed by numerous multiseriate medullary rays, usually of uniform width or variable in *R. canina*. In all cases, the rays consist of radially elongated cells with relatively thin but lignified walls. In all species, the oldest ring of secondary xylem is thicker than the subsequent ones, in which large-diameter vessels predominate.

The pith exhibits a network-like appearance, in which very large, thin-walled parenchyma cells are separated by much smaller cells with moderately thickened but strongly lignified walls.

Key words: anatomy, earlywood, latewood, perennial stem, ring-porous xylem, *Rosa* spp.

Introduction

Species of the genus *Rosa* are shrubs and therefore possess a woody, aerial, multi-stemmed system with sympodial (anisotonic) branching, exhibiting an orthotropic growth direction in most cases [BUIA, 1956] and more rarely a plagiotropic one, with mesotonic branching [KAUSSMANN & SCHIEWER, 1989]. This is complemented by a basitonic branching pattern that appears with variable periodicity, depending on a series of extrinsic factors such as climatically favourable years and an intrinsic hormonal signal that triggers the rejuvenation of the shrub through the formation of new shoots that replace the old ones [ADUMITRESEI, 2011].

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The stem originates in the hypocotyl axis and the gemmule of the seed embryo. After seed germination and throughout the plant's life, the stem grows in length and thickness through the activity of the initial (primary) meristematic tissues and, more importantly, through the activity of the lateral meristems – the cambium and phellogen – of secondary origin, which appear later. These secondary meristems act as two generative zones, giving rise to the definitive secondary tissues [TOMA & GOSTIN, 2000].

In exceptional cases, the stem may reach remarkable dimensions; the largest recorded specimen is a *R. canina* from the Cathedral of Hildesheim, with stem circumferences of 7-8 m and a crown projection of 13 m, its age exceeding 300 years [HEGI, 1923].

The oldest stem was 13-year-old [MESTRE & al. 2014], most of them though stems within a *Rosa* shrub are 4-5 years old. For this reason, we analysed their anatomical structure, especially since such studies are scarce in the specialized literature [PASHINA, 2021] maybe because many of the rose species are difficult to cut using classical methods. The majority of anatomical investigations focus on the primary and secondary structure of the annual shoot, both in classical works [PARMENTIER, 1898; FAHN, 1968; METCALFE & CHALK, 1988] and in modern studies, addressing all aspects from the histological structure of the stem [TOMA & al. 1997; ADUMITRESEI & TĂNĂSESCU, 2005; TÜRKER & al. 2005], to that of the leaf [ADUMITRESEI & al. 2005; ADUMITRESEI & al. 2006; AL-DOSKEY, 2023; ADUMITRESEI & al. 2024], fruit and seed [STARIKOVA, 1973; CHURIKOVA & BARYKINA, 2005], and appendages [ZHOU & al. 2021; ADUMITRESEI & al. 2024].

Materials and methods

Plant material

A comparative study of the anatomical structure of the perennial stem was conducted on *Rosa canina* L., *R. damascena* Mill., *R. rubiginosa* L., and *R. rugosa* Thunb., all cultivated in the Botanical Garden of Iași. Stem fragments were collected from mature shrubs and used for anatomical analysis.

Sample preparation

The samples, consisting of small stem segments, were rinsed several times with distilled water to remove residual ethyl alcohol from preservation. The material was subsequently kept in clean water for a minimum of 2 hours to ensure full rehydration.

Sectioning procedure

Stem fragments approximately 1.5 cm in length were sectioned using a Slee cryotome equipped with a Peltier-based freezing system. The material was frozen at -26 to -28 °C until completely embedded in ice. Transverse sections were obtained at thicknesses of 30-40 µm.

Staining

Histological staining was performed with iodine green for 30 seconds. Sections were then rinsed with ethyl alcohol to remove excess stain, followed by a final rinse in distilled water.

Microscopy and Imaging

Prepared sections were examined using an Olympus BX41 light microscope. Photomicrographs were captured with an Olympus C330 digital camera.

Results and discussion

The secondary structure is especially evident at the level of the central cylinder [TOMA & al. 1997]. The vascular bundles (xylem-phloem bundles) persist within the secondary structure, arranged in a ring and separated by typically narrow medullary rays typical of many *Rosaceae* [FLORIA, 1998; NIJSSE & PUT, 2007; NIKITIN & PANKOVA, 1982].

The phloem contains sieve tubes, companion cells, and few phloem parenchyma cells. The xylem shows vessels arranged in radial rows, separated by cellulosic parenchyma cells toward the pith and by lignified parenchyma and libriform fibres toward the phloem. At the periphery of each vascular bundle, adjacent to the phloem, there is a thick band of sclerenchymatous fibres with very thick but only weakly lignified walls, similar to those of the libriform elements. The vessels typically exhibit simple perforations. According to METCALFE & CHALK (1988), some rose species may show ring-porous or semi-ring-porous xylem, as well as spiral thickenings on the vessels. The paratracheal (vasicentric) parenchyma cells are palisade-like and elongated, with thin walls; their radial walls contain numerous simple, elliptical pits.

In *Rosa*, the multiseriate rays are partly homogeneous, composed of nearly square cells, and partly heterogeneous of the Kribs II type (marked heterogeneity), consisting of square to rectangular cells. Typically, *Rosa* presents 3-5 rays per mm. Growth rings were described by various authors [COSTER, 1927; METCALFE & CHALK, 1988; SCHOCH & al. 2004] as being present, but without distinctive features.

The pith is fully developed even during the herbaceous stages of the annual shoot. It is composed of cells of varying size – large and small – alternating to form a network-like structure, as reported for all species studied by various authors [ADUMITRESEI, 2011; DELINSCHI & al. 2009; LOTOVA & TIMONIN, 1999].

Macroscopically, in transverse section, the "wood" structure of shrubs resembles that of trees; however, clear distinctions between heartwood and sapwood are not evident. Microscopically, it is composed of cellulose, hemicellulose, or lignin fibres that provide mechanical strength and rigidity, and of vascular tissue arranged in a specific configuration through which crude and elaborated sap circulate. These tissues are often associated with parenchyma cells with thickened walls, which contribute to both support and nutrient transport [GLEMEZIU & SUCIU, 1959; ZHANG, 1992].

The following characteristics were observed in transverse sections of the perennial stem and are described below.

***Rosa damascena* Mill.**

Cambial activity gives rise to a relatively thin outer ring of secondary phloem and a number of secondary xylem rings, which indicate the age of the analysed branch.

Within the phloem ring, conducting elements (sieve tubes and companion cells), phloem parenchyma cells (some containing idioblasts), and irregularly dispersed libriform fibers with thickened, lignified walls can be distinguished. At the periphery of the phloem ring, in contact with the inner layers of the primary cortex, thick bands of sclerenchymatous fibres with very thick, heavily lignified walls (with a punctiform lumen) are visible.

Analysis of the secondary xylem shows that it is ring-porous. Each growth ring exhibits vessels of larger diameter on the inner side (representing earlywood), separated by libriform fibers with strongly thickened, intensely lignified walls. On the outer side, each ring contains fewer and smaller vessels, with libriform fibres predominating (Plate II, Figures 7-9).

In a transverse section of a 4-year-old branch, the oldest ring, located adjacent to the pith, contains the smallest-diameter vessels. In this ring, early and latewood are difficult to distinguish, the main difference being the large proportion of libriform fibres in the latewood.

In contact with the pith, remnants of primary xylem are still visible, fully lignified and protruding as small bundles into the pith.

All annual rings of secondary xylem are traversed by numerous multi-seriate medullary rays, composed of radially elongated cells with relatively thin but lignified walls.

The pith exhibits a network-like structure in which very large parenchyma cells with extremely thin walls are interspersed with very small cells with moderately thickened, heavily lignified walls, many of which function as true hydrocysts [ADUMITRESEI & al. 2024] as described by CHURIKOVA & BARYKINA (2005).

***Rosa rubiginosa* L.**

Compared to the previous species (*R. damascena*), only the observed differences are highlighted. In *R. rubiginosa*, within the thickness of the secondary phloem ring, the libriform fibers are arranged in two layers forming tangential chains, separated by phloem parenchyma cells (Plate I, Figures 4-6).

Regarding the secondary xylem, it is noteworthy that the oldest ring, adjacent to the pith, is considerably thicker than the subsequent four rings. In these younger rings, earlywood and latewood are more easily distinguished due to the differences in the transverse size of the vessels, which are otherwise irregularly distributed within the fundamental mass of libriform fibres.

***Rosa canina* L.**

In the secondary phloem ring of *R. canina*, very few libriform fibres are present, while the periphloemic bands of sclerenchymatous elements are thicker (Plate I, Figures 1-3).

In this species too, the internal secondary wood ring is thicker than the others and has smaller diameter vessels throughout its thickness.

In fact, in all species studied, the oldest ring of secondary xylem is thicker than the following rings, in which large-diameter vessels predominate.

The medullary rays vary in width, ranging from biseriate to multiseriate [ADUMITRESEI & al. 2006] as opposed to SCHOCH & al. (2004).

***Rosa rugosa* Thunb.**

The structure of *R. rugosa* is more similar to that of *R. damascena*. Within the thickness of the secondary phloem ring, numerous libriform fibres are present, though irregularly dispersed (Plate II, Figures 10-12).

The borders between annual rings are much clearer, with wider multiseriate rays predominating compared to the other species. In this case as well, the first annual ring is considerably thicker; within it, early and late wood can still be distinguished due to the marked difference in vessel diameter.

Conclusions

The secondary xylem is ring-porous in all species studied.

The annual rings of secondary xylem are traversed predominantly by multiseriate medullary rays. *Rosa canina* is notable for also exhibiting biseriate medullary rays.

The distinction between earlywood and latewood is determined both by vessel diameter and by the amount of libriform fibres. Latewood contains vessels of smaller diameter and a correspondingly higher proportion of libriform fibres.

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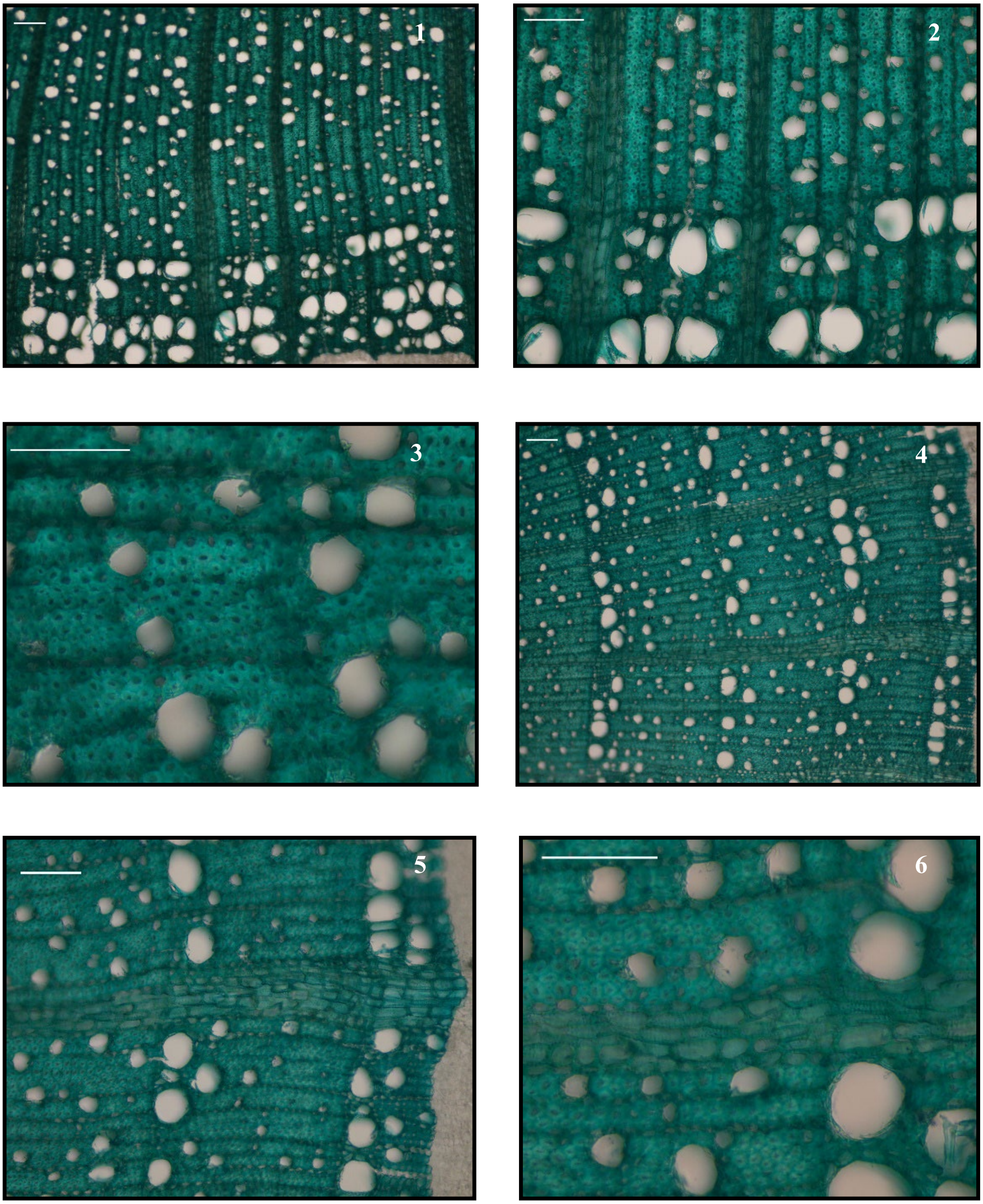
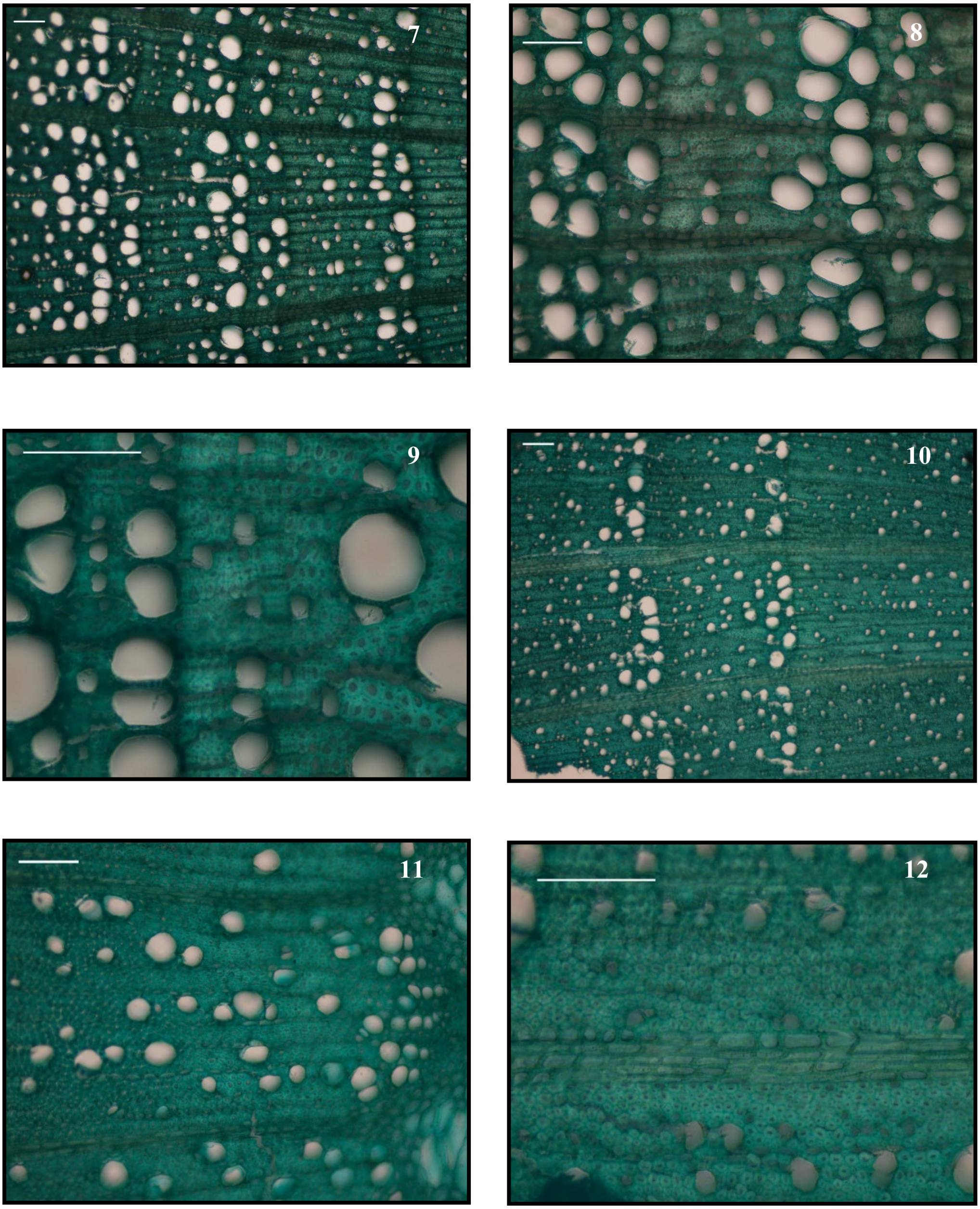
Plate I



Figures 1-6. Anatomical aspects of the upper third of the stem in *Rosa* L. species – cross section through the stem of: 1-3 *Rosa canina*; 4-6 *Rosa rubiginosa* (scale = 100 µm)

Plate II



Figures 7-12. Anatomical aspects of the upper third of the stem in *Rosa* L. species – cross section through the stem of: 7-9 *Rosa damascena*; 10-12 *Rosa rugosa* (scale = 100 µm)

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Research Article

ASSESSMENT AND SUSTAINABLE CONTROL OF WHITE RUST (*PUCCINIA HORIANA* HENN.) IN THE *CHRYSANTHEMUM* CULTIVARS COLLECTION OF THE IAȘI BOTANICAL GARDEN (ROMANIA)

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Abstract: Chrysanthemum white rust (CWR), caused by *Puccinia horiana* Henn., is a quarantine disease that poses a serious threat to chrysanthemum production under protected cultivation. This study aimed to evaluate the incidence, severity, and cultivar-specific responses to CWR in a greenhouse collection of *Chrysanthemum* × *grandiflorum* Ramat. at the "Anastasie Fătu" Botanical Garden of Iași, Romania. A total of 35 cultivars (1,120 plants) were monitored under uniform greenhouse conditions between 2020 and 2023 growing seasons. Disease assessment was based on systematic visual inspections, microscopic confirmation of the pathogen, and quantitative evaluation using Disease Incidence, Disease Severity Index, and standardized Combined Score integrating both parameters. Cultivars were classified into susceptibility categories ranging from tolerant to highly susceptible. In 2020, disease incidence ranged from 0% in resistant cultivars to over 90% in highly susceptible ones, with corresponding variations in disease severity. The implementation of integrated management strategies, including cultural practices, curative fungicide applications at 13 and 15 weeks after planting, and preventive treatments at 5 weeks after planting, resulted in a significant reduction in disease incidence and severity in 2021. Continuous monitoring combined with preventive treatments in 2022 and 2023 led to the complete suppression of CWR, with no visible symptoms observed across all cultivars. Several cultivars consistently exhibited resistance throughout the study, while others showed high susceptibility under initial disease pressure. The findings demonstrate that the integration of resistant cultivars with early preventive measures and continuous monitoring is essential for the sustainable management of *P. horiana* in greenhouse chrysanthemum production. This study provides a practical framework for disease assessment and supports informed cultivar selection and integrated disease control strategies for ornamental crops.

Keywords: chrysanthemum horticultural varieties, disease management, *Puccinia horiana*, resistance, white rust.

Introduction

Chrysanthemum is an important traditional ornamental crop, ranking among the four most widely produced cut flowers globally, ranking second globally in the cut flower trade, after rose [MEKAPOGU & al. 2022, SUN & al. 2011]. The crop is highly susceptible to a range of diseases, including *Alternaria* leaf blight, brown rust, white rust, wilt, bacterial blight, and various viral and viroid infections [TROLINGER & al. 2018]. Among these, *Chrysanthemum* white rust (CWR), caused by *Puccinia horiana* Henn., is particularly destructive, posing a major threat to commercial cultivation. This disease has a significant impact on chrysanthemum production worldwide [DICKENS, 1990] and is recognized as a quarantine pest in many

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countries, often triggering strict eradication programs upon detection. Characterized by its rapid spread and severe effects on both ornamental quality and market value, *P. horiana* represents a major challenge for growers and botanical collections [ZENG & al. 2013].

Chrysanthemum white rust (CWR), caused by *P. horiana* Henn., is one of the most destructive fungal diseases affecting chrysanthemum crops worldwide. The pathogen is indigenous to Japan, where it was first reported in 1895, and remained confined to China and Japan until 1963. Since 1964, it has spread rapidly through the international trade of infected cuttings and is now established across Europe, Africa, Australia, Central and South America, and the Far East. The global dissemination of *P. horiana* is facilitated by the high susceptibility of chrysanthemum cultivars and favorable microclimatic conditions that promote epidemic development [BONDE & al. 2015; EPPO, 2025; USDA-APHIS, 2020, 2025].

The disease significantly impacts both ornamental quality and commercial value, leading to major economic losses in the floriculture industry. As a quarantine pathogen in many countries, its detection triggers strict regulatory measures and eradication programs to prevent further spread. The obligate biotrophic nature of *P. horiana* makes early detection and accurate diagnosis critical for effective management. Botanical gardens, nurseries, and commercial cultivations are particularly vulnerable due to dense plantings and the presence of susceptible cultivars. Understanding the pathogen's biology, epidemiology, and host interactions is therefore essential to develop sustainable control strategies and to safeguard genetic diversity in chrysanthemum collections [ZENG & al. 2013; KUMAR & al. 2021].

In Romania, the growing popularity of chrysanthemum cultivars in horticulture and public gardens increases the necessity for rigorous disease monitoring and management - especially within conservation-oriented institutions. The *Chrysanthemum* collection of the "Anastasie Fătu" Botanical Garden of Iași represents a valuable scientific, educational, and ornamental resource, a reservoir of genetic diversity and a national reference for horticultural practices. Safeguarding the health of this collection demands precise detection, thorough characterization, and the development of sustainable control strategies against pathogens such as *P. horiana*.

According to the EPPO Global Database (2025), *P. horiana* is reported in Romania with the status "present, restricted distribution." The first documented occurrence was recorded in 1995 in Vidra, Romania [COSTACHE & COSTACHE, 1995], with subsequent reports appearing in later studies, including NEGREAN (2003), and NEGREAN & ANASTASIU (2009). No recent peer-reviewed scientific articles from Romania reporting experimental data on *P. horiana* were found in accessible databases, and most sources consist of internal reports, popular articles, control guides, or phytosanitary observation records. Overall, the situation in Romania appears to reflect mainly presence and risk assessments rather than systematic research [BUTA & al. 2011], indicating a lack of recent experimental studies or limited publication in international journals.

Puccinia horiana Henn. is a rust fungus belonging to the order Pucciniales (formerly Uredinales), family Pucciniaceae. Unlike many rusts that complete their life cycle on two unrelated hosts, *P. horiana* is an autoecious microcyclic rust fungus, completing all developmental stages on chrysanthemum hosts [BONDE & al. 2015]. The pathogen forms characteristic buff-white telia that differentiates it from other rust fungi, notably *Puccinia chrysanthemi* Roze, which produces orange-brown uredinia [DEMERS & al. 2015; CUMMINS & HIRATSUKA, 2003].

The pathogen is an obligate parasite and cannot be cultured on artificial media. Infection begins when basidiospores, produced from germinating teliospores, penetrate leaf tissues through stomata. High humidity (>95%) and temperatures between 10-20°C are key factors supporting infection, sporulation and secondary spread [RHS, 2025]. After penetration, the fungus develops an intercellular mycelium that forms haustoria in host cells, enabling nutrient uptake and systemic spread within plant tissues.

Typical symptoms include pale yellow chlorotic spots on the upper leaf surface; white to cream-coloured pustules (telia) on the underside of leaves; premature defoliation and chlorosis; stunted growth, deformed shoots and compromised flowering.

Under favorable conditions of high humidity and persistent leaf wetness, the disease can spread rapidly, leading to severe damage: defoliation, stunted growth, and loss of ornamental and commercial value. In advanced infections, systemic colonization may occur, leading to severe plant decline or death [BONDE & al. 2015].

Given these risks, accurate and early detection of *P. horiana*, especially before visible symptoms appear, is essential. Effective and quick detection methodologies are required to mitigate yield loss and time constraints associated with monitoring and management of CWR.

The scientific literature demonstrates that *P. horiana* remains a major phytosanitary threat to chrysanthemum collections globally. Modern diagnostics, especially molecular techniques, are essential for early detection and effective management. Sustainable control strategies integrate cultural, ecological and minimal chemical methods, aligning with the mission of botanical gardens to preserve biodiversity while ensuring environmental safety.

Ensuring phytosanitary stability within such collections requires rigorous monitoring of plant pathogens, with *P. horiana* representing a major threat due to its rapid spread and capacity to remain latent in infected tissues [ALAEI & al. 2009; PEDLEY, 2009]. This study aims to assess the presence and progression of white rust within *Chrysanthemum* collection, to characterize the pathogen and to identify environmentally responsible methods for its long-term management. Through these objectives, the research contributes to improved plant protection protocols and the preservation of chrysanthemum genetic resources in Romania.

In this context, the present work aims to: (1) detect the presence of *P. horiana* in the *Chrysanthemum* cultivars collection of the Iași Botanical Garden, using traditional mycological methods; (2) characterize the pathogen (morphology, infection patterns, possibly cultivar susceptibility); (3) propose and evaluate sustainable control strategies, combining good horticultural practices, early detection, sanitation, and minimal targeted intervention, to safeguard the collection's health and preserve its ornamental and genetic value. By achieving these aims, this study will contribute to improved phytosanitary management of ornamental chrysanthemum germplasm in Romania and support broader efforts to monitor and control white rust in botanical gardens and horticultural settings.

Material and methods

Study site and plant material

The study was conducted at the „Anastasie Fătu" Botanical Garden of Iași - Romania, under protected greenhouse conditions (47°11'17.69" N, 27°33'22.8" E - DMS system, 150 m a.s.l.). The observation took place in a single greenhouse with a total area of 50 m², designed to ensure uniform growing conditions and to minimize the influence of external climatic factors.

The plant material consisted of 35 *Chrysanthemum × grandiflorum* cultivars, selected for their horticultural relevance and phenotypic diversity: cv. *Alec Bedser* (cv.1), *Astro* (cv.2), *Axillia* (cv.3), *Blanche* (cv.4), *Cassandra* (cv.5), *Crimson Robe* (cv.6), *Cristal* (cv.7), *Diplomate Orange* (cv.8), *Escort Gelb* (cv.9), *Escort Orange* (cv.10), *Escort Roth* (cv.11), *Evelyn Busch* (cv.12), *Flame Blaier* (cv.13), *Good Bust* (cv.14), *Hannenburg* (cv.15), *Hagoromo* (cv.16), *Homaro* (cv.17), *Inga* (cv.18), *Jonson* (cv.19), *Margaret* (cv.20), *Marielle Purple* (cv.21), *Marielle Red* (cv.22), *Nob Hill* (cv.23), *Nyll Zwager* (cv.24), *Pink Always* (cv.25), *Prince de Monaco* (cv.26), *Princess Armgard* (cv.27), *Promenade* (cv.28), *Sheer Purple* (cv.29), *Sterling* (cv.30), *Stramer* (cv.31), *Taylor* (cv.32), *Tom Pierce* (cv.33), *Vienna Cooper* (cv.34), *Vienna White* (cv.35).

For each cultivar, 32 individual plants were included in the observation, resulting in a total of 1,120 plants analyzed throughout the study. All plants were cultivated in greenhouse compartments under identical horticultural practices, including substrate composition, irrigation regime, fertilization, and plant protection measures. Environmental parameters such as temperature, relative humidity, and light conditions were maintained at optimal levels for chrysanthemum growth and development. The experimental design allowed for comparative evaluation among cultivars under uniform protected conditions, ensuring the reliability of the obtained results.

The cultivars used in this study originate from the living chrysanthemum collection of the Botanical Garden of Iași, which comprises over 450 cultivars of *Chrysanthemum* × *grandiflorum* Ramat. and *Chrysanthemum indicum* L., representing the most extensive and important chrysanthemum collection in Romania and an essential resource for research, conservation, and ornamental plant breeding [COJOCARIU, 2016; PETRE & al. 2017; COJOCARIU & al. 2018].

CWR monitoring and visual detection

Monitoring of Chrysanthemum white rust (CWR), caused by *P. horiana*, was performed between 2020-2023, through systematic visual inspections of chrysanthemum plants cultivated under protected greenhouse conditions. The monitoring aimed to identify the onset, spread, and severity of disease symptoms, as recommended for quarantine pathogens affecting ornamental crops [EPPO, 2023].

Visual detection focused on the identification of typical CWR symptoms, including chlorotic or necrotic spots on the adaxial leaf surface and the development of white to pinkish pustules (telia) on the abaxial surface [O'KEEFE & DAVIS, 2015; PEDLEY, 2009]. Individual plants were inspected at regular intervals (one week, in August-October) to ensure detection of infection and accurate assessment of disease progression.

Assessment of CWR on *Chrysanthemum*

The incidence and severity of Chrysanthemum white rust (*Puccinia horiana* Henn.) on chrysanthemum cultivars were evaluated using a combination of quantitative and semi-quantitative parameters. For each plant, control strategies against Chrysanthemum white rust (CWR) were implemented using an integrated approach, combining cultural and chemical measures, in accordance with good horticultural practices for protected ornamental crops.

Cultural control strategies were applied to reduce disease pressure and limit the spread of *P. horiana* within the greenhouse. These measures included: plant spacing (*Chrysanthemum* plants were arranged at appropriate distances to prevent canopy overlap and to reduce leaf-to-leaf contact, thereby limiting the conditions favorable for pathogen transmission and sporulation), sanitation practices (regular sanitation measures were strictly applied, infected leaves and plant debris showing visible CWR symptoms were promptly removed and destroyed to reduce the inoculum source, tools and equipment used during plant handling were disinfected to prevent mechanical dissemination of the pathogen), ventilation management (natural ventilation was used to maintain air circulation and to create unfavorable conditions for fungal growth, to reduce relative humidity and leaf wetness duration, both of which are critical factors for CWR infection and disease development).

Chemical Control Measures for CWR was carried out using fungicide treatments based on azoxystrobin, a systemic fungicide belonging to the strobilurin (Q_oI) group, known for its preventive and curative activity against rust pathogens [BARTLETT & al. 2002].

Fungicide applications were performed according to the manufacturer's recommendations, considering the appropriate dosage, application interval, safety regulations for greenhouse use, and to ensure complete foliar coverage. Treatments were applied at the early stages of symptom appearance to ensure maximum efficacy. Treatments were applied twice per growing season (2020, 14 and 28 September, and 2021, 13 and 27 September, representing a latent period of 13 and 15 weeks after planting, WAP) at a 0.1% recommended concentration, and also as preventive treatment in 2021 and 2022 at 5 WAP (2021, 19 July and 2022, 18 July).

Data collection

The collected data on CWR were analyzed to evaluate disease incidence, severity, and cultivar responses under greenhouse conditions. The following procedures were applied for calculation of disease parameters:

Disease Incidence (DI, %)

Disease incidence was calculated as the proportion of infected plants in each cultivar, expressed as a percentage:

$$DI(\%) = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Disease Severity Index (DSI, %)

Disease severity was assessed using a standardized visual scoring scale (1-5) and expressed as a disease severity index (DSI, %), calculated as follows [CHIANG & al. 2017]:

$$DSI(\%) = \frac{\sum(n_i \times v_i)}{N \times V} \times 100$$

where: n_i = number of plants in each severity category; v_i = numerical value of each category (1-5); N = total number of plants assessed; V = maximum disease score (5).

All 1,120 plants, representing 35 chrysanthemum cultivars, were individually evaluated using a standardized visual scoring scale (1-5), *VSS*, based on symptom severity, adapted from commonly used disease assessment protocols [EPPO, 1990], where:

1 = no visible symptoms; 2 = very mild infection (few isolated lesions); 3 = moderate infection (multiple lesions on several leaves); 4 = severe infection (numerous lesions, chlorosis, leaf deformation); 5 = very severe infection (extensive pustule coverage, necrosis, premature senescence).

This index provides a quantitative measure of disease intensity, facilitating comparison between cultivars. Disease incidence (%) and severity scores were calculated for each cultivar. This combined approach allowed for an objective evaluation of the pathogen's impact while providing a standardized framework for comparing susceptibility among chrysanthemum cultivars. This monitoring approach enabled the identification of tolerant and susceptible cultivars and supported the evaluation of host–pathogen interactions involving *P. horiana* under greenhouse conditions.

To classify chrysanthemum cultivars according to their susceptibility to CWR, both Disease Incidence (DI) and Disease Severity Index (DSI) were integrated into a *standardized*

Combined Score (CS). First, the individual values of DI and DSI were normalized by dividing each percentage by 100, yielding values between 0 and 1: $DI_{norm} = DI/100$, $DSI_{norm} = DSI/100$

Normalization ensures that both variables contribute equally to the combined evaluation. The Combined Score (CS) was then calculated as the average of the normalized values: $CS = (DI_{norm}+DSI_{norm})/2$

The CS integrates both the proportion of infected plants and the intensity of symptoms, providing a comprehensive measure of cultivar response. Based on the CS value, cultivars were assigned to susceptibility categories as shown in Table 1:

Table 1. Susceptibility categories of *Chrysanthemum* cultivars based on Combined Score (CS) of Disease Incidence and Severity

CS range values (0-1)	Susceptibility Category
0 – 0.20	Tolerant
0.21 – 0.40	Moderately Tolerant
0.41 – 0.60	Moderately Susceptible
0.61 – 0.80	Susceptible
0.81 – 1.00	Highly Susceptible

This method allows for a quantitative and integrative assessment of all 35 cultivars included in the study, facilitating comparisons and identification of tolerant, moderately susceptible, and highly susceptible genotypes.

Results and discussions

Detection and Identification of Chrysanthemum White Rust (CWR)

Chrysanthemum white rust (CWR), caused by *P. horiana*, was first detected in September 2020 in the protected greenhouse environment of the "Anastasie Fătu" Botanical Garden of Iași and subsequently monitored throughout four consecutive growing seasons (2020-2023). The occurrence of the disease was identified through regular visual inspections, which revealed the characteristic symptoms of CWR, including chlorotic to necrotic lesions on the adaxial (upper) leaf surface and the development of white to pinkish spore-producing pustules (telia) on the abaxial (lower) surface (Figures 1, 2A-D).

Symptom appearance and progression were systematically recorded during the critical period from August to October, when environmental conditions within the greenhouse were favorable for pathogen development. Weekly monitoring allowed for the detailed tracking of disease dynamics, including the timing of initial symptom expression, the rate of disease spread, and the progression of symptom severity across cultivars. This intensive surveillance enabled early detection of infection foci, which proved essential for the prompt implementation of integrated control measures.

The early identification of CWR symptoms, combined with consistent monitoring, facilitated an accurate assessment of cultivar-specific responses to *P. horiana* and supported timely disease management decisions. This approach not only limited further disease development within the greenhouse but also provided a reliable framework for evaluating host susceptibility and the effectiveness of applied control strategies over multiple growing seasons.

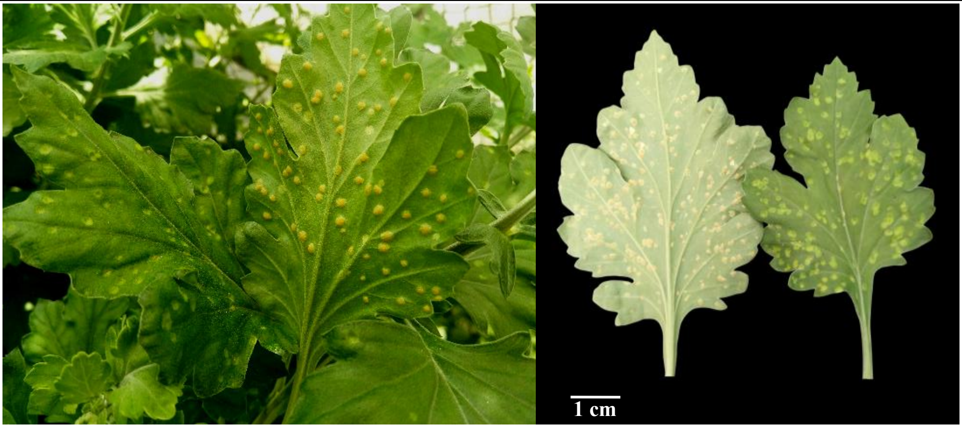

Figure 1. Typical symptoms of Chrysanthemum white rust (*Puccinia horiana*): chlorotic spots on the adaxial leaf surface and white to pinkish pustules (telia) on the abaxial surface (original).

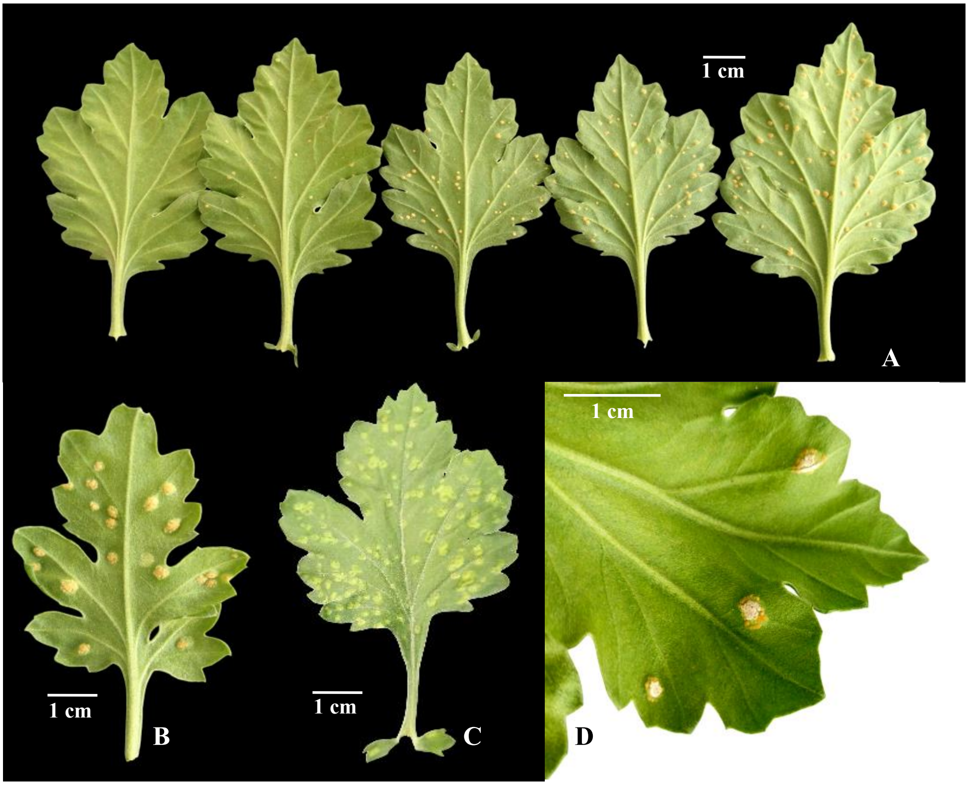

Figure 2. Different stages of Chrysanthemum white rust (*Puccinia horiana*) infection on chrysanthemum leaves (A); abaxial (lower) leaf surface with white to pinkish telial pustules (B); adaxial (upper) leaf surface showing chlorotic to necrotic lesions (C); detailed view of pustules illustrating infection and spore-producing structures (D) (original).

Disease symptoms were observed on different plant organs, with the leaves being predominantly affected. Initial symptoms appeared as pale green to yellow chlorotic spots, up to 5 mm in diameter, on the adaxial surface of infected leaves. As the disease progressed, these lesions darkened, becoming brown and necrotic. On the abaxial leaf surface, spore-producing pustules developed, initially buff to pink in color, later turning white upon maturation. Microscopic examination of these pustules revealed the presence of teliospores borne on pedicels up to 52 µm in length (Figure 3). Teliospores were pale yellow, two-celled, oblong to oblong-clavate, slightly constricted, measuring 30-52 x 11-18 µm, with thin walls (1-2 µm) that were distinctly thickened at the apex (4-9 µm). These morphological features are characteristic of *P. horiana*, confirming the identity of the causal agent.

Figure 3. Characteristic teliospores of *Puccinia horiana* observed microscopically (200x, 400x) (original)

Leaf samples of *Chrysanthemum × grandiflorum* showing characteristic symptoms of Chrysanthemum white rust were collected and deposited as voucher material in the Herbarium of the Faculty of Biology, "Alexandru Ioan Cuza" University of Iași (Index Herbariorum acronym: I), under voucher number **I207021**.

Disease Incidence and Severity

Quantitative evaluation of Chrysanthemum white rust revealed a wide spectrum of responses among the 35 *Chrysanthemum × grandiflorum* cultivars evaluated under protected greenhouse conditions. Disease Incidence (DI, %) varied considerably, ranging from 0% in resistant cultivars to over 90% in highly susceptible genotypes, while the Disease Severity Index (DSI, %) showed comparable variability across cultivars and growing seasons (Table 2). These results highlight pronounced differences in host response to *P. horiana* infection.

The application of a standardized visual scoring system (1-5) enabled an objective and reproducible assessment of symptom intensity, facilitating reliable comparisons among cultivars and between growing seasons. Cultivars exhibiting complete resistance showed no visible symptoms throughout the evaluation period, resulting in DI and DSI values of 0%. In contrast, susceptible cultivars developed extensive chlorosis, necrosis, and abundant telial pustules, leading to high DI and DSI values.

During the 2020 growing season, several cultivars, including *Axillia*, *Diplomate Orange*, *Marielle Purple*, *Marielle Red*, *Nob Hill*, *Vienna Cooper*, *Vienna White*, and *Taylor*, displayed very high disease incidence (≥ 75%), accompanied by increased severity levels (DSI > 55%). Conversely, cultivars such as *Blanche*, *Escort Gelb*, *Escort Roth*, *Evelyn Busch*, *Hannenburg*, *Jonson*, *Princess Armgard*, and *Stramer* remained symptomless, indicating strong resistance under the experimental conditions.

In the 2021 growing season, a general reduction in both DI and DSI values was observed across most cultivars. This decrease may be attributed to the implementation of preventive fungicide treatments at 5 weeks after planting (WAP), combined with curative applications at 13 and 15 WAP, as well as the consistent application of cultural control measures. Despite this overall reduction, cultivar-specific differences in susceptibility were maintained, suggesting that genetic factors played a dominant role in determining disease response.

Normalized values of disease incidence (DI_{norm}) and disease severity (DSI_{norm}), expressed on a 0-1 scale, and enabled direct integration of both parameters into a standardized Combined Score (CS). This approach allowed for a more comprehensive evaluation of cultivar susceptibility by accounting for both the proportion of infected plants and the intensity of symptoms. As shown in Table 2, CS values ranged from 0.00 in resistant cultivars to values exceeding 0.80 in highly susceptible ones.

Overall, the combined analysis of DI, DSI, and CS demonstrates substantial variability in cultivar response to *P. horiana*. These findings emphasize the importance of integrating multiple disease parameters when evaluating resistance and provide a basis for the classification of chrysanthemum cultivars into susceptibility categories. The results also underscore the potential of tolerant and resistant cultivars for use in breeding programs and sustainable chrysanthemum production under protected cultivation systems.

Sustainable control and integrated management

Across cultivars, a consistent downward shift in DI, DSI, and CS values was observed in 2021 compared with 2020, indicating a general reduction in disease pressure between growing seasons. This trend was particularly pronounced in cultivars that exhibited high disease levels in 2020, where large absolute and relative decreases were recorded. Cultivars such as *Axillia*, *Diplomate Orange*, *Marielle Purple*, *Marielle Red*, *Nob Hill*, *Taylor*, *Vienna Cooper*, and *Vienna White* showed substantial reductions in DI and DSI, with corresponding decreases in normalized indices and combined scores, suggesting a strong seasonal effect on disease expression.

The distribution of normalized disease indices in 2021 was narrower and shifted toward lower values compared with 2020, reflecting reduced variability and lower overall disease intensity across the cultivar set. This pattern is further supported by the decrease in CS values for most cultivars, indicating an overall improvement in health status under the applied management regime. In contrast, cultivars that remained symptom-free in both seasons (*Blanche*, *Escort Gelb*, *Escort Roth*, *Evelyn Busch*, *Hannenburg*, *Jonson*, *Princess Armgard*, *Stramer*) consistently exhibited zero variance for all disease parameters, highlighting stable resistance under the experimental conditions.

The observed seasonal differences are attributed to the disease control strategy implemented prior to and during the assessment period. The two treatments applied in 2020 likely resulted in a statistically meaningful reduction of the initial inoculum load, which was translated into lower baseline DI and DSI values at the beginning of 2021 growing season. Moreover, the preventive treatment program applied in 2021 appears to have further constrained early disease development, as indicated by systematically lower normalized values and reduced combined scores across susceptible and moderately susceptible cultivars.

Taken together, the data indicates a strong treatment effect, with disease parameters in 2021 consistently lower than those recorded in 2020. Although no formal inferential statistics are presented, the magnitude, consistency, and directionality of the changes across multiple cultivars and disease metrics support the conclusion that the treatment programs applied in 2020 and preventively in 2021 had a significant impact on reducing initial disease incidence and severity in *Chrysanthemum* × *grandiflorum*.

Several cultivars (*Blanche*, *Escort Gelb*, *Escort Roth*, *Evelyn Busch*, *Hannenburg*, *Jonson*, *Princess Armgard*, and *Stramer*) showed no disease symptoms in either year, indicating a high level of tolerance or resistance under the applied management conditions. Conversely, cultivars with intermediate susceptibility (e.g., *Alec Bedser*, *Cassandra*, *Homaro*, *Inga*, *Pink Always*, *Prince de Monaco*) also benefited from the treatment programs, displaying moderate but consistent reductions in disease parameters in 2021.

The comparative analysis between the two growing seasons highlights the cumulative effect of disease control measures, demonstrating that the curative treatments applied in 2020, together with the preventive strategy adopted in 2021, played a decisive role in reducing initial disease incidence and severity across *Chrysanthemum* × *grandiflorum* cultivars. Importantly, the monitoring program was continued in 2022 and 2023, during which preventive fungicide treatment was systematically applied at 5 weeks after planting (WAP) each year. Under these conditions, no visible symptoms of CWR were recorded during the subsequent growing seasons, indicating the effective suppression and eventual eradication of *P. horiana* from the chrysanthemum crop within the Botanical Garden of Iași. This outcome underscores the critical importance of early preventive interventions, continuous monitoring, and the integration of cultural and chemical control measures in managing quarantine pathogens under protected cultivation. The sustained absence of disease symptoms in 2022 and 2023 further confirms that the implemented management strategy was not only effective in reducing disease pressure but also successful in breaking the pathogen's infection cycle, thereby ensuring long-term phytosanitary safety of the chrysanthemum collection.

The results obtained in the present study are consistent with previous research highlighting the high variability in host response to *P. horiana* among chrysanthemum cultivars and related taxa. Similar patterns of differential susceptibility were reported by ZENG & al. (2013), who evaluated resistance to chrysanthemum white rust in 19 accessions of *Ajania* and *Chrysanthemum* species using artificial inoculation. Their comprehensive assessment, based on latent period, infection type, disease incidence, and severity, demonstrated substantial genetic variation in resistance, supporting the cultivar-dependent responses observed in our study under natural greenhouse infection conditions.

More recently, KUMAR & al. (2021) screened multiple *Dendranthema grandiflora* genotypes for resistance to *P. horiana* and confirmed infections using microscopic diagnostic techniques. Their findings emphasized the rapid spread of white rust in greenhouse and nursery environments, particularly under cool and humid conditions, and documented symptom development remarkably like those recorded in the present investigation. The progression from chlorotic spots on the adaxial leaf surface to necrotic lesions and the formation of buff to pinkish pustules on the abaxial surface, which later turn white upon maturation, closely matches the symptomatology observed in our monitored cultivars. These parallels further validate the accuracy of visual and microscopic diagnosis applied in the current study.

The wide range of Disease Incidence (DI) and Disease Severity Index (DSI) values recorded among cultivars also aligns with findings by SRIRAM & al. (2020), who quantified the effectiveness of fungicides against chrysanthemum white rust under controlled conditions.

Their results demonstrated that although chemical control can significantly reduce disease incidence and severity, cultivar susceptibility remains a critical factor influencing overall disease expression. In the present study, the marked reduction in disease parameters following the application of azoxystrobin-based treatments, particularly when combined with preventive applications at early growth stages, supports the effectiveness of integrated disease management strategies, as also recommended by SRIRAM & al. (2020).

Morphological confirmation of *P. horiana* based on teliospore characteristics observed in this study agrees with recent micromorphological investigations conducted on naturally infected chrysanthemum plants. Detailed descriptions of teliospore size, shape, wall thickness, and pedicel length reported in these studies closely correspond to our observations, reinforcing the reliability of classical morphological criteria for pathogen identification. Similarly, O'KEEFE & DAVIS (2015) provided an in-depth characterization of *P. horiana* teliospores from naturally infected hosts, emphasizing the importance of combining field symptomatology with microscopic examination to ensure accurate diagnosis.

Importantly, the findings of BONDE & al. (2015) regarding the systemic nature of *P. horiana* infections have significant implications for disease monitoring and management. Their demonstration that the pathogen can colonize vascular tissues and persist in asymptomatic stems and crowns highlights the potential for latent infections and disease re-emergence. This systemic behavior underscores the necessity of long-term monitoring, even in the absence of visible symptoms, and supports the preventive strategy adopted in the later years of the present study. Continued monitoring during 2022 and 2023, combined with early preventive fungicide applications, likely contributed to the successful suppression and eventual eradication of the disease within the chrysanthemum collection of the Botanical Garden of Iași.

The findings of RAHARDJO & al. (2019), who evaluated both biological and chemical fungicides for controlling white rust in chrysanthemums grown under open-field conditions, highlight the effectiveness of combining chemical and biocontrol measures. Their results reinforce the concept that integrated management strategies, tailored to environmental conditions and host susceptibility, can substantially reduce disease incidence and severity, complementing observations from greenhouse-based studies.

The results of this study on the application of azoxystrobin-based fungicide treatments align with earlier research demonstrating the effectiveness of Q_oI (quinone outside inhibitor) fungicides in managing *P. horiana*, the causal agent of chrysanthemum white rust. Previous work has shown that various strobilurin compounds, including formulations like azoxystrobin, significantly reduced pustule formation and disease development when applied preventively or curatively on chrysanthemum leaves, resulting in visibly lower pathogen activity compared to untreated controls. In these experiments, chrysanthemums protected with strobilurin fungicides exhibited substantially fewer active pustules and symptoms than non-treated plants, indicating that the mode of action of azoxystrobin effectively disrupts the infection cycle of *P. horiana* at key stages of spore germination and early colonization [WOJDYŁA, 2007]. These findings support the use of azoxystrobin as an important tool within integrated chemical control programs for chrysanthemum white rust under greenhouse conditions. However, reports have also identified strains of *P. horiana* with reduced sensitivity to Q_oI (quinone outside inhibitor) fungicides, suggesting that reliance on a single mode of action can lead to tolerance development in pathogen populations, and highlighting the need for careful fungicide rotation and integration with cultural practices to preserve long-term efficacy.

Overall, the present study corroborates existing evidence that chrysanthemum white rust is a highly aggressive disease under favorable environmental conditions, particularly in protected cultivation systems. While fungicides remain an effective component of disease management, their repeated use raises concerns related to environmental impact and production costs, as previously noted by WAARD & al. (1993). Therefore, the identification and utilization of resistant or tolerant cultivars, as demonstrated in this study, represent a sustainable and economically viable approach for long-term management of chrysanthemum white rust. The integration of host resistance, preventive chemical treatments, and strict cultural practices emerges as a strategy for controlling *P. horiana* in greenhouse-grown chrysanthemums.

Conclusions

The present study provides a comprehensive evaluation of Chrysanthemum white rust (*Puccinia horiana* Henn.) under protected greenhouse conditions, based on systematic monitoring, quantitative disease assessment, and integrated disease management strategies. The investigation, conducted on 35 *Chrysanthemum* × *grandiflorum* cultivars from the living collection of the "Anastasie Fătu" Botanical Garden of Iași, revealed pronounced variability in cultivar response to CWR, highlighting the importance of host genotype in determining disease incidence and severity.

Visual detection, supported by microscopic examination of teliospores, allowed for accurate identification of *P. horiana* and confirmed the presence of the pathogen during the 2020 growing season. The use of standardized disease parameters such as Disease Incidence (DI), Disease Severity Index (DSI), and a normalized Combined Score (CS), proved effective in integrating both the proportion of infected plants and symptom intensity, enabling classification of cultivars into susceptibility categories ranging from tolerant to highly susceptible.

Results demonstrated that several cultivars exhibited complete resistance, remaining symptom-free throughout the monitoring period, while others showed high susceptibility, with DI values exceeding 90% and severe symptom expression. The comparative analysis between growing seasons highlighted a consistent reduction in disease incidence and severity in 2021, attributable to the combined effect of cultural practices, curative fungicide applications at 13 and 15 weeks after planting (WAP), and the introduction of a preventive treatment at 5 WAP.

Continued monitoring in 2022 and 2023, combined with the systematic application of preventive treatments at early growth stages, resulted in the absence of visible CWR symptoms, indicating the successful suppression and eradication of *P. horiana* from the chrysanthemum crop within the Botanical Garden of Iași. This outcome emphasizes the critical role of early detection, continuous monitoring, and integrated disease management in controlling quarantine pathogens under protected cultivation.

Overall, the study underscores the value of resistant and tolerant cultivars for sustainable chrysanthemum production and provides a practical framework for disease assessment and management. The findings contribute valuable information for breeding programs, greenhouse production systems, and phytosanitary management, supporting long-term biosecurity and the preservation of ornamental plant collections.

Table 2. Disease Incidence (DI, %) and Disease Severity Index (DSI, %) of *Chrysanthemum* × *grandiflorum* cultivars evaluated at 13 Weeks After Planting (WAP) each growing season (2020, 2021), under two treatments (azoxystrobin) applied at 13 and 15 WAP in 2020 and a preventive treatment at 5 WAP in 2021, including Normalized Values (DI_{norm}, DSI_{norm}) and Combined Score (CS)

Legend: DI (%) = *Disease Incidence*; DSI (%) = *Disease Severity Index*; DI_{norm} = *Normalized Disease Incidence*; DSI_{norm} = *Normalized Disease Severity Index*; CS = standardized Combined Score.

Chrysanthemum x *grandiflorum* cv.	Growing season/2020					Growing season/2021*				
	DI (%)	DSI (%)	DI_{norm}	DSI_{norm}	CS	DI (%)	DSI (%)	DI_{norm}	DSI_{norm}	CS
cv.1 Alec Bedser	25.00	35.62	0.25	0.36	**0.30**	15.63	28.12	0.16	0.28	**0.22**
cv.2 Astro	28.13	32.50	0.28	0.33	**0.30**	28.13	31.87	0.28	0.32	**0.30**
cv.3 Axillia	93.75	64.37	0.94	0.64	**0.79**	37.50	36.87	0.38	0.37	**0.37**
cv.4 Blanche	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00	**0.00**
cv.5 Cassandra	34.38	35.63	0.34	0.36	**0.35**	18.75	33.75	0.19	0.34	**0.26**
cv.6 Crimson Robe	37.50	37.50	0.38	0.38	**0.38**	31.25	31.87	0.31	0.32	**0.32**
cv.7 Cristal	31.25	36.88	0.31	0.37	**0.34**	31.25	33.75	0.31	0.34	**0.33**
cv.8 Diplomate Orange	93.75	70.63	0.94	0.71	**0.82**	50.00	45.63	0.50	0.46	**0.48**
cv.9 Escort Gelb	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00	**0.00**
cv.10 Escort Orange	28.13	33.12	0.28	0.33	**0.31**	28.13	32.50	0.28	0.33	**0.30**
cv.11 Escort Roth	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00	**0.00**
cv.12 Evelyn Busch	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00	**0.00**
cv.13 Flame Blaier	6.25	23.13	0.06	0.23	**0.15**	0.00	0.00	0.00	0.00	**0.00**
cv.14 Good Bust	25.00	28.12	0.25	0.28	**0.27**	25.00	28.13	0.25	0.28	**0.27**
cv.15 Hannenburg	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00	**0.00**
cv.16 Hagoromo	18.75	28.75	0.19	0.29	**0.24**	15.63	26.25	0.16	0.26	**0.21**
cv.17 Homaro	37.50	36.25	0.38	0.36	**0.37**	25.00	30.63	0.25	0.31	**0.28**
cv.18 Inga	56.25	47.50	0.56	0.48	**0.52**	28.13	30.63	0.28	0.31	**0.29**
cv.19 Jonson	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00	**0.00**
cv.20 Margaret	37.50	41.62	0.38	0.42	**0.40**	0.00	0.00	0.00	0.00	**0.00**
cv.21 Marielle Purple	81.25	60.62	0.81	0.61	**0.71**	37.50	34.38	0.38	0.34	**0.36**
cv.22 Marielle Red	87.50	67.50	0.88	0.68	**0.78**	43.75	38.13	0.44	0.38	**0.41**
cv.23 Nob Hill	81.25	65.62	0.81	0.66	**0.73**	40.63	37.50	0.41	0.38	**0.39**
cv.24 Nyll Zwager	21.88	33.75	0.22	0.34	**0.28**	0.00	0.00	0.00	0.00	**0.00**
cv.25 Pink Always	68.75	55.62	0.69	0.56	**0.62**	34.38	33.75	0.34	0.34	**0.34**
cv.26 Prince de Monaco	75.00	53.75	0.75	0.54	**0.64**	31.25	31.88	0.31	0.32	**0.32**
cv.27 Princess Armgard	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00	**0.00**
cv.28 Promenade	25.00	31.87	0.25	0.32	**0.28**	21.88	29.38	0.22	0.29	**0.26**
cv.29 Sheer Purple	6.25	23.75	0.06	0.24	**0.15**	6.25	21.88	0.06	0.22	**0.14**
cv.30 Sterling	18.75	28.75	0.19	0.29	**0.24**	9.38	23.13	0.09	0.23	**0.16**
cv.31 Stramer	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00	**0.00**
cv.32 Taylor	81.25	65.00	0.81	0.65	**0.73**	37.50	36.25	0.38	0.36	**0.37**
cv.33 Tom Pierce	50.00	43.75	0.50	0.44	**0.47**	37.50	37.50	0.38	0.38	**0.38**
cv.34 Vienna Cooper	81.25	56.88	0.81	0.57	**0.69**	40.63	40.00	0.41	0.40	**0.40**
cv.35 Vienna White	87.50	67.50	0.88	0.68	**0.78**	46.88	41.25	0.47	0.41	**0.44**

Legend: DI (%) = *Disease Incidence*; DSI (%) = *Disease Severity Index*; DI_{norm} = *Normalized Disease Incidence*; DSI_{norm} = *Normalized Disease Severity Index*; CS = standardized Combined Score (interval 0-1).

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Research Article

DIVERSITY OF WOOD-INHABITING FUNGI IN TĂTĂRUŞI FOREST RESERVE

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Abstract: The article presents the results of a mycological study carried out in the Tătăruși Forest Reserve, where there were recorded 44 species of wood-inhabiting macromycetes, in 31 plots with beech trunks in different stages of decomposition. The relationships between fungal diversity and trunk parameters, forest cover were analyzed using multivariate statistical methods. The number of species ranged between 5-19 species per sample plot. The highest diversity was recorded in plots with trunks without bark, with larger volume, not exposed to the sun. Most fungal taxa were recorded on woody debris in the IV degree of decomposition. The lignicolous macromycetes were separated into two groups mainly depending on the degree of substrate decomposition.

Keywords: diversity, ecology, *Fagus sylvatica* forest, lignicolous fungi, wood decomposition.

Introduction

The beech (*Fagus sylvatica* L.) is a tree species that occupies large areas in the northern hemisphere, forming forests in Europe, both in pure stands and in various combinations with conifers at higher altitudes. The beech forests of Europe represent some of the most valuable ecosystems on the continent, highlighted both for their unique beauty and for the essential roles they play in the balance of the environment. Beyond their aesthetic appearance, these forests are true sanctuaries of biodiversity, protecting an impressive variety of plant, animal and micro-organism species that depend on the stability and resources they offer. At the same time, *Fagus* sp. forests are subjected to great anthropogenic pressures. Their importance is therefore complex and significant. Preserving these forests means preserving a priceless natural heritage, and treasure of biodiversity.

A significant number of beech forests are included in protected areas or even UNESCO World Heritage Sites due to their unique value and high degree of naturalness. However, beech forests are currently facing numerous challenges. Human activities, such as intensive logging or habitat destruction through fragmentation, reduce the capacity of these ecosystems to regenerate and maintain their natural functions. The ecological amplitude of beech is greater compared to other deciduous species in Europe in the context of natural regeneration [AXER & al. 2021; FANG & LECHOWICZ, 2006; WAGNER & al. 2010].

In Romania, protection of beech forests is a major priority, focused on their conservation, in order to maintain this natural heritage, a refuge for countless species and an essential factor for the stability of the environment. The beech forests are not just natural

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resources, but they are part of Romania's identity, history and future, and their protection represents an indispensable commitment for next generations [MILESCU & al. 1967; BIRIȘ & al. 2016].

The beech forests in the northeastern part of Moldova (Romania) represent valuable temperate ecosystems, with remarkable biodiversity, including the Tătăruşi forest (Photo 1) as well as those in the surrounding areas such as Humosu and Codrii Paşcanilor. These habitats offer optimal conditions for the development of a large number of fungi species due to the slightly acidic soil, consistent litter of decaying leaves, and a significant quantity of dead wood. The Tătăruşi Forest (Iaşi County) was declared a protected area of national interest (according to the decision of the County Council no. 8/1994) for the conservation of more than 130 years old *Fagus sylvatica* specimens and the presence of *Fagus taurica* individuals with impressive dimensions (heights of 32-33 m and diameters of over 50 cm). The protected area has 49.9 ha [NICOARĂ & BOMHER, 2004].

Photo 1. Aerial view of *Fagus sylvatica* phytocoenoses in Tătăruși Forest Reserve (original)

Fungi species are fundamental components of forest ecosystems with essential ecological roles in the decomposition of organic matter, formation of mycorrhizae with host trees, and contributions to the soil nutrients cycles. At the same time, the presence of dead wood, characteristic of natural and old-growth forests, creates microhabitats necessary for many rare species of insects, birds and fungi. Forest microhabitats create conditions for increased biodiversity; thus they are important for the complexity of forest habitats, whose structural diversity is enriched. In general, for the existence of microhabitats, large trees (especially of large diameters), but also an adequate density of these large trees are required. [LARRIEU & al. 2014].

There are numerous studies focused on the influence of quantity and quality of dead wood on wood inhabiting fungi diversity, on the influence of the contact area of dead wood with the soil, on the humidity and light exposure of wood residues and the importance of the accessibility of the wood surface for the colonization of fungi [ABREGO & SALCEDO, 2011; HEILMANN-CLAUSEN, 2001; HEILMANN-CLAUSEN & al. 2014]. Similar studies on fungi from beech forests in northeastern Romania have been carried out in order to identify the main environmental factors influencing the diversity of these groups of organisms [BÎRSAN & al. 2014; COPOŢ & al. 2018; COPOŢ & al. 2020]. The current contribution was focused on the diversity and on the influence of wood characteristics and forest cover on wood-inhabiting fungi in Tătăruși forest.

Material and methods

The mycological investigations were carried out over two successive years, in 31 sample plots, each containing *Fagus sylvatica* trunks of different diameters and sizes. For all plots were registered the geographical coordinates, altitude, forest (canopy) cover, degree of bark coverage and contact with the soil of each trunk, because they are among the most important factors influencing the composition of wood-inhabiting fungi (Table 1). The stage of wood decay was estimated in accordance with the scale defined by HEILMANN-CLAUSEN (2001) and summarily presented below:

1 - fallen or standing trunks with no visible signs of decay, hard wood, intact bark (0-20%);

2 - trunks with minor signs of decay, wood still quite hard, bark starting to break (21-40%);

3 - trunks with moderate signs of decay, with distinctly weak surface of wood, bark partially lost (41-60%);

4 - trunks with strong signs of decay, but still with ± original shape, with heavily decayed surface of wood, and bark lost in most places (61-80%);

5 - completely rotted, wood very strongly decayed, either to a very soft brittle substance or to a very fragile structure (81-100%).

Table 1. Characteristics of studied plots used in the analysis of lignicolous fungal assemblages in *Fagus sylvatica* stands from Tătăruşi Forest Reserve

Plots	Altitude m a.s.l.	Wood decay (%)	Forest cover (%)	Bark cover (%)	Contact with soil
S1	352	80	85	40	100
S2	352	60	85	60	100
S3	352	85	85	50	100
S4	352	65	85	40	100
S5	352	80	85	40	65
S6	352	60	85	60	100
S7	352	40	85	80	75
S8	352	30	85	80	80
S9	376	80	90	40	100
S10	376	80	90	40	80
S11	376	80	90	40	100
S12	376	60	90	60	60
S13	376	70	90	40	100
S14	376	60	90	60	80
S15	376	75	90	40	90
S16	430	20	70	90	10
S17	430	60	70	60	70

S18	430	30	70	80	10
S19	430	80	70	40	100
S20	430	60	70	60	80
S21	430	30	70	80	10
S22	430	30	70	80	10
S23	430	30	70	100	10
S24	390	75	80	40	90
S25	390	60	80	40	80
S26	390	80	80	40	100
S27	390	60	80	60	80
S28	390	60	80	60	80
S29	390	80	80	40	100
S30	390	75	80	40	90
S31	390	60	80	60	80

The fungi species were identified based on macroscopic and microscopic characters, both in the field and in the laboratory, using well-known monographs [SĂLĂGEANU & SĂLĂGEANU, 1985; BREITENBACH & KRÄNZLIN, 1986; GERHARDT, 1999; BERNICCHIA, 2005; TĂNASE & al. 2009; COURTECUISSE & DUHEM, 2013]. The scientific names were updated according to INDEX FUNGORUM database [http://www.indexfungorum.org/Names/Names.asp]. The species statistically significant associated to the clusters were identified based on indicator value index [DUFRÊNE & LEGENDRE, 1997; DE CÁCERES & LEGENDRE, 2009].

In order to observe similarities among plots, a hierarchical agglomerative clustering procedure was used, based on the Sorensen presence-absence index, in GINKGO software [DE CÁCERES & al. 2003; BOUXIN, 2005]. In addition, in order to identify the ecological factors influencing the composition of wood-inhabiting fungi, a detrended correspondence analysis was performed, in CANOCO 5 program [TER BRAAK & ŠMILAUER, 2012].

Results and discussions

A number of 44 species of lignicolous fungi species, classified into 36 genera, were identified in the investigated sample plots with beech trunks. The species list includes: *Auricularia auricula-judae* (Bull.) Quél., *Auricularia mesenterica* (Dicks.) Pers., *Bjerkandera adusta* (Willd.) P. Karst., *Calycina citrina* (Hedw.) Gray, *Chlorociboria aeruginascens* (Nyl.) Kanouse, *Chondrostereum purpureum* (Pers.) Pouzar, *Clavulina coralloides* (L.) J. Schröt., *Exidia glandulosa* (Bull.) Fr., *Flammulina velutipes* (Curtis) Singer, *Fomes fomentarius* (L.) Fr., *Gymnopus dryophilus* (Bull.) Murrill, *Hericium coralloides* (Scop.) Pers., *Hymenopellis radicata* (Relhan) R.H. Petersen, *Hypholoma fasciculare* (Huds.) P. Kumm., *Hypholoma lateritium* (Schaeff.) P. Kumm., *Hypoxylon fragiforme* (Pers.) J. Kickx f., *Jackrogersella cohaerens* (Pers.) L. Wendt, Kuhnert & M. Stadler, *Legaliana badia* (Pers.) Van Vooren, *Lentinus arcularius* (Batsch) Zmitr., *Lycogala epidendrum* (J.C. Buxb. ex L.) Fr., *Meripilus giganteus* (Pers.) P. Karst., *Mucidula mucida* (Schrad.) Pat., *Mycena aetites* (Fr.) Quél., *Mycena haematopus* (Pers.) P. Kumm., *Mycoacia livida* (Pers.) Zmitr., *Nectria cinnabarina* (Tode) Fr., *Peziza varia* (Hedw.) Alb. & Schwein., *Pleurotus cornucopiae* (Paulet) Quél., *Plicaturopsis crispa* (Pers.) D.A. Reid, *Pluteus cervinus* (Schaeff.) P. Kumm., *Pluteus salicinus* (Pers.) P. Kumm., *Ramaria stricta* (Pers.) Quél., *Sarcoscypha coccinea* (Jacq.) Lambotte, *Schizophyllum* *commune* Fr., *Scutellinia scutellata* (L.) Lambotte, *Stereum hirsutum* (Willd.) Pers., *Trametes hirsuta* (Wulfen) Lloyd, *Trametes pubescens* (Schumach.) Pilát, *Trametes versicolor* (L.) Lloyd, *Tremella mesenterica* (Schaeff.) Pers., *Typhula fistulosa* (Holmsk.) Olariaga, *Ustulina deusta* (Hoffm.) Maire, *Volvariella bombycina* (Schaeff.) Singer, *Vuilleminia comedens* (Nees) Maire.

The most frequent species were *Fomes fomentarius*, *Schizophyllum commune*, *Mucidula mucida*, and *Hypholoma fasciculare*, species found on over 80% of the trunks.

On individual trunks were identified from 5 to 19 fungal species. The beech trunk with the highest number of lignicolous taxa (19 species), presented significant dimensions, with a soil contact of 60% and a bark coverage of 60%. On trunks with higher soil contact, more species of fungi were identified compared to trunks with lower soil contact, because the humidity is maintained for a longer time. The less frequent species, occurring on 1-4 trunks, represented 13.63% of the total identified species.

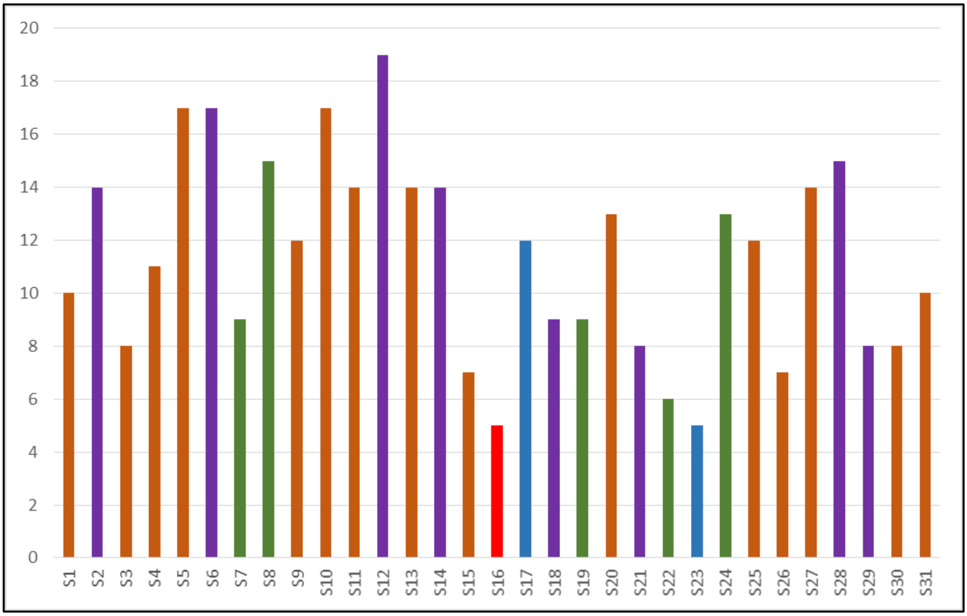

Figure 1. Diversity of lignicolous fungi depending on sample plot and degree of decomposition of dead wood (class I – blue, II – green, III – violet, IV – orange and V – red)

Trunks in different degrees of decomposition and which come from different tree species can provide a different quality of substrate, sustaining different fungal communities. The number of logs per individual decomposition class was as follows: class I – 2 logs, II – 5 logs, III – 8 logs, IV – 15 logs and V – 1 log. Differences regarding the number of fungal species depending on the degree of decomposition of trunks were also recorded. Thus, on trunks in stage IV of decomposition were identified 17 species of lignicolous fungi each (among the most frequent species there were *Bjerkandera adusta*, *Exidia glandulosa*, *Pluteus cervinus*, *Trametes pubescens*, *Fomes fomentarius*, etc.). The total and average richness of wood-inhabiting fungi taxa on trunks in stage V of decomposition as well as on those in stages I and II of decomposition was lower compared to trunks in stage IV of decomposition (Figure 1).

In both clustering procedure (Figure 2) and detrended correspondence analysis (Figure 3) two major groups of lignicolous fungi can be observed, depending on the decomposition stage of the studied trunks, on the degree of bark coverage, on the forest canopy cover and the area of contact with the soil of trunks. Some fungi species were more common (e.g. *Fomes* sp., *Trametes* sp. and *Stereum* sp., etc.) in the early stages of wood decay, while in the advanced decomposition stages, species of *Mycena*, *Hypholoma* and corticioide species were more frequent. These differences in respect to wood properties and characteristics favor the existence of different microhabitats, with a higher species richness and specialization of wood fungal communities. In addition, a higher degree of insolation due to some gaps in forest canopy can inhibit fungal species development and favor a richer herbaceous layer. Wood degradation is also influenced by the tree species and the fungal species that initially colonize the log. This process is mediated by multiple biotic interactions and environmental factors [MÜLLER-USING & BARTSCH, 2009].

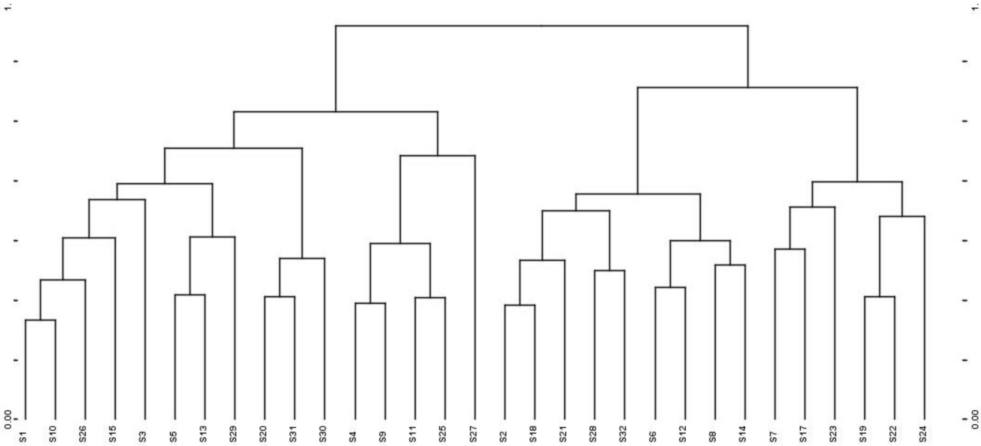

Figure 2. Dendrogram showing the results of the cluster analysis of the 32 trunks based on the Flexible β and Bray-Curtis distance.

Dead woody debris of different sizes, abundantly present in protected forest areas, is a determining factor for the biodiversity of lignocellulosic fungi by providing a wide range of niches. A higher variability of trunks, branches or small woody debris tends to provide more favorable conditions for the establishment of different species-rich lignocellulosic fungal communities [HEILMANN-CLAUSEN & CHRISTENSEN, 2003; HEILMANN-CLAUSEN & CHRISTENSEN, 2004; KÜFFER & al. 2004; KÜFFER & SENN-IRLET, 2005]. The beech forests in protected natural areas harbor numerous fungal species in their mycobiota, some of them rare in Western Europe due to high anthropogenic pressures, such as *Hericium* sp. Species that develop on old and well-rotted trunks, in advanced stages of decomposition, such as *Volvariella bombycina*, are particularly sensitive to forest management activities [TĂNASE & POP, 2005]. Moreover, numerous wood-inhabiting fungi are indicator species for the conditions in old-growth forests, the availability of dead wood, as well as for conservation value of beech forests. Species such as *Hericium coralloides* or *Volvariella bombycina* often signal long-term ecological continuity.

Cluster 1 – this group includes fungal species that prefer wood in advanced stages of decay (stages III-IV), the trunks have a high degree of contact with the ground, and a low degree of bark cover. These trunks are located under trees that provide a high degree of shade. The species significantly associated are: *Mucidula mucida* (0.696*), *Pleurotus cornucopiae* (0.683**), *Trametes pubescens* (0.632**), *Hypoxylon fragiforme* (0.577*), *Nectria cinnabarina* (0.577*), *Chondrostereum purpureum* (0.516*) - (*$p < 0.05$; **$p < 0.01$). In this group of wood-inhabiting fungi, are highlighted the species preferring substratum in IV degree of decomposition. In addition, due to the higher degree of canopy cover and the high contact of the logs with the soil, the humidity conditions are maintained for a longer time, favoring the development of the fungi.

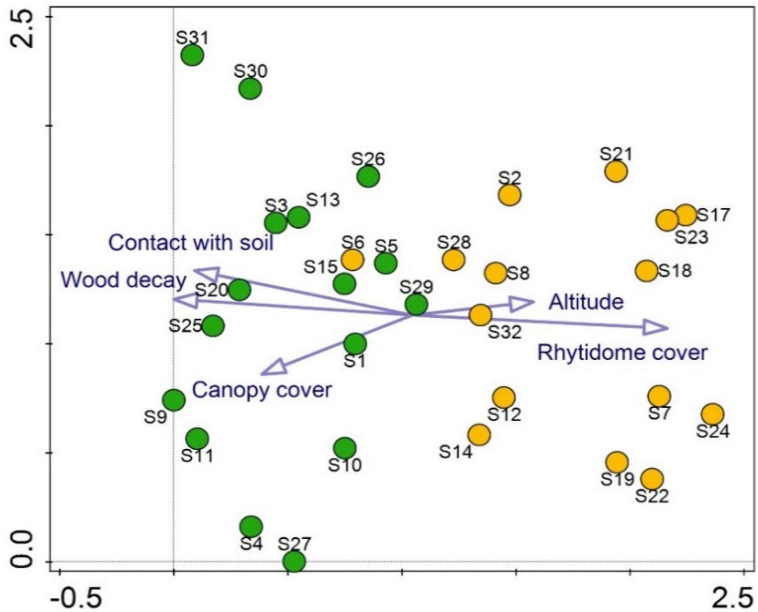

Figure 3. DCA ordination diagram of the 32 investigated plots. Only first two axes are presented. Explained variation: 44.1%. Eigenvalues: Axis 1 – 0.412, Axis 2 – 0.223. Fungal assemblages resulted from hierarchical clustering are: cluster 1 – green circles, cluster 2 – yellow circles.

Cluster 2 – this group includes species of fungi that prefer to colonize trunks in an incipient state of decomposition (stages I-II-III), with more or less intact rhytidome, reduced contact with the soil, and located in areas of the forest more exposed to solar radiation. The species significantly associated are: *Legaliana badia* (0.707**), *Hymenopellis radicata* (0.707**), *Hypholoma lateririum* (0.688*), *Calycina citrina* (0.612*), *Meripilus giganteus* (0.559*) - (*$p < 0.05$; **$p < 0.01$). In the second group it is highlighted the presence of fungi colonizing trunks in early decay stages, trunks broken by mechanical factors, etc. Richness of fungal species is low compared to the first cluster. Also, due to the occurrence of gaps in the forest canopy, the degree of isolation is higher and the number of fungi species per sampled plot was lower.

Conclusions

In beech forests, the wood-inhabiting fungi play an essential role by recycling nutrients, stimulating wood decomposition, supporting biodiversity, influencing carbon storage, shaping forest structure and regeneration, and serving as ecological indicators. In the Tătăruși Forest Reserve were identified numerous lignicolous fungi species developed on beech trunks, with the highest richness on trunks characterized by high wood decay and with higher contact with soil, distributed in shaded places. The lignicolous macromycetes were separated into two groups mainly depending on the degree of substrate decomposition. This contribution improves the knowledge of this group of organisms in studied area, because wood-inhabiting fungi were insufficiently considered in the biodiversity studies carried out in this forest reserve.

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Research Article

DRYOPTERIS DILATATA (HOFFM.) A.GRAY (DRYOPTERIDACEAE HERTER) IN THE FLORA OF THE REPUBLIC OF MOLDOVA

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Abstract: As a result of the new floristic investigations in the Republic of Moldova, the species *Dryopteris dilatata* (Hoffm.) A.Gray was recorded for the first time. It was found in the lower part of a forested valley near the Unguri comune of Ocnița district. This species is proposed to be included in the Red Book of the Republic of Moldova, the 4th edition, Critically Endangered (CR) category.

Key words: habitat, morphological description, rare species, Red Book of Republic of Moldova.

Introduction

In previous works [GEJDEMAN, 1986; KIRTOKA & ISTRATI, 1987; CHIRTOACĂ & al. 1992; NEGRU & al. 2002; PÎNZARU & al. 2002; CHIRTOACĂ & al. 2005; CHIRTOACĂ & PÎNZARU, 2005; NEGRU & CHIRTOACĂ, 2011; POSTOLACHE & al. 2018] has been cited only the species *D. dilatata* (Hoffm.) A.Gray [= D. *austriaca* (Jacq.) Woynar ex Schinz et Thell] from the "Plaiul Fagului" Scientific Reserve (plot no. 40), included in the Red Book of the Republic of Moldova [LAZU, 2015]. The specimens collected and determined as *D. austriaca* (Jacq.) Woynar ex Schinz et Thell, from plot 38 and 16, by V. Kirtoka and A. Istrati CHGB 226754, 226755, 226756; coll. 23.05.1985; 10.09.1987) actually belong to the species *D. carthusiana* (Vill.) H.P. Fuchs [PÎNZARU, 2019]. In the summer of this year, we conducted new floristic field research, during which we once again found *D. carthusiana* and not *D. dilatata* vegetates in the plot no. 38 and 16.

This work confirms the presence of the species *D. dilatata* (Hoffm.) A.Gray in the local flora, although from around Unguri commune, Ocnița district.

Materials and metods

The article is based on the floristic researches carried out in natural habitats in the Republic of Moldova, but also in Italy (Piemonte region). The exsiccatae are stored in Herbarium of the "Alexandru Ciubotaru" National Botanical Garden (Institute) Moldova State University (CHGB) and in the Herbarium of the botanist Pavel Pînzaru at the "Ion Creangă" State Pedagogical University of Chișinău (CH-HBPP). The identification of the species was carried out with the help of guides, taking into account the morphological and ecological peculiarities.

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The list of exsiccatae of *Dryopteris dilatata* (Hoffm.) A.Gray analysed by the author:

Republic of Moldova:

- Ocnița district, Unguri commune, "Rudi-Arionești" Landscape Reserve, plot 62, 14.09.2025, leg. P. Pînzaru (CHGB 240421; CH-HBPP 12018).

Italy, Piemonte region:

- Verceli province, Piode commune, beech forest, 15.07.2004, leg. P. Pînzaru (CH-HBPP 9940);
- Asti province, Tonengo commune, beech forest, 24.07.2004, leg. P. Pînzaru (CH-HBPP 9938, 9937);
- Torino province, Pont-Canavese commune, chestnut forest, 16.11.2006, 22.05.2007, leg. P. Pînzaru; (CH-HBPP 9934, 9932); Valle Soana, beech forest, alt. 650 m, 25.02.2007, leg. P. Pînzaru (CH-HBPP 9935); Torino commune, Colline Torinese, chestnut forest with beech, 24.05.2005, leg. P. Pînzaru (CH-HBPP 9936, 9937).

Ukraine:

- Reg. Cernăuți, Hotin district, village Blișceadi, beech forest, 30.06.1996, collected P. Pînzaru, determined A. Negru 09.02.2007 (*D. austriaca* (Jacq.) Woyner ex Schinz et Thell.), redetermined 03.12.2018 P. Pînzaru (CHGB 23617, 23618); village Ruhotin, beech forest, 30.06.1996, leg. P. Pînzaru (CHGB 236128).

Russia:

- Sibiri, Western Sayans, Kantegir left tributary of the Enisei River, 17.08.1931, leg. M. Ilin, B. Obrunikov and A. Ivanova (CHGB 116009);
- **Caucasus,** leg. I. Abramov, 15.07.1937 (CHGB 116007).

Results and discussions

As a result of the new floristic investigations in the Republic of Moldova, the species *Dryopteris dilatata* (Hoffm.) A.Gray was recorded for the first time, near the Unguri commune, Ocnița district. A single plant was observed, from which were collected spores for *in vitro* cultivation. The species *D. dilatata* differs from *D. carthusiana* by the color of the scales on the petiole: bicolored scales, with a blackish-brown longitudinal stripe in the middle, pale brown on the edges (not pale brown scales, uniformly colored); the rachis, the abaxial leaf blade and the indusium with glands (not without glands).

Dropteris dilatata (Hoffm.) A.Gray, 1848, Man. Bot.: 631; Fraser-Jenkins, 1993, in Fl. Europ., ed. 2, 1: 29; Lauber & Wagner, 2001, Fl. Helvetica, 2[e] ed.: 54; Prelli, 2001, Les Fougères et plantes alliées de France et d'Europe occidentale: 352; Aeschimann & al. 2004, Fl. Alpina 1: 96; Tzvelev, 2012, Conspectus florae Europae Orientalis, 1: 35; Sârbu, Ștefan & Oprea, 2013, Pl. Vasc. România: 44. – *Polypodium dilatatum* Hoffm. 1796, Deutschl. Fl. 2:7. – *D. austriaca* (Jacq.) Woynar ex Schinz et Thell. 1915, Viert. Naturf. Ges. Zürich, 60: 339; Grințescu, 1952, Fl. R.P.R., 1: 108; Protopopova, 1999, Opred. Vysših. Rast. Ukr., 2[e] izd.: 31. **– Broad Buckler-fern.** – Figure 1-2.

Rhizome ascending. Leaves about 70 cm long. Petiole 26-28 cm long, with light brown scales, in the middle with a blackish longitudinal stripe. Lamina ± 40 cm long and ± 30 cm wide, triangular-ovate, triple-pinnatisectat, dark green, on the underside with glandular hairs. First leaf segments: oblong-ovate, asymmetrical. First and lower secondary segments: shorter than ½ of the length of the primary segment, the segments of the last order with appressed, curved and acute teeth. Sori about 1 mm in diameter, not overlapping. Persistent indusium, dentate on the edges, with stalked glands. Dark brown spores, verrucous.

Biological and ecological characteristics. Perennial, hemicryptophyte, geophyte, VII-IX, 2n = 82, 164, mesohygrophilous (mesophilous), (hilly-) montain-subalpine (-alpine), Eurosiberian species [AESCHIMAN & al. 2004].

Habitat. In the Republic of Moldova, it grows at an altitude of 88 m, in the lower part of a deep, canyon-type valley, on purple schists with a little soil mixed with shale fragments, covered with moss, under the crown of hazelnut shrubs (*Corylus avellana* L.). The herbaceous cover is scattered, accompanying species: *Chaerophyllum temulum* L., *Glechoma hederacea* L., *Lamium galeobdolon* L., *Salvia glutinosa* L., *Scrophularia nodosa* L., *Urtica dioica* L., *Viola reichenbachiana* Jord. ex Boreau; *p*H = 6.5.

Figure 1. *Dryopteris dilatata* (Hoffm.) A.Gray, 14.09.2025, Unguri commune, Ocnița district

Quantitative aspects. In the landscape "Rudi-Arionești" a single plant was recorded (plot 62).

Limitation factors. Aridization of climate, at the limit of the spreading area, destabilization of forest ecosystems through irrational management.

Local distribution (Figure 2). "Rudi-Arionești" landscape reserve, plot 62, near the village of Unguri, Ocnița district.

Figure 2. Local distribution of the species *Dryopteris dilatata* (Hoffm.) A.Gray

Protection status. Territorially protected in the landscape "Rudi-Arionești" (plot 62).

Protection measures. Inclusion in the *List of species protected by the state* and in *The Red Book of the Republic of Moldova* (ed. IV). It is proposed to multiply the plant under *in vitro* conditions.

Status. Criticallly Endangered species (CR) Category.

General distribution. Eurosiberian species: Europe, Asia-Temperate (Caucasus North, Transcaucasus, Mongolia, Altay, Iran, Turkey), Africa (Macaronesia Azores), introduced into: Subantarctic Islands Falkland Is, Southern America (Argentina South) [***].

Conclusions

In the local flora, the genus *Dryopteris* Adans. includes 3 rare species: *D. carthusiana* (Vill.) H. P. Fuchs [Endangered (EN)], *D. dilatata* (Hoffm.) A.Gray [(Critically Endangered (CR)], and *D. filix-mas* (L.) Schott [(Vulnerable (VU)] and is proposed to be included in the 4th edition of the Red Book of the Republic of Moldova.

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***Distribution *Dryopteris dilatata* (Hoffm.) A.Gray. www.worldfloraonline.org/taxon/wfo-0001108304

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Research Article

OTHER CONTRIBUTIONS TO THE KNOWLEDGE OF THE DISTRIBUTION OF SOME ALIEN SPECIES IN ROMANIA'S FLORA

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Abstract: Alien plant species can significantly affect the structure and functioning of natural habitats, especially under anthropogenic conditions. In this context, this study provides new information on the spread of alien plant species in the flora of Romania, including *Amaranthus palmeri*, *Azolla filiculoides*, *Datura wrightii*, *Dittrichia graveolens*, *Eclipta prostrata*, *Erigeron sumatrensis*, *E. bonariensis*, *Euphorbia prostrata*, *Oenothera depressa* and *Paspalum distichum* subsp. *paucispicatum*, etc. Most of these species, already naturalized in anthropically disturbed areas, affect biodiversity in natural habitats, in some cases.
Some species studied show varied ecological adaptations, occupying different niches. For example, *Eriochloa villosa* spreads in agricultural lands, *Oenothera depressa* along rivers, *Azolla filiculoides* and *Heteranthera limosa* in puddles and ponds, and *Amaranthus palmeri* in ruderal lands associated with railways and bus stations, etc. Many of the species analysed show a tendency to expand their distribution areas in Romania.
There is a lack of national-level management strategies to eradicate or control alien plant species, including those with invasive potential, thereby allowing their uncontrolled expansion.
Voucher specimens collected in the field are deposited in the Herbarium of the University of Life Sciences Iaşi (IASI), the Herbarium of the "Alexandru Ioan Cuza" University, "Anastasie Fătu" Botanic Garden Iaşi (IAGB), the Herbarium of the University of Craiova (CRA) or in the personal herbaria.

Keywords: alien plants, invasive species, new species in Romania's flora.

Introduction

One of the first attempts to count the alien plant species in Romania's flora registered 435 species, of which 88.3% were neophytes and 11.7% archaeophytes [ANASTASIU & NEGREAN, 2005]. In one of the most recent syntheses upon the alien plant species, it was reported a continuous enrichment of Romania's flora with new aliens, recording approximately 670 species [SÎRBU & OPREA, 2011].

Some of these alien species can become invasive, threatening natural habitats and crops, and causing severe damage to the economy and human health [PIMENTEL & al. 2000; McNEELY, 2001; WITTENBERG & COCK, 2001; WITTENBERG, 2005].

New or rare alien species recorded within the flora of different historical regions of Romania, some of which are invasive, are reported in this paper.

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Material and methods

All species recorded in this paper were collected during our field trips in the historical regions of Transylvania, Crişana, Banat, Oltenia, Muntenia, Moldavia and Dobrudja (Romania), during the years of 2023, 2024 and 2025. The geographic coordinates were recorded using OsmAnd version 4.8.6, installed on a Xiaomi smartphone. Some of the voucher specimens collected during the field surveys have been lodged in the herbaria of the University of Life Sciences "Ion Ionescu de la Brad" in Iaşi (IASI), the Herbarium of the Botanic Garden "Anastasie Fătu"/University "Alexandru Ioan Cuza", Iaşi (IAGB), and the Herbarium of the University of Craiova (CRA). Morphological features of species were analyzed on the specimens collected from the field and compared with the data from relevant literature sources [TUTIN & al. 1964-1980, 1993; CIOCÂRLAN, 2009; SÂRBU & al. 2013], and, with the existing herbarium sheets in IASI, IAGB and CRA herbaria. The nomenclature of plant species is that adopted by the work *Flora Europaea* [TUTIN & al. 1964-1980, 1993] and by the *Flora Europaea* database [https://europlusmed.org/]. The plant family nomenclature follows the Euro+Med database [https://europlusmed.org/].

The abbreviations for herbarium collections follow the Global Herbarium Index [THIERS, 2025+; https://sweetgum.nybg.org/science/ih/herbarium-list].

The abbreviations for authors' plant names follow BRUMMITT & POWELL (eds.) (1992).

Citations of authors/works within this paper are done in chronological order.

Results and discussion

During our recent field research, focused on alien plant species, in 2023, 2024 and 2025, two new alien taxa were recorded for the flora of Romania (*Heteranthera limosa* and *Paspalum distichum* subsp. *paucispicatum*), and a few others for the historical provinces of Transylvania, Crişana, Banat, Oltenia, Muntenia, Moldavia and Dobrudja (e.g. *Amaranthus palmeri*, *Azolla filiculoides*, *Datura wrightii*, *Dittrichia graveolens*, *Eclipta prostrata*, *Erigeron sumatrensis*, *E. bonariensis*, *Euphorbia prostrata*, *Oenothera depressa*, etc.); other species were identified in new localities in the same regions (e.g. *Dittrichia graveolens*, *Euphorbia prostrata*, etc.), some of them showing an obvious invasive character (e.g. *Azolla filiculoides*, *Erigeron sumatrensis*, *E. bonariensis*, *Dittrichia graveolens*, etc.).

a) New alien taxa identified in the flora of Romania

Heteranthera limosa (Sw.) Willd. [Syn. *Pontederia limosa* Sw.] – Pontederiaceae Kunth

It is a species native to North, Central, and South America [ESPEJO SERNA & LÓPEZ-FERRARIA, 2000; ACEVEDO-RODRÍGUEZ & STRONG, 2005, 2012; HOKCHE & al. 2008; DIAMOND & WOODS, 2009; BERNAL & al. 2015; POWELL & WORTHINGTON, 2018], where it occurs as an aquatic or semi-aquatic species in shallow wetlands, ephemeral pools, and disturbed hydric habitats [SMITH & al. 1977; SMITH, 1988; GLEASON & CRONQUIST, 1991]. It has been reported as an invasive weed in rice-growing regions of Portugal [VASCONCELOS & al. 1999], Spain [GUARA REQUENA & al. 2003; CASTROVIEJO & al. 2008], Greece [DIMOPOULOS & al. 2013], and the Island of Sardegna/Italy [COSSU & al. 2014], Japan [IWATSUKI & al. 2016]. The species exhibits a high ecological plasticity and readily colonises anthropogenic aquatic environments, including roadside ditches, pond margins, irrigation and drainage channels, and rice-field systems [DILDAY & al. 1991].

In Romania, *H. limosa* is documented here as a new alien species, with confirmed occurrences in several localities in the southern and southwestern regions, as follows:

1. Timiș County, at Partoș, in southern part of Timiș County, about 7-8 km south-southwest of the town of Deta, close to the Romania-Serbia border; the GPS coordinates are: N 45° 20' 04.54″ / E 21° 09' 32.27″
2. Dolj County, Craiova, on the northern outskirts of Craiova, just a few kilometers from the city center; the GPS coordinates are: N 44° 20’ 47.19″ / E 23° 47’ 19.30″
3. Dolj County, north of Craiova (Tanchiştilor lake); practically, the species was identified in the same area as the previous one, also in the northern part of Craiova, just a few hundred meters away; the GPS coordinates are: N 44° 20' 41.47″ / E 23° 47' 21.63″
4. Dolj County, at Rojiște: in a rural area, south-east of Craiova, close to the border area between Dolj and Olt counties, in an agricultural / village landscape; the GPS coordinates are: N 44° 06' 19.50″ / E 24° 00' 25.77″
5. Brăila County, at Stăncuța: on the Danube floodplain in eastern Romania, in the area between Giurgeni (Ialomița County) and Hârșova / Vadu Oii (Constanța County) – essentially near the Giurgeni-Vadu Oii section of the Danube River; the GPS coordinates are: N 44° 43' 46.74″ / E 27° 48' 34.18″

These are, to date, the only known Romanian records. Its introduction pathway remains uncertain; however, escape from rice-cultivation systems or horticultural use as a decorative aquatic plant should be considered among the most plausible vectors. Because no herbarium vouchers were previously available in Romanian collections and all detections are recent (2019-2024), the taxon's presence in the country was long overlooked or considered doubtful.

This annual species typically grows immersed or stranded in very shallow water, preferably at depths below 5 cm [GUARA REQUENA & al. 2003]. It persists in human-modified habitats with permanent or nearly permanent water, including watercourses that do not freeze or freeze only briefly during winter. In rice fields, *H. limosa* behaves as a competitive weed; studies from North America indicate that it may reduce rice yields by 27-30% under water-seeded culture [SMITH & al. 1977; SMITH, 1988]. Given this potential, the species should be prioritised for monitoring in Romanian rice-growing areas, which may represent its primary introduction corridor.

Morphologically, *H. limosa* is characterised by ephemeral 6-merous blue to purple flowers subtended by bladeless sheaths, with distinctly dimorphic stamens - two short lateral yellow stamens with ovate anthers and a central, longer filament bearing a purple or white oblong anther [BASKIN & al. 2003]. Vegetative identification is unreliable because leaf morphology varies with moisture conditions; however, leaves are consistently oblong, with obtuse apices and parallel venation. Reliable identification requires flowering material, which is usually available in early to mid-summer. Field observations indicate that populations fluctuate dramatically with annual hydrological conditions. As in North American habitats, the species may appear abundant in wet years but absent in dry years, due to its dependence on shallow, temporary water bodies that can support germination and flowering [SMITH, 1988]. Seed-bank longevity is presumed to be multi-year, though exact viability remains unknown. Voucher specimens of Romanian populations should be deposited in national herbaria to support the confirmation of their alien status and facilitate future floristic treatments. Further investigations, including potential distribution modelling and targeted surveys in rice-growing regions, are strongly recommended (Figures 1 & 2).

The voucher specimens are in stored the personal herbarium of Mihai Doroftei.


Figure 1. *Heteranthera limosa* – Craiova outskirts


Figure 2. *Heteranthera limosa* (details)

Paspalum distichum L. (Syn. *P. paspalodes* (Michx.) Scribn., *P. digitaria* Poir., *Digitaria paspalodes* Michx.) subsp. ***paucispicatum*** (Vasey) Verloove & Reynders (Syn. *P. paucispicatum* Vasey) – Poaceae Barnhart (pro parte majore)

P. distichum subsp. *paucispicatum* was intitially described as *P. paucispicatum* by VASEY (1893). Later on, other authors categorize this taxa as a subspecies [VERLOOVE & REYNDERS, 2007], as it is, also, considered within this paper.

It can be distinguished as follows:

- robust plants, ± glaucous appearance, 50-60 (-70) cm high, with ascending to erect stems;
- densely hairy (pubescence) on leaf blades, sheaths and nodes, bulbous-based hairs;
- leaf blades up to 20 cm long;
- inflorescence racemose with (2-) 3 (-4) branches;
- spikes bear 2 rows of paired spikelets at each node (thus spikes appear having 4 longitudinal rows of spikelets) [KUNEV, in: RAAB-STRAUBE & RAUS, 2023].

This taxon is native to Mexico - on a vast area between Sonora and Oaxaca [VASEY, 1893; HITCHCOCK, 1950; VERLOOVE & REYNDERS, 2007].

In Europe, it is also an alien taxon, in:

- France: close to Montreux, along the Loire River [VERLOOVE & REYNDERS, 2007; VALDÉS & SCHOLZ, 2009+].
- Italy [GALASSO & al. 2018; https://europlusmed.org/cdm_dataportal]
- Bulgaria (**1**. Danubian Plaine, Pleven province, Belene municipality, Hisarlaka – about 4 km NW of Belene municipality; **2**. Limana – about 6 km NW of Belene municipality [leg. I. G. Kunev, in: RAAB-STRAUBE & RAUS, 2023].

In Romania, *P. distichum* subsp. *paucispicatum* has been recorded in **Dobrudja** region, Tulcea County, Danube Delta, as in: 1) Cardon village – along a canal from Cardon toward Sfiştofca; 2) Sfiştofca village – close to the Monastery “Buna Vestire Deltă”; 3) downstream of

Crișan village – at the pier for picking up waste; 4) Mila 23; 5) one Mile upstream of Vulturu; 6) Sulina town – eastern canal from Sulina to the Black Sea (no name of it); 7) entrance canal of Perivolovca; 8) canal of Perivolovca; 9) Ostrov Dranov I; 10) canal of Dranov II; 11) a canal derived from "Gârla Turcească" toward Meleaua Sacalin; 11) Isaccea town (along a loan canal, GPS coordinates N 45.28182 / E 28.44189); 12) northern parts of Sulina town – on the left bank of Sulina arm (also, along a loan canal, GPS coordinates N 45.16215 / E 29.63737); 13) in the channels of the southern part of Furtuna polder (leg. S. Covaliov, August, 7th, 2025).

A voucher specimen was lodged in Herbarium of the Botanic Garden "Anastasie Fătu"/University "Alexandru Ioan Cuza", Iaşi (IAGB, no. 47916).

b) New records in the alien flora of various historical provinces of Romania

Azolla filiculoides Lam. [Syn. *A. caroliniana* auct., non Willd.; *A. microphylla* Kaulf.] – Salviniaceae Martinov

A species native to North, Central, and South America [http://efloras.org/*Flora of North America*; https://powo.science.kew.org] was introduced to Europe in the second half of the 19th century. WEST (1953), as cited by HUSSNER (2006), reports that *A. filiculoides* was first recorded in Europe in 1880, in France (Bordeaux). Thus, according to JERMY [in: TUTIN & al. 1993], the current range of this species in Europe includes the western, central and southern regions of the continent, within the following countries: Belgium, Great Britain, Bulgaria, Czech Republic, France, Germany, and Greece [DIMOPOULOS & al. 2013], Netherlands, Spain, Hungary, Italy (incl. the Island of Sardegna), Portugal, and Romania. Later, this species was also reported from Turkey [ÜNAL & ÜZEN, 1996]. It is also present in Asia and Africa [https://powo.science.kew.org].

The presence of the species *A. filiculoides* has been reported since the first decades of the last century in Romania's flora, being identified for the first time by PALLIS (1916), in the Danube Delta, information later taken up also by PRODAN (1939 a, b), ŢOPA (1952), and others. Later on, *A. filiculoides* was identified (under this name!) in many other localities in the southern parts of the country, mainly along the Danube River, in puddles and oxbows, as it is in the counties of Caraș-Severin, Mehedinți, Dolj, Giurgiu, Teleorman, Ilfov, Călăraşi, Brăila, Tulcea and Constanța [SÎRBU & OPREA, 2011], Olt and Gorj [RĂDUŢOIU, 2024].

This is the first record of this species in the **Moldavia** region flora, namely in Galaţi County – in the puddle of Lozova, west of Braniştea village (GPS coordinates: N 46.44027 / E 27.82898).

Voucher specimens were lodged in the Herbarium of the Botanic Garden "Anastasie Fătu"/University "Alexandru Ioan Cuza", Iaşi (IAGB, no. 47910).

Amaranthus palmeri S. Watson – Amaranthaceae Juss.

It is a native species in Southern California, Texas, and Mexico [BRENAN, 1961; https://powo.science.kew.org; http://efloras.org/*Flora of North America*]. Nowadays, *A. palmeri* is an invasive species, also in North America, but also on other continents, such as South America, Europe, Asia, Africa [https://powo.science.kew.org], and Australia [ROBERTSON, 1981].

In Europe it is known from the Czech Republic (registered since 1908) [in: PYŠEK & al. 2003], Great Britany [BRENAN, 1961], Sweden, Netherlands, Germany, Austria, Luxembourg, Switzerland, and France [AELLEN, 1960; SAUER, 1967], Poland [FREY, 1974], Spain [CARRETERO, 1990], Ukraine [MOSYAKIN, 1995], Romania [COSTEA, 1996, 1997, 1998], Republic of Moldova [COSTEA & NEGREAN, 1997, in: COSTEA, 1998]. More recently, *A. palmeri* was cited from Greece [RAUS & RAABE, in: GREUTER & RAUS, 2006; DIMOPOULOS & al. 2013].

A. palmeri was previously cited from Romania, as follows: the railway station of Bucharest city [leg. G. Negrean, 1981, in: COSTEA, 1998; NEGREAN & CONSTANTIN, 1999]; Constanţa harbour and surroundings [leg. M. Costea, 1995-1996, in: COSTEA, 1996, 1997, 1998; CIOCÂRLAN & al. 2004]; railway stations of Timişoara, Oradea, Focşani, Bacău, and Dorohoi [OPREA & al. 2021]; Giurgiu County - various localities [NAGODĂ & al. 2023].

A. palmeri is reported here for the first time in the **Oltenia** region: Dolj County – railway station of Craiova (GPS coordinates N 44.32851 / E 23.81909), the railway station of Băileşti (leg. A. Oprea, August, 17th, 2024), among the railway tracks and along the edges of the surrounding streets (GPS coordinates N 44.03369 / E 23.34526), and the railway station of Drăgăneşti-Olt (leg. D. Răduţoiu, September, 3rd, 2025), among the railway tracks (GPS coordinates N 44.15475 / E 24.54411).

Voucher specimens were lodged in the Herbarium of the Botanic Garden "Anastasie Fătu"/University "Alexandru Ioan Cuza", Iaşi (IAGB, no. 47920).

Dittrichia graveolens (L.) Greuter [Syn. *Erigeron graveolens* L., *Inula graveolens* (L.) Desf., *I. quadridentata* Lag.] – Compositae Giseke (pro parte majore)

It is a native species in the terrestrial areas around the Mediterranean Sea basin [BALL, 1976; BRULLO & DE MARCO, 2000; KIRÁLY & al. 2014; PAROLIN & al. 2014], including Greece [DIMOPOULOS & al. 2013]. It is also an alien species in Africa, Asia, Australia, New Zealand, and North America [https://powo.science.kew.org].

In Romania, it has been identified, firstly, in the **Transylvania** region, in Braşov County, between the villages of Viștea de Jos and Ucea de Jos, along a stream called "Corbul Ucii" [SZATMARI & HURDU, 2020] and in Tohanu Nou village [SÎRBU & al. 2023]; Sibiu County - between the villages of Arpașul de Jos and Scoreiu [SZATMARI & HURDU, 2020]. Other records of the species in the Transylvania region are as follows: Braşov County – from Vlădeni village (GPS coordinates N 45.75206 / E 25.39513) to Perşani village (GPS coordinates N 45.76014 / E 25.23663), on both sides of the National Road no. 1 / European Road E68.

D. graveolens is reported now for the first time in **Crişana** region: Bihor County – Cornițel, Borod, Groşi, Tinăud, Oşorhei, and Aleşd villages; Cluj County – Bucea village (GPS coordinates N 46.95479 / E 24.68192).

This species tends to become an invasive weed in these new localities, growing in hundreds, if not thousands, of individuals and invading meadows along roads.

A voucher have been lodged in the Herbarium of the Botanic Garden "Anastasie Fătu"/University "Alexandru Ioan Cuza", Iaşi (IAGB, no. 47918).

Erigeron bonariensis L. [Syn. *Conyza bonariensis* (L.) Cronquist, *C. ambigua* DC., *Erigeron crispus* Pourr., *E. linifolius* Willd., ? *E. transsilvanicus* Schur] – Compositae Giseke (pro parte majore)

It is a native species in Central and South America [WURZELL, 1994; ŠÍDA, 2003; https://powo.science.kew.org], widely distributed today in North America and Europe [CRONQUIST, in: TUTIN & al. 1976; WURZELL, 1994; ŠÍDA, 2003; MILOVIĆ, 2004], including Greece [DIMOPOULOS & al. 2013], Asia, Africa, Australia, Tasmania, and New Zealand [https://powo.science.kew.org].

The old locations, already known in Romania, are in **Oltenia**: Mehedinţi County – S-W of Cireşu village – along Bahna stream valley and in the railway station of Drobeta-Turnu Severin [NEGREAN & CIORTAN, 2012]; Olt County – along Olteţ River valley, at Osica de Sus, Bobu, Ulmet and Pârşcoveni villages [BURDUŞEL & al. 2020]. Another locality where *E. bonariensis* has been recorded is in: Dolj County – outskirts of Dăbuleni town toward the agricultural farm "Ianca", on ruderal sandy places (GPS coordinates N 43.79767 / E 24.10982).

Here are the first records of *E. bonariensis* in **Dobrudja** region: Tulcea County – Malcoci village (GPS coordinates N 45.14323 / E 28.8818), and Nufăru village, in ruderal places, in ditches, the verges of the county road no. 222C Tulcea toward Murighiol village, on places where people deposit manure and other household waste, or next to fences (at Nufăru).

The vouchers were lodged in the Herbarium of the Botanic Garden "Anastasie Fătu"/University "Alexandru Ioan Cuza", Iaşi (IAGB, no. 47913) and the Herbarium of the University of Craiova (CRA, no. 72121-72122).

Erigeron sumatrensis Retz. [Syn. *Conyza sumatrensis* (Retz.) E. Walker; *C. albida* Spreng.; *C. bonariensis* (L.) Cronquist var. *microcephala* (Cabrera) Cabrera; *Erigeron bonariensis* L. var. *microcephala* Cabrera] – Compositae Giseke (pro parte majore)

It originated in Central and South America and has since spread to warm regions worldwide, including Africa, South Asia, Taiwan, Tasmania, and New Zealand [PIGNATTI, 1982; HUANG, 1994-2003; MILOVIĆ, 2004; VLADIMIROV, 2009; https://powo.science.kew.org]. In Europe, it is distributed as an alien species, mainly in the southern and western regions [https://europlusmed.org/cdm_dataportal], including Greece [DIMOPOULOS & al. 2013].

Known distribution in Romania: Constanţa County – Constanţa harbor [ANASTASIU & MEMEDEMIN, 2012; MEMEDEMIN & al. 2016], Corbu village – on the coast of the Black Sea, at an abandoned industrial site [OPREA & al. 2021], from the area of Corbu-Midia toward Năvodari [OPREA & al. 2021]; Timişoara County – Timişoara railway station [OPREA & al. 2021]; Mehedinţi County – Drobeta-Turnu Severin railway station and various places around the town [OPREA & al. 2021], Orşova town – on the left bank of the Danube River and in the railway station [OPREA & al. 2021]; Gorj County – railway station of Târgu Jiu [OPREA & al. 2021]; Dâmboviţa County – Târgoviște in the railway station and around "Curtea Domnească" [SÎRBU & al. 2023]; Giurgiu County: between Falaştoaca and Prundu villages, between Băneasa and Pietrele villages, between Izvoarele and Radu Vodă villages, Varlaam village, Adunații-Copăceni village and in Giurgiu town [NAGODĂ & al. 2023]; Bucharest City (leg. M. Urziceanu, University of Bucharest in 2023, pers. comm.); Buzău County: railway station of Buzău [SÎRBU & al. 2023]; Vrancea County – railway station of Focșani [OPREA & al. 2021]; Bacău County: Oneşti – on the banks of Trotuş River [OPREA & al. 2021]; Iaşi County: Miroslava village and the city of Iași – at "Palace of Culture" [SÎRBU & al. 2023].

There are new records of this species, such as:

i) the first record in **Crişana** province, in Bihor County – Băile 1 Mai – on the banks of the Hidișel stream and ruderal places along the roadsides.

ii) new records in:

- Caraş-Severin County: Băile Herculane, along Cerna River banks (GPS coordinates N 44.86739 / E 22.40666);

- Dolj County: railway station of Craiova, among railway tracks (GPS coordinates N 44.32853 / E 23.81936); Rastu Vechi - ditches along the village streets;
- Gorj County: Rovinari-Gară, among railway tracks (GPS coordinates N 44.89953 / E 23.16706);
- Vâlcea County: railway station of Râmnicu Vâlcea, among railway tracks (GPS coordinates N 45.10098 / E 24.3707);
- Constanţa County: Cape Midia, on the sands of the Black Sea beach (GPS coordinates N 44.34772 / E 28.69149).

Euphorbia prostrata Aiton [Syn. *E. perforata* Guss., *Tithymalus prostratus* (Aiton) Samp., *Chamaesyce prostrata* (Aiton) Small] – Euphorbiaceae Juss.

Origin and ecology of the species: southern parts of the USA, Central America, South America, and the West Indies [https://powo.science.kew.org], on disturbed areas, fields, gardens, sidewalks, sandy places or ballast piles, between 0 and 1,400 m a. s. l. (above sea level) [http://efloras.org/*Flora of North America*]. It is also present in South Asia, Africa, and Australia [https://powo.science.kew.org].

Distribution in Europe: Spain, Portugal, Italy, Sicily, France, and Greece [SMITH & TUTIN, in: TUTIN & al. 1968; RAUS, in: GREUTER & RAUS, 1998; BERGMEIER, in: GREUTER & RAUS, 2007; DIMOPOULOS & al. 2013; https://europlusmed.org/cdm_dataportal], Croatia [PANDŽA & al. 2001].

Distribution and habitats already known in Romania: Bucharest – along I. Budişteanu St. [leg. G. Negrean, in: ANASTASIU & NEGREAN, 2008] and in the park of “National Opera” house (leg. C. Sîrbu, 2022); Dolj County – Craiova [RĂDUŢOIU & STAN, 2013]; Iaşi County: Iaşi – “Pallas Public Garden” and surroundings [OPREA & al. 2021]; Caraş-Severin County - Baziaș [OPREA & al. 2021]; Galați County – Galați (at the confluence of the Danube River with the Siret River) [ŞUŞNIA, 2022]; Giurgiu County [NAGODĂ & al. 2023].

This is the first record in the **Dobrudja** region: Tulcea County – Tulcea town, on the right bank of the Danube River, on the gravel and in the concrete cracks, located between a sidewalk along Tulco St. and a slope of the Danube River (GPS coordinates N 45.18224 / E 28.80573).

A voucher was lodged in the Herbarium of the Botanic Garden “Anastasie Fătu”/University “Alexandru Ioan Cuza”, Iaşi (IAGB, no. 47919).

Senecio inaequidens DC. [Syn. *Senecio burchellii* DC., *S. vimineus* Harv., *S. harveianus* MacOwan] – Compositae Giseke (pro parte majore)

It is a species native to Mozambique and South Africa [https://powo.science.kew.org]. It was accidentally introduced into East Asia [MITO & UESUGI, 2004], South America, North America and Central America [HEGER & BÖHMER, 2006], Hawaii and Australia [EPPO Global Database]. In Europe, *S. inaequidens* is present in: Germany [BRANDES, 1910, in: BORNKAMM, 2002], France, Italy, and Belgium [CHATER & WALTERS, in: TUTIN & al. 1976; WITTENBERG, 2005; LÓPEZ-GARCÍA & MAILLET, 2005; HEGER & BÖHMER, 2006; EPPO Global Database], Slovenia [KALIGARIČ, 1992, in: KIRÁLY & al. 2007], Slovakia [JEHLÍK, 1998; FERÁKOVÁ, 2002], Austria [ESSL & RABITSCH, 2002], Czech Republic [PYŠEK & al. 2002], Switzerland [WITTENBERG, 2005], Great Britain, Spain, Netherlands, Denmark, Finland, Sweden, Norway, Poland, and Hungary [EPPO Global

Database, 2006; DAISIE, 2009], Baleares, Belarus, Bulgaria, Corse, Ireland, Portugal, Sardegna, Sicilia, ex-Yugoslavia [https://powo.science.kew.org].

In Romania's vascular flora, *S. inaequidens* was collected for the first time by G. Negrean from Bucharest - the railway station of "Triaj" [leg. G. Negrean, 2005, in: ANASTASIU & NEGREAN, 2008].

Later, this alien species was collected in Iaşi County, in the city of Iaşi, near a commercial trade centre [leg. C. Sîrbu, 2009, in: SÎRBU & OPREA, 2011]; Arad County – in Macea village [I. Don, Macea Botanic Garden, pers. comm., 2018]; Buzău County – Mânzălești village, on Meledic Plateau [CAMEN-COMĂNESCU & al. 2023].

S. inaequidens was collected for the third time in **Muntenia** region, in 2023, the county of Vâlcea, between the villages of Păuşa and Jiblea Veche, on the left bank of Olt River, in a ditch along the National Road no. 7 (European Road no. 15A), along a bypass road of the well-known balneo-climatic resorts of Călimănești and Căciulata, on September 8-9, 2023 (GPS coordinates N 45.25087 / E 24.33994).

c) Alien plant species found in new localities in Romania

Anisantha diandra (Roth) Tutin Roth (Syn. *Bromus diandrus* Roth) – Poaceae Barnhart (pro parte majore)

A. diandra is a native species to the Irano-Turanian, Pontic, and Mediterranean regions [CABI, 2020]. Still, it has been widely introduced elsewhere in the world, as in: Europe, Asia, and North Africa [https://europlusmed.org/cdm_dataportal], North America, Central America and South America, Australia, Tasmania, and New Zealand as an alien weed [KON & BLACKLOW, 1989; CABI, 2020; BORGER & al. 2021; KUNEV, 2021; https://powo.science.kew.org], as well as in North India [MALIK & MOHAMMAD, 2015]. Closer to Romania, *A. diandra* has been reported as a naturalised species in Bulgaria [KUNEV, 2021; STOYANOV & al. 2022], and in Hungary, as doubtfully introduced there, perhaps only cultivated [https://europlusmed.org/cdm_dataportal], Ukraine – on "Insula Şerpilor" and in Crimea, as well as in southwestern Russia and Georgia [GBIF, 2023; https://www.gbif.org/species/2703760], the European part of Turkey [WEBB, 1966], and Greece [DIMOPOULOS & al. 2013].

A. diandra is already known in Romania, being recorded up to now in: Timiş County: Timișoara North railway station (leg. G. Negrean, Herb. I); Constanța County – the harbour of Constanța [Herb. SOM, no. 177292], in: STOYANOV & al. 2022]: Mehedinţi County – Drobeta-Turnu Severin railway station [Herb. CL, no. 665457, in: STOYANOV & al. 2022]; Buzău County – Buzău railway station [OPREA & SÎRBU, 2023].

This is the second record in the **Oltenia** region, namely at the Craiova railway station, Dolj County, among the railway tracks (GPS coordinates: N 44.32853 / E 23.81936).

It is also the second and third records of this species in **Dobrudja** province, in Constanţa County, as they are.

i. town of Năvodari, ruderal places on dry sands [leg. I. Gaţu, Botanic Garden "Anastasie Fătu"/University "Alexandru Ioan Cuza" Iaşi, in: INDEX SEMINUM ET SPORARUM, **CI**/2024, no. 1790];

ii. Mamaia - between the Fishery House "Pontica" and the Cape of Singol (wasteland, roadsides, dry sands), very abundant; GPS coordinates: N 44.217719 / E 28.642929; leg. C. Sîrbu, 19th of May, 2025.

A voucher specimen was lodged in the Herbarium of the Botanic Garden "Anastasie Fătu"/University "Alexandru Ioan Cuza", Iaşi (IAGB. no. 47917).

Asclepias syriaca L. [Syn. *Asclepias cornuti* Decne.] – Apocynaceae Juss.

It is a species originating in Eastern-Central and East Canada to Central and East of the USA [FRANKTON & MULLIGAN, 1974; https://powo.science.kew.org], at latitudes 35 °N to 50 °N, from where it was introduced to Europe around the year of 1629 [BAGI, in: BOTTA-DUKÁT & BALOGH, 2008], being first cultivated in Paris. It was first cited as a weed from the Mediterranean regions, then from Central Europe, and mentioned from Hungary (Transdanubia) during the years of 1736 and 1737 [BAGI, in: BOTTA-DUKÁT & BALOGH, 2008]. It is currently naturalized in southern, central and eastern Europe - Austria, Bulgaria, former Czechoslovakia, France, Switzerland, Hungary, Italy, former Yugoslavia, Poland, Romania, Russia, and Crimea [MARKGRAF, in: TUTIN & al. 1972], Montenegro [STEŠEVIĆ & al. 2008], Germany, Ukraine, and the Baltic region [BAGI, in: BOTTA-DUKÁT & BALOGH, 2008], as well as in Asia - in Iraq [PHELOUNG & al. 1999; BAGI, in: BOTTA-DUKÁT & BALOGH, 2008], and in the former USSR. It was naturalised in the forest-steppe regions of Ukraine and in the Caucasus [POBEDIMOVA, in: SCHISHKIN & BOBROV 1967/1952].

It is also present in other parts of Asia [https://powo.science.kew.org].

In Romania, *A. syriaca* was cited for the first time by Czihack (in 1836) from Moldavia (as a cultivated species, cited in [KANITZ, 1879-1881]) and Transylvania – Sibiu [SCHUR, 1866], Cluj-Napoca [ŢOPA, 1947] and so on.

A. syriaca was previously recorded in the Moldavia region, as in: Vrancea County – Lespezi village [ŞUŞNIA, 2022]; Neamţ County – Başta and Ion Creangă villages [MONAH, 2001]; Iaşi County – the city of Iaşi, at "Copou" and "Mihail Sadoveanu" Memorial House [SÎRBU, 2006].

Here is a new record of *A. syriaca* in **Moldavia** province, between Grivița village (Vaslui County) and Bălăbănești village (Galaţi County), along the National Road no. 24D (GPS coordinates: N 46.13741 / E 27.68421), in ruderal habitats, in a population of ca 50-100 individuals.

Coreopsis tinctoria Nutt. [Syn. *Calliopsis tinctoria* (Nutt.) DC., *Bidens tinctoria* (Nutt.) E. H. L. Krause] – Compositae Giseke (pro parte majore)

It is a North American species (native from Canada to the USA and East of Mexico) [BRITTON & BROWN, 1970; https://powo.science.kew.org], cultivated for ornamental purposes and sometimes occurring spontaneously. It is an alien species across much of Europe, Mozambique, and South-East Africa [https://powo.science.kew.org].

It was previously mentioned in Romania from: Braşov County – Braşov city (as *Calliopsis bicolor* Rchb.) [SCHUR, 1866]; Sibiu County: Sibiu city (as *Calliopsis bicolor* Rchb.) [SCHUR, 1866] and Păuca village [DRĂGULESCU, 2010]; Bucharest City – subspontaneous in the Botanic Garden "Dimitrie Brândză" [DIACONESCU, 1961; leg. E. Nagodă, University of Bucharest, 2013, pers. comm.]; Alba County – Lupşa village [SORAN, 1962]; Galaţi County: Covurlui Plain - with no location [PĂTRAȘC, 1974], Viile village [SÂRBU, 1978], Galați West [SÎRBU & OPREA, 2011]; Neamţ County: Piatra Neamţ - along Cuejdi River banks [SÎRBU & OPREA, 2008], Agapia [SÎRBU & OPREA, 2010]; Iași County - Iaşi city (around Ciric lake) [SÎRBU & OPREA, 2010]; Tulcea County – Sulina [SÎRBU &

OPREA, 2011]; Constanţa County: Constanţa city – "Dolphinarium" area [SÎRBU & OPREA, 2011], Vrancea County - Suraia village (along the Siret River banks) [ŞUŞNIA, 2022].

There are new records of *Coreopsis tinctoria* in:

- Cluj County: Cluj-Napoca city – in a small green area along the Someşul Mic canal (GPS coordinates N 46.76637 / E 23.55510);
- Botoşani County – the village of Răchiţi, on ruderal places (GPS coordinates N 47.76227 / E 26.69547).

Datura wrightii Regel [Syn. *D*. *meteloides* auth. tex.] – Solanaceae Juss.

It is a species native to the southwestern United States and Mexico [CORREL & JOHNSTON, 1970; LUNA-CAVAZOS & BYE, 2011]. It is cultivated as an ornamental plant in Europe, but locally escaped from cultivation, as in Austria [ESSL & RABITSCH, 2002], France and Corsica [LAMBINON, 2006, in: VERLOOVE, 2008], Spain [VERLOOVE, 2008], and Hungary [KIRÁLY & al. 2009]. It was also cited from Tunisia and Australia [https://powo.science.kew.org].

In Romania, *D*. *wrightii* was firstly identified in ruderal places (vacant lands, garbage storage places) in the southern parts of Moldavia, in Galaţi County – Folteşti and Umbrăreşti villages and Târgu Bujor town, also in the Danube Delta – Crişan, Caraorman and Letea villages, Tulcea County [SÎRBU & OPREA, 2011].

Later on, *D*. *wrightii* was identified in many places in Romania, as they are: Tulcea County [TĂNASE & al. 2011, 2012; OPREA & al. 2015]; Galaţi County [SÎRBU & OPREA, 2011; ŞUŞNIA, 2022]; Brăila County [CAMEN-COMĂNESCU & MIHAI, 2022]; Vrancea County [ŞUŞNIA, 2022]; Dolj and Vâlcea Counties [NICULESCU, 2022]; Giurgiu County [NAGODĂ & al. 2023].

There are other records in **the Oltenia** region, including Vâlcea County – Drăgăşani town (on ruderal sites) (GPS coordinates N 44.66605 / E 24.26398); Olt County – Balş town (GPS coordinates N 44.3398 / E 24.11731).

New records of the species were made in the **Dobrudja** region, as in Tulcea County – Rachelu village (GPS coordinates N 45.29088 / E 28.32117) and Periprava village (GPS coordinates N 45.39116 / E 29.54113).

Dysphania pumilio (R. Br.) Mosyakin & Clemants [Syn. *Chenopodium pumilio* R. Br.] – Chenopodiaceae Vent. (pro parte majore)

This species originates in tropical regions (Australia, New Zealand, New Caledonia) and was unintentionally introduced to Europe. Greece [DIMOPOULOS & al. 2013], by importing wool from Australia [AELLEN, 1979, in: CHYTRY, 1993]. It is also present in Africa, South Asia, North America and South America [https://powo.science.kew.org].

In Romania, it was first mentioned by CHYTRY (leg. M. Chytry, 1989) and COSTEA (1994) from the Danube Delta, Tulcea County (e.g. Crişan, Partizani, Sulina, Maliuc, Crapina, Mila 28, and Caraorman villages), growing on sandy river banks influenced by the human activities, but also in southern parts of Moldavia, on the left bank of the Danube River, in Galaţi town and downstream of Galaţi, along the banks of Danube River, at a point called "Cotul Pisicii" [SÎRBU & OPREA, 2011].

There are new records in Tulcea County – along the Danube River banks, on wet sands at Grindu village (GPS coordinates N 45.41112 / E 28.19145), Mila 64 toward Mila 65, and Tulcea town (GPS coordinates N 45.18224 / E 28.80573).

Eclipta prostrata (L.) L. [Syn. *Verbesina prostrata* L.] – Compositae Giseke (pro parte majore)

It is a native species in Temperate, Tropical, and Subtropical America [TUTIN, in: TUTIN & al. 1976; STROTHER, 2006; https://powo.science.kew.org], or in Asia (Iran, South Asia?) [VASSILCZENKO, 1999/1959; TZONEV, 2007]. As an alien species in Europe, *E. prostrata* has been recorded mainly in the Southwestern, Southern, and Eastern regions, in Greece [DIMOPOULOS & al. 2013; https://europlusmed.org/cdm_dataportal], Montenegro and Western Balkans [CAKOVIĆ & al. 2014].

It is also present in Asia, Africa, Australia, and Tasmania [https://powo.science.kew.org].

It is distributed in many places and habitats in Romania, as they are: Brăila County - "Insula Mică a Brăilei" [DIHORU & SÂRBU, 1998; CAMEN-COMĂNESCU & MIHAI, 2022]; Tulcea County – in Danube Delta [I. SÂRBU, Botanic Garden "Anastasie Fătu"/University "Alexandru Ioan Cuza" Iaşi, pers. comm. 2005; OPREA & SÎRBU, 2011; ANASTASIU, 2011; TĂNASE & al. 2011]; Constanţa County [OPREA, 2005]; Galaţi County [OPREA & al. 2011]; Olt County [P. ANASTASIU, University of Bucharest, pers. comm., 2018]; Ilfov County [P. ANASTASIU, University of Bucharest, pers. comm., 2018]; Iaşi County [OPREA & al. 2021]; Mehedinţi County [OPREA & al. 2021]; Bucharest [ANASTASIU & al. 2023], and Călăraşi County [URZICEANU & al. 2023]. It seems that it is a naturally distributed species along the whole area of the Danube River banks in Romania [P. ANASTASIU, University of Bucharest, pers. comm., 2020].

There are new records in historically regions of Romania, as they are:

i) **Moldavia**: Galaţi County – Şerbeştii Vechi village, on wet sands along the Siret River (GPS coordinates N 45.41781 / E 27.88573);

ii) **Dobrudja**: Tulcea County - Grindu village and Isaccea town, growing on wet sands along the Danube River (GPS coordinates N 45.41112 / E 28.19145).

Eriochloa villosa (Thunb.) Kunth [Syn. *Paspalum villosum* Thunb., *P. japonicum* Thunb.] – Poaceae Barnhart (pro parte majore)

The woolly cupgrass is a native species to Temperate and Subtropical Eastern Asia (Eastern Russia, Eastern China, Mongolia, Japan, Korean Republic, Korean Democratic People's Republic, Taiwan, Vietnam) [DARBYSHIRE & al. 2003; EPPO-IAP, 2024; EPPO Global Database … *Eriochloa villosa*; WFO, 2024; https://powo.science.kew.org/]. From here it spread, as an alien plant, to the Caucasus region, Western Siberia, Iran and South Asia [TSVELEV, 1984, in: DARBYSHIRE & al. 2003], in Europe, as in Ukraine [CLAYTON, in: TUTIN & al. 1980], France [RIVIÈRE & al. 1992, in: DARBYSHIRE & al. 2003], Hungary [PARTOSFALVI & al. 2008; TAKÁCS & al. 2014; SZILÁGYI & al. 2015, 2019], European Russia [SUKHORUKOV, 2011; MIKHAJLOVICH & al. 2015], Czech Republic [PAULIČ & NĚMEC, 2014; FOLLAK & al. 2020], Belgium, France and Great Britain [VERLOOVE, 2019], Austria [FOLLAK & al. 2020], Germany [https://powo.science.kew.org], as well as in USA and Canada [HITCHCOCK, 1950; DARBYSHIRE & al. 2003; SIMARD & al. 2015].

In Romania, *E. villos*a was identified in: Satu Mare County [CIOCÂRLAN & SIKE, 2006; SZATMARI, 2011, 2016; NEGREAN, 2012]; Timiş County [FĂRCĂŞESCU & al. 2007, 2008; NEGREAN, 2011, 2012]; Arad County [ARDELEAN & al. 2009, 2018; PELE, 2009]; Caraş-Severin County [NEGREAN, 2011]; Bihor County [NEGREAN, 2012]; Maramureş County [NEGREAN, 2012]; Sălaj County [NEGREAN & al. 2017]; Alba County [SÎRBU & al. 2023].

There are new records of *E. villosa* in:

- Alba County: surroundings of the railway station of Alba Iulia, in maize crops and along maize crop field margins tracks (GPS coordinates N 46.05779 / E 23.58356);

- Sibiu County: Bradu, also in maize crops and along maize crop field margins (this is the easternmost record in Romania !) (GPS coordinates N 45.71509 / E 24.32164).

A voucher was lodged in Herbarium of the Botanic Garden "Anastasie Fătu"/University "Alexandru Ioan Cuza", Iaşi (IAGB, no. 47915).

Lindernia dubia (L.) Pennell (Syn. *Gratiola dubia* L.] – Linderniaceae Borsch & al.

Species originating in North, Central and South America, naturalized in much of Europe [WEBB & PHILCOX, in: TUTIN & al. 1972; https://powo.science.kew.org], incl. Greece [DIMOPOULOS & al. 2013], and Asia [HUANG, 1998; https://powo.science.kew.org]. In Romania, *L. dubia* was first reported by CIOCÂRLAN & COSTEA (1994), on wet alluvia from the Danube Delta – Sacalin Island toward Sfântu Gheorghe arm; subsequently, it was, also, cited from Periprava and Chilia Veche villages [CIOCÂRLAN, 1994], Sulina arm at Mila 28 – west of Maliuc and Crişan villages [SÎRBU & OPREA, 2011], Tătaru forest and Chilia Veche canal [ANASTASIU, 2011], as well as within the city of Galaţi, on the left bank of Danube River [SÎRBU & OPREA, 2011]. It was also cited around the lake of Surduc (Timiş County) [NEACȘU & al. 2021], various localities along the Danube River in Giurgiu County [NAGODĂ & al. 2023], and along the Borcea arm of the Danube River (Călăraşi County) [URZICEANU & al. 2023].

There are two new records in Tulcea County: 1) Grindu village, on wet sands and small waters of the right bank of the Danube River (GPS coordinates N 45.39408 / E 28.12182), and 2) Tatanir point (downhill of Pardina village / uphill of Tătaru Mare Sand Islet) (GPS coordinates N 45.35646 / E 29.03904).

A voucher specimens was lodged in Herbarium of the Botanic Garden "Anastasie Fătu"/University "Alexandru Ioan Cuza", Iaşi (IAGB, no. 47923).

Oenothera depressa Greene [Syn. *Oe. salicifolia* G. Don, *Oe. hungarica* Borbás, *Oe. baurii* Boedijn, *Oe. strigosa* (Rydberg) Mackenzie & Bush var. *depressa* (Greene) Gates, *Oe. villosa* Thunberg ssp. *villosa* Dietrich & Raven, *Onagra salicifolia* (G. Don) Spach] – Onagraceae Juss.

It is originated in North America [DIETRICH & al. 1997] and currently is widespread as an alien species in a large part of Europe [https://europlusmed.org/cdm_dataportal], Asia, South Africa, and South America [https://powo.science.kew.org].

The old known distribution and habitats in Romania are: Harghita County – railway station of Miercurea-Ciuc [1. SÎRBU & OPREA, 2017; 2. leg. G. Negrean, University of Bucharest, 2021, as *Oe.* ? *biennis* L., in: herbarium BUC – *Flora Romaniae Herbarium G. Negrean*, no. 1824]; Galaţi County – along Siret River sands at Șendrenii Vechi, Movilenii de Jos and Bucești-Iveşti villages [SÂRBU & OPREA, 2017], Mălureni village [ŞUȘNIA & al. 2020], Condrea village [ŞUŞNIA, 2022], Cosmești village [ŞUŞNIA, 2022], Lungoci village [leg. C. Sîrbu, in: ŞUŞNIA, 2022], Smârdan village [SÎRBU & al. 2023], Tecuci town [SÎRBU & al. 2023]; Vrancea County – Biliești, Suraia, Ciușlea (Garoafa), Ploscuțeni and Vadu Roșca villages [ŞUŞNIA, 2022]; Dolj County – along the Jiu River valley [leg. C. Sîrbu, 2023]; Bacău County: Cornățel village – along the Trotuș River banks [SÎRBU & al. 2023]; Iaşi – railway station of Socola [SÎRBU & al. 2023]; Suceava County – Bogata village – along Moldova River banks [SÎRBU & al. 2023]; Tulcea County – Sulina [OPREA & al. 2021].

There are new records of *Oe. depressa* in Romania, as they are:

- **Transylvania**: Covasna County – East of Lemnia village, on banks of River Negru (GPS coordinates N 46.04503 / E 26.27363); Braşov County – Rupea-Gară (GPS coordinates N 46.01912 / E 25.2787); Sibiu County – Turnu Roşu village (GPS coordinates N 45.6432 / E 24.29455) and Avrig town (GPS coordinates N 45.71915 / E 24.37375);
- **Oltenia**: Gorj County – Rovinari-Gară, among railway tracks (GPS coordinates N 44.89953 / E 23.16706);
- **Moldavia**: Vrancea County – Ţifeşti village (the meadow of Putna River) (GPS coordinates N 45.85869 / E 27.06697); Galaţi County – Lieşti and Traian (Şerbeştii Vechi) villages – along the Siret River banks (GPS coordinates N 45.41781 / E 27.88573).

A voucher specimen was lodged in Herbarium of the Botanic Garden "Anastasie Fătu"/University "Alexandru Ioan Cuza", Iaşi (IAGB, no. 47922).

Oenothera parviflora L. [Syn. *Oe. muricata* auct., non L., *Oe. biennis* L. var. *parviflora* (L.) Torrey & A. Gray] – Onagraceae Juss.

It is a native species from East Canada to North and Central-East of USA [RAVEN, in: TUTIN & al. 1968; https://powo.science.kew.org]. It was also introduced as an alien species in East Asia and South Africa [https://powo.science.kew.org] and New Zealand [HOCH & WAGNER, 2007].

In Europe, it was introduced in England, in 1871 [MIHULKA & PYŠEK, 2001], as an alien species in ex-Czechoslovakia, France, Germany, Netherlands, Hungary, Italy, Norway, and Poland [RAVEN, in: TUTIN & al. 1968], Great Britany [ROSTAŃSKI, 1982], Romania [CIOCÂRLAN, 1994, 2009], Republic of Moldova [BURAC & MITITELU, 1995], Greece [DIMOPOULOS & al. 2013]. It grows in open, disturbed habitats [WEAKLEY, 2007], along roads, from sea level up to ca 1,000 m a.s.l. as in China [HOCH & WAGNER, 2007].

In Romania, *Oe. parviflora* was cited at the end of the 19th century, from the Danube Delta – Caraorman village, Tulcea County (? under the name of *Oe. biennis* L. var. *parviflora* Koch) [UECHTRITZ & brother SINTENIS, 1876, in: KANITZ, 1879-1881; BRÂNDZĂ, 1898; CĂLINESCU, 1942; DOLTU & al. 1983; CIOCÂRLAN, 1994]. It was also cited from: Caraş-Severin County [DOLTU & al. 1983; FĂGĂRAȘ & COVALIOV, 2021]; Maramureş County [MITITELU & DORCA, 1987]; Nemira Mountains [MITITELU & al. 1987; MITITELU & BARABAŞ, 1993]; Suceava County [MITITELU & al. 1987, 1989; TOMESCU, 2016]; Bacău County [CĂLIN & MITITELU, 1992; MITITELU & al. 1994; EPURAN, 2000; MONAH, 2001; GURĂU, 2003]; Iaşi County [MITITELU & al. 1995]; Vrancea County [COROI & COROI, 1999; A. M. COROI, 2001]; Galaţi County [M. COROI, 2001]; Sibiu County [DRĂGULESCU, 2003; SÎRBU & OPREA, 2017]; Vâlcea County [leg. C. Sîrbu & A. Oprea, 2012]; Teleorman County [DIHORU & SCHNEIDER-BINDER, 2024].

However, in some references in Romanian literature, the name *Oe. parviflora* was considered synonymous to *Oe. muricata*, which is why the presence and distribution of these two species in our country is still uncertain.

New records of *Oe. parviflora* are in **Transylvania**, as they are: Braşov County – Rupea-Gară (GPS coordinates N 46.01912 / E 25.2787); Sibiu county – Avrig town (the down part of the Brukenthal Palace park) (GPS coordinates N 45.73054 / E 24.37389); Alba County – Teiuș-Gară (GPS coordinates N 46.20309 / E 23.69148).

A voucher was lodged in Herbarium of the Botanic Garden "Anastasie Fătu"/University "Alexandru Ioan Cuza", Iaşi (IAGB, no. 47921).

Conclusions

Nineteen alien plant species are presented in this paper, six of them being newly registered in the alien flora of different historical regions of Romania, while eleven of them are registered in new localities in several counties of Romania, some in of these in the 2nd, 3rd or 4th locality. Two taxa are newly recorded for the flora of Romania, namely *Heteranthera limosa* and *Paspalum distichum* subsp. *paucispicatum*.

Some of these species have a remarkable tendency to spread, expanding their invasion area in Romania, for example, *Dittrichia graveolens*, *Paspalum distichum* subsp. *paucispicatum*, *Eriochloa villosa*, *Amaranthus palmeri*, and *Asclepias syriaca*. Other species are still rare in Romania's flora, e.g. *Heteranthera limosa*, *Coreopsis lanceolata*, *Erigeron bonariensis*, *Erigeron sumatrensis*, *Datura wrightii*, *Anisantha diandra*, *Senecio inaequidens*, etc., but they have a high reproductive capacity, with real possibilities of invasion, being able to cause significant damage to agriculture, infrastructure and even become harmful to human health.

Regarding the origin of the taxa, they are as the next: North American (8), South American (2), Mediterranean (1), North and South American (1), temperate and subtropical Asian (1), subtropical American (1), North American - South American - Central American (2), Iranian-Turanian - Ponto-Mediterranean (1), Australian-New Zealand-New Caledonian (1), and South Africa (1).

Regarding the bioforms to which they belong, the taxa are grouped as follows: annual (13), perennial (2), biennial (2), annual-biennial (1) and chamaephyte (1).

As for the habitats in which the taxa were identified, they are: vacant lots (7), riverbanks (4), road embankments (3), railway embankments (2), crops (1), and permanent waters (2).

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Research Article

TILIO TOMENTOSAE-QUERCENION PETRAEAE (PÎNZARU ET CANTEMIR 2023) PÎNZARU, SFECLĂ ET CANTEMIR STAT. NOV. HOC LOCO (*QUERCION PETRAEAE* ISSLER 1931) IN THE REPUBLIC OF MOLDOVA

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Abstract: This paper describes the slightly acidic hill forests of *Quercus petraea* (Matt.) Liebl. with *Tilia tomentosa* Mill. from the Republic of Moldova, grouped into 3 associations: *Carpino orientalis-Quercetum petraeae* Pînzaru, Sfeclă et Cantemir, ass. nov. hoc loco, *Tilio tomentosae-Quercetum petraeae* Sârbu 1979 corr. Pînzaru, Sfeclă et Cantemir hoc loco, *Hieracio umbrosi-Quercetum petraeae* Pînzaru, Cantemir, Manic & Popescu 2017 from the suballiance *Tilio tomentosae-Quercion petraeae* (Pînzaru & Cantemir 2023) Pînzaru, Sfeclă et Cantemir, stat. nov. hoc loco. These associations occur on plateaus, in the upper part of the hills, at an altitude of 138-367 m, on yellow sands, devoid of soil, or on virgin brown or gray luvic soils, formed on sandy, loamy-clayey rocks, with the pH value ranging from 4.5 to 6.0. The phytocoenoses of the associations *Carpino orientalis-Quercetum petraeae*, *Hieracio umbrosi-Quercetum petraeae* are rare and it is proposed to include them in the future *List of rare associations in the Republic of Moldova*.

Keywords: distribution range, ecology, phytocoenoses, rare forest associations, sessile oak, silver lime, Republic of Moldova.

Introduction

Forest stands of sessile oak (*Quercus petraea*) with silver lime (*Tilia tomentosa*), or oriental hornbeam (*Carpinus orientalis*) in the Republic of Moldova, in previous publications, they were described using the methods of the Soviet School and grouped into several associations according to the dominant species by GEJDEMAN & al. (1964) and POSTOLACHE (1995). Later, using the methods of the Central-European School [BRAUN-BLANQUET, 1964], some sessile-oak stands were assigned to the associations *Querco–Carpinetum orientalis* [GANCEV, 1961] Csűrös et al. 1968 [LAZU & al. 2007; POSTOLACHE, 2024], while others were attributed to *Hieracio umbrosi–Quercetum petraeae* Pînzaru, Cantemir, Manicé & Popescu, 2017. Next, the sessile oak forests with sliver lime, or oriental hornbeam are grouped in the *Tilio tomentosae-Quercenion petraeae* (Pînzaru et Cantemir 2023) Pînzaru, Sfeclă et Cantemir, stat. nov. hoc loco.

In this paper, on the basis of new phytocoenological investigations, the authors present three associations of sessile-oak stands: *Carpino orientalis-Quercetum petraeae* Pînzaru, Sfeclă et Cantemir, ass. nov., hoc loco; *Tilio tomentosae-Quercetum petraeae* Sârbu 1979 corr.

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Pînzaru, Sfeclă et Cantemir, hoc loco, and *Hieracio umbrosi-Quercetum petraeae* Pînzaru et al. 2017, grouped within the suballiance *Tilio tomentosae-Quercenion petraeae* (Pînzaru et Cantemir 2023) Pănzaru, Sfeclă et Cantemir, stat. nov., hoc loco, in the alliance *Quercion petraeae* Issler 1931, order *Quercetalia pubescenti-petraeae* Klika 1933, class QUERCO-FAGETEA SYLVATICAE Br.-Bl. Et Vlieger in Vlieger 1937.

Material and methods

Floristic and phytosociological surveys were carried out during 1995-2025, from spring and summer where spring data were missing, the symbol 'x' was used in the synoptic table. In total, 74 phytosociological relevés were recorded according to the Central-European School [BRAUN-BLANQUET, 1964]. Relevé was 600 m² [CRISTEA & al. 2004]. Species cover-abundance was estimated on the scale proposed by J. Braun-Blanquet and J. Pavillard (1928); for species occurring in sparse clumps, the cover-abundance entries are underlined in the table.

Floristic nomenclature follows the monograph *Vascular Flora of the Republic of Moldova* (*species list and ecology*) [PÎNZARU, 2023]. State-protected species are indicated according to [Law No. 1538/1998; *Red Book of the Republic of Moldova*, 2015]. For the floristic analysis, we followed [SANDA & al. 1983; SÂRBU & al. 2013]. Mean annual temperature and precipitation were taken from the *Atlas of Climate Resources of the Republic of Moldova* [NEDEALCOV & al. 2013], and soil types from *The Soils of Moldova* [URSU, 2011]. Soil reaction (pH) was determined in situ by the authors using *p*H indicator paper and distilled water (Coralclub pH Test Paper 4.5-7.5). Tree stem diameter was measured at 1.30 m above ground (DBH) with a diameter tape (D-tape), and tree height with a Vertex V hypsometer.

Results and discussions

Forests dominated by sessile oak (*Quercus petraea*) mixed with silver lime (*Tilia tomentosa*) are most widespread on the Central Moldavian Plateau, and are less frequent on the Tigheci Hills, the Dniester Plateau, and the southern part of the North Moldavian Plain. They occur on upper hill slopes and plateaus at 138-360 m, under a temperate-continental climate; mean annual air temperature ranges from 10.5 °C in the south to 9.0 °C in the north, and mean annual precipitation is 500-650 mm. The substrate includes yellow sands with little or no developed soil cover, as well as virgin brown or gray luvic soils, clay-illuviated chernozems, and grey soils developed on sandy to loamy-clayey parent materials, soil *p*H ranges from 4.5 to 6.0.

The association *Querco-Carpinetum orientalis* (Gancev, 1961) Csűrös et al. 1968, also reported by some authors from the Republic of Moldova [LAZU & al. 2007; POSTOLACHE, 2024], is treated as a synonym of the association *Carpinetum orientalis* Rudski apud Horvat 1946 [SANDA & al. 2008; CĂRĂBUȘ & ȘOFLETEA, 2014]. Stands of this association are dominated by *Carpinus orientalis*, accompanied by *Cotinus coggygria*, *Fraxinus ornus* and *Prunus mahaleb*, whereas the phytocoenoses with *Carpinus orientalis* in the Republic of Moldova have the tree layer dominated by *Quercus petraea*, with oriental hornbeam occurring only sporadically in the second canopy layer. For this reason, a new association is proposed: *Carpino orientalis-Quercetum petraeae*, ass. nova.

The new suballiance has many species in common with the suballiance *Carpino-Tilenion* (*tomentosae*) Doniță 1970 prov. cited by its author for the Babadag Plateau (Dihoru et Doniță 1970) but is not valid [Art. 3b].

The xeromesophilous, acidophilous oaks from the north of the Republic of Moldova, grouped in the *alliance Quercion petraeae* Issler 1931, differ from those from the center and south of the republic by the presence of the following characteristic species: *Carex montana*, *Laserpitium latifolium*, *Iris graminea*, *Genista elata*, *Hieracium umbellatum*, *Potentilla alba*, *Pulmonaria mollis*, *Serratula tinctoria*, *Silphiodaucus prutenicus*, *Stachys officinalis*, *Veratrum nigrum,* for which reason a new suballiance is proposed

Suball. ***Tilio tomentosae-Quercenion petraeae*** (Pînzaru et Cantemir 2023) Pînzaru, Sfeclă et Cantemir, stat. nov., hoc loco

Syn.: *Tilio tomentosae-Quercion petraeae* Pînzaru et Cantemir 2023, International Scientific-Practical Conference, 18-19 March 2023, vol. 1, p. 201; *Carpino orientalis–Tilenion tomentosae* Doniță *prov*. in Dihoru & Doniță, 1970 [Art. 3b].

N o m e n c l a t u r a l t y p e: ass. *Hieracio umbrosi-Quercetum petraeae* Pînzaru et al. 2017, *Journal of Plant Development*, **24**: 103-116.

It includes West-Pontic, thermophilic, xeromesophilic, slightly acidophilic forest phytocenoses, found in the hilly area.

<u>Diagnostic species:</u> *Carpinus orientalis*, *Quercus petraea*, *Q. robur* subsp. *pedunculiflora*, *Tilia tomentosa*, *Crataegus pentagyna*, *Allium siculum* subsp. *dioscoridis*, *Coronilla elegans*, *Digitalis grandiflora*, *Doronicum hungaricum*, *Fritillaria montana*, *Galanthus plicatus*, *Hieracium robustum*, *H. sabaudum*, *H. umbrosum*, *Laser trilobum*, *Lathyrus aureus*, *Loranthus europaeus*, *Potentilla micrantha*, *Tulipa biebersteiniana* var. *biebersteiniana*, *Vicia cassubica*.

As a result of the floristic and ecological analysis of the phytocoenoses in the Republic of Moldova, three associations are recognized within this suballiance:

1. ***Carpino orientalis-Quercetum petraeae*** Pînzaru, Sfeclă et Cantemir, ass. nova, hoc loco
2. ***Tilio tomentosae-Quercetum petraeae*** Sârbu 1979 corr. Pînzaru, Sfeclă et Cantemir, hoc loco
    - subass. ***typicum*** Sârbu 1979
    - subass. ***cotinetosum coggygriae*** Sârbu 1979
    - subass. ***cornetosum maris*** (Sârbu 1979) Pînzaru et al., stat. nov. h. l.
3. ***Hieracio umbrosi-Quercetum petraeae*** Pînzaru, Cantemir, Manic et Popescu 2017

1. Ass. *Carpino orientalis-Quercetum petraeae*
Pînzaru, Sfeclă et Cantemir, ass. nova. hoc loco

Syn.: *Fraxineto* (*excelsioris*)-*Quercetum* (*petraeae*) *carpinulosum* (*orientalis*) Gejdeman et al. 1964, *Tipy lesa i lesnye assotziatzii MSSR*: 194; Postolache, 1995. – *Querceto* (*petraeae*)-*Carpinetum* (*orientalis*) *cotinosum* (*coggygriae*) Gejdeman et al. 1964, *work cited*: 195; Postolache 1995. – *Fraxineto* (*excelsioris*)-*Quercetum* (*petraeae*) *carpinuloso* (*orientalis*) *hederosum* (*helicis*) Gejdeman et Simonov 1971, *Izvestia AN MSSR. Ser. biol. i him. nauk*, 1: 83. – *Querco petraeae-Carpinetum orientalis* auct. non (Gancev 1961) Csüros et al. 1968: Lazu et al. 2007; Postolache, 2024.

Typus hoc loco: Tab. 1, rel. 4.
Table synthetic hoc loco: Tab. 1, 25relevés.

Figure 1. *Carpinus orientalis* Mill. 17.04.2016, Cărbuna commune, Ialoveni district

Characteristic species: *Carpinus orientalis* (Figure 1), *Quercus petraea*.

Constant species: *Acer campestre*, *Allium siculum* subsp. *dioscoridis*, *Anemonoides ranunculoides*, *Arum orientale*, *Asparagus tenuifolius*, *Carex brevicollis*, *Corydalis solida*, *Crataegus monogyna*, *Fraxinus excelsior*, *Geum urbanum*, *Glechoma hirsuta*, *Lathyrus niger*, *Melica uniflora*, *Mercurialis ovata*, *Polygonatum hirtum*, *Scilla bifolia*, *Stellaria holostea*, *Viburnum lantana*.

Chorology and site conditions. The phytocoenoses of the association are distributed on the Central Moldavian Plateau, in Cimișlia District (Zloți village) and Ialoveni District (the communes of Cărbuna, Răzeni, and Suruceni). They adjoin the phytocoenoses of the associations *Iridio variegatae-Quercetum pubescentis* Pînzaru, Cantemir & Belous 2022, *Tilio tomentosae-Quercetum petraeae* Sârbu 1979, corr. hoc loco, and *Carici pilosae-Carpinetum betuli* Neuhausl et Neuhauslova-Novotna 1964. They occur at 150-239 m a.s.l., on hills with various aspects and gentle slopes 5-25(35)°, on grey soils developed over sandy to loamy-clayey parent materials; soil *p*H ranges from 5.0 to 6.0.

Floristic composition and phytocoenotic structure (Table 1: 25 relevés; association no. 1). The floristic composition of the 25 phytosociological relevés comprises 132 vascular plant species, of which: 12 are assigned to the suballiance *Tilio tomentosae-Quercenion petraeae* (Pînzaru et Cantemir 2023) *stat. nov. hoc loco*; 5 to the alliance *Quercion petraeae* Issler 1931; and 27 to the order *Quercetalia pubescenti-petraeae* Klika 1933; 5 – all. *Carpinion betuli* Issler 1931; 29 – ord. *Fagetalia sylvaticae* Passarge 1928; 27 – cl. QUERCO-FAGETEA Br.-Bl. et Vlieger in Vlieger 1937; 6 – cl. CRATAEGO-PRUNETEA Tx. 1962; 5 – cl. TRIFOLIO-GERANIETEA SANGUINEI T. Müller 1962 and 19 to other syntaxa (Aliae).

The tree layer is two-layred. The upper tree layer (A1) is 18-24 m tall with 60-85% canopy cover and is dominated by *Quercus petraea*; stems are 25-40 (-50) cm in diametr, with admixtures of *Fraxinus excelsior* and, locally, *Tilia tomentosa* and *Acer platanoides*. In the lower tree layer (A2), *Carpinus orientalis* occurs sporadically to frequently (7-12 m tall, 7-15 cm in diametr), together with *Acer campestre* and *A. tataricum*; *Sorbus torminalis*, *S. aucuparia*, *S. domestica*, and *Quercus pubescens* are rare.

The shrub layer is unevenly developed, depending on canopy closure and the extent of anthropogenic disturbance; its cover ranges from 10–50% (occasionally up to 70%) and is composed of *Crataegus monogyna*, *Viburnum lantana*, *Euonymus verrucosus*, and *Cotinus coggygria*.

The herb layer in spring, with 40-100% cover, is composed of *Corydalis solida*, *Anemonoides ranunculoides*, *Scilla bifolia*, *Allium siculum* subsp. *dioscoridis*, *Anthriscus longirostris*, *Veronica hederifolia*; sporadically present are *Arum orientale*, *Corydalis cava*, *Corydalis marschalliana*, *Allium ursinum*, *Fritillaria montana*, *Tulipa biebersteiniana* var. *biebersteiniana* or var. *tricolor*, and *Ficaria verna*. In summer, the herb-layer cover generally ranges from 40-80%, with more abundant species including *Aegonychon purpurocaeruleum*, *Poa nemoralis*, *Polygonatum hirtum*, and *Stellaria holostea*; *Asparagus tenuifolius*, *Carex brevicollis*, *Geum urbanum*, *Lathyrus niger*, *Melica uniflora*, and *Mercurialis ovata* occur only sporadically. Species richness per relevé ranges from 28 to 53.

Protected species. Species included in the Red Book of the Republic of Moldova (2015): *Allium siculum* subsp. *dioscoridis* (= *Nectaroscordum bulgaricum*) [Vulnerable (VU)]; *Carpinus orientalis* [Vulnerable (VU)]; *Cephalanthera longifolia* [Vulnerable (VU)]; *Chaerophyllum nodosum* (= *Physocaulis nodosus*) [Critically Endangered (CR)]; *Digitalis lanata* [Critically Endangered (CR)]; *Fritillaria montana* [Vulnerable (VU)]; *Sorbus domestica* [Endangered (EN)]. Species at low risk of extinction [Near Threatened (NT)] (Law No. 1538/1998): *Asparagus tenuifolius*, *Doronicum hungaricum*, *Epipactis helleborine*, *Lilium martagon*, *Tulipa biebersteiniana* var. *biebersteiniana*.

The bioforms spectrum: hemicryptophytes (H) 37.1%; geophytes (G) 21.2%; microphanerophytes (M) 9.8%; nanophanerophytes (N) and annual therophytes (Th) 9.1% each; biennial therophytes (TH) 6.8%; megaphanerophytes (MM) 5.3%; chamaephytes (Ch) and lianas (L) 0.8% each.

The floristic elements spectrum. Eurasian elements (Eua) are most frequent (33.3%), followed by European (Eur) – 16.6% and Central-European (Euc) – 8.3%. Mediterranean (M), Pontic-Mediterranean (PM) and Circumpolar (Circ) elements each account for 5.3%; the remaining elements are represented by 1-4 species.

The spectrum of the edaphic humidity. Xero-mesophilous species (class 2) predominate at 51.5%, versus mesophilous species (class 3) at 48.5%.

Conservation status. At the territorial level, the phytocoenoses are protected within the “Cărbuna” Landscape Reserve and the “Molești–Răzeni” Forest Reserve.

2. Ass. *Tilio tomentosae-Quercetum petraeae*

Sârbu 1979 corr. Pînzaru, Sfeclă et Cantemir, hoc loco (Figure 2)

Syn.: *Tilio tomentosae-Quercetum dalechampii* Sârbu 1979, *Culeg. Șt. Art. Biol.*, *1. Grăd. Bot. Iași*, 173-176; CHIFU & al. 2006; GAFTA & al. 2008; SANDA & al. 2008; CHIFU & IRIMIA, 2014; INDREICA, 2015; *Tilieto* (*tomentosae*)-*Quercetum* (*petraeae*) *caricosum*

Gejdeman et al. 1964, *work cited*.: p. 144; *Tilieto* (*tomentosae*)-*Carpineto*-*Quercetum* (*petraeae*) *caricosum* Ghejdeman et al. 1964, *work cited*.: p. 142; *Piptathero virescentis-Quercetum dalechampii* Chifu, Sârbu et Ştefan (1998) 2004.

N o m e n c l a t u r a l t y p e: Sârbu 1979, single Table, rel. no. 2, lectotypus Chifu, Mânzu et Zamfirescu 2006.

Table synthetic h. l.: Tab. 2, 1-18 relevés; Tab. 3 (subass. *cotinetosum coggygriae* Sârbu 1979– rel. no. 19-23; subass. *cornetosum maris* (Sârbu 1979) stat. nov. h.l. – rel. 24-36).

Characteristic species: *Tilia tomentosa*, *Quercus petraea* [= *Q. dalechampii* auct rom., non Ten.].

Constant species: *Acer campestre*, *Carex brevicollis*, *C. pilosa*, *Convallaria majalis*, *Cornus mas*, *Euonymus verrucosus*, *Fraxinus excelsior*, *Geum urbanum*, *Glechoma hirsuta*, *Stellaria holostea*, *Viburnum lantana*.

Chorology and site conditions. The association comprises phytocoenoses of sessile oak with silver lime, typically on the Central Moldavian Plateau, more rarely on the Middle Prut Plain, the Dniester Plateau, and the Tigheci Hills. Its phytocoenoses adjoin those of the associations *Carici pilosae-Carpinetum betuli* Neuhausl et Neuhauslova-Novotna 1964; *Carpino orientalis-Quercetum petraeae* Pînzaru et al. *ass. nov.. hoc loco*, and *Hieracio umbrosi-Quercetum petraeae* Pînzaru et al. 2017. They occur at 138-367 m, on plateaus or the upper parts of hills, on clay-illuviated chernozems or grey soils developed on sandy clays or yellow sands. The slope exposure is diverse, with a predominance of north-western and north-eastern exposures; slopes are 5-15(25)°. Soil *p*H values are: in subass. *typicum*, 5.0–5.5; in subass. *cotinetosum coggygriae*, 5.0-6.0; and in subass. *cornetosum maris*, 5.5-6.0.

Floristic composition and phytocoenotic structure. In 36 phytosociological relevés, 149 vascular plant species were recorded, including 9 character species of the suballiance *Tilio tomentosae-Quercenion petraea* (Pînzaru et Cantemir 2023) Pînzaru, Sfeclă et Cantemir *stat. nov. hoc loco*; 7 of the alliance *Quercion petraeae* Issler 1931; 25 of the order *Quercetalia pubescenti-petraeae* Klika 1933; 9 of the alliance *Carpinion betuli* Issler 1931; 39 of the order *Fagetalia sylvaticae* Pawłowski 1928; 36 of the class *QUERCO-FAGETEA SYLVATICAE* Br.-Bl. et Vlieger in Vlieger 1937; 5 of the class *CRATAEGO-PRUNETEA* Tx. 1962; 5 of the class *TRIFOLIO-GERANIETEA SANGUINEI* T. Müller 1962; and 14 belonging to other syntaxa (Aliae). The vertical structure comprises 2-3 layers. The tree layer is 18-24 m tall with 75-85% canopy cover, dominated by *Quercus petraea* (stem diameter 30-56 cm), accompanied by *Tilia tomentosa*, *Acer campestre*, *A. platanoides*, *Carpinus betulus*, *Fraxinus excelsior*, and *Sorbus torminalis* occur sporadically. The shrub layer is absent or scattered in places, or else well developed and dominated by *Cotinus coggygria* or *Cornus mas*. The herb layer is unevenly developed – sparser in phytocoenoses dominated by *Cotinus coggygria*, which forms large patches at the herb-layer level with cover – abundance 3-5 (scale Braun-Blanquet et Pavillard, 1928) – and better developed in phytocoenoses with *Cornus mas*.

Protected species. Species included in the *Red Book of the Republic of Moldova* (2015): *Allium siculum* subsp. *dioscoridis* (= *Nectaroscordum bulgaricum*) [Vulnerable (VU)]; *Cephalanthera damasonium* [Vulnerable (VU)]; *Epipactis purpurata* [Critically Endangered (CR)]; *Fritillaria montana* [Vulnerable (VU)]; *Galanthus plicatus* [Critically Endangered (CR)]; *Ornithogalum pyrenaicum* [Endangered (EN)]; *Scopolia carniolica* [Vulnerable (VU)]. Species at low risk of extinction [Near Threatened (NT)] (Law No. 1538/1998): *Asparagus tenuifolius*, *Epipactis helleborine*, *Lathyrus venetus*, *Lilium martagon*, *Sorbus torminalis*, *Staphylea pinnata*, *Tulipa biebersteiniana* var. *biebersteiniana*.

The bioforms spectrum: hemicryptophytes (H) predominate at 41.0%, followed by geophytes (G) at 24.1%; nanophanerophytes (N) and annual therophytes (Th) at 7.7% each; megaphanerophytes (MM) at 6.2%; microphanerophytes (M) at 5.6%; biennial therophytes (TH) at 4.9%; epiphytes (Ep) at 1.4%; and chamaephytes (Ch) and lianas (L) at 0.7% each.

The floristic elements spectrum. The Eurasian element (Eua) is dominant – 33.3%, followed by the European (Eur) – 22.7%, Central European (Euc) – 12.5%, Circumpolar (Circ) – 5.5%, Pontic-Mediterranean (PM) – 4.1%, the other floristic elements are present with few species (1-4).

The spectrum of the edaphic humidity: Numerically, mesophilic species predominate (61.2%), followed by xeromesophilic ones (38.8%).

Figure 2. Ass. *Tilio tomentosae-Quercetum petraeae* Sârbu 1979 Pînzaru et al. corr. h. l., 05.06.2025, Malcoci commune, Ialoveni district

Phytocoenotic diversity. The phytocoenoses of this association, from a floristic and ecological point of view, are grouped into 3 sub-associations:

- subass. ***typicum*** Sârbu 1979 (tab. 1, 26 rel.); CHIFU & al., 1996 (tab. 2, 15 rel.); CHIFU & al. 2006 (tab. 27, col. 1a, 68 rel.); CHIFU & IRIMIA, 2014 (tab. 29, col. 8a, 132 rel.)

Table synthetic h. l.: Tab. 2, rel. 1-18

Mesophilous phytocoenoses; shrub layer absent or scattered; in the herb layer, more abundant species include *Corydalis solida*, *Carex pilosa*, *Stellaria holostea*, *Glechoma hirsuta*, and *Convallaria majalis*. Species richness per relevé ranges from 26 to 62.

Local distribution. Chişinău municipality (Dumbrava), Ialoveni (Ulmu), Cantemir Districts (Capaclia and Hănăseni villages), Hîncești (Logănești), Strășeni (Căpriana, Stejăreni), Călărași (Bahmut), Nisporeni (Boldurești), Șoldănești (Olișcani), Rezina (Saharna) and Edineț (Brînzeni);

- subass. ***cotinetosum coggygriae*** Sârbu 1979 (tab. 1, rel. 27-31); CHIFU & al. 2006 (tab. 27, col. 1b, 33 rel.); CHIFU & IRIMIA, 2014 (tab. 29, col. 8c, 33 rel.)

Syn.: *Querco petraeae-carpinetum cotinetosum coggygriae* Horeanu 1981; *Cotino-Quercetum petraeae* Borza 1937 (phantome): POSTOLACHE, 2024; *Quercetum* (*petraeae*) *cotinosum* Gejdeman et al. 1964, *work cited*.: p. 199.

Lectotypus hoc loco: I. Sârbu, 1979, tab. 1, rel. 27

Table synthetic h. l.: Tab. 3, rel. no. 19-23.

Xero-mesophilous phytocoenoses exhibiting a summer water deficit, typically developing on yellow sands. The shrub layer is well developed and dominated by *Cotinus coggygria* with cover-abundance 3-5 (Braun–Blanquet scale). The herb layer is poorly expressed or absent; vernal ephemeroids are practically lacking. In summer, *Convallaria majalis*, *Poa nemoralis*, *Glechoma hirsuta*, *Lathyrus niger*, *Vincetoxicum hirundinaria*, and *Stellaria holostea* occur sporadically or in small patches. Species richness per relevé ranges from 26 to 45.

Local distribution. Ialoveni District (Malcoci) and Hîncești District (Mereșeni);

- *subass.* ***cornetosum maris*** (Sârbu 1979) stat. nova h.l.

Syn.: *Tilio tomentosae-Quercetum dalechampii* Sârbu 1979, facies cu *Cornus mas* (tab. 1, rel. 32-35); *Carpineto-Quercetum* (*petraeae*) *cornosum* Gejdeman et al. 1964, p.p. *work cited*.: p.155; *Quercetum* (*petraeae*) *cornosum* Gejdeman et al. 1964, p.p. *work cited*.: p. 136.

Lectotypus hoc loco: Sârbu 1979, tab. 1, rel. 34,

Table synthetic h. l.: Tab. 3, rel. no. 24-36

They occur on forest chernozem soils of high fertility; the substrate is drier in summer than in spring. The shrub layer is dominated by *Cornus mas*. The herb layer is generally well developed throughout the growing season: in spring, vernal ephemeroids include *Corydalis solida*, *Anemonoides ranunculoides*, *Gagea lutea*, *Scilla bifolia*, *Allium siculum* subsp. *dioscoridis*, and *Allium ursinum* s.l. in summer, the more abundant species are *Carex pilosa*, *Carex brevicollis*, *Mercurialis perennis*, and *Stellaria holostea*. Species richness per relevé ranges from 34 to 65.

Local distribution. Chişinău municipality (Condrița), Ialoveni (Malcoci) and Hincești districts (Drăgușenii Noi, Horodiște, Logănești).

Territorial protection of the association. The phytocenoses of the association are included in the scientific reserves “Codru”, “Plaiul Fagului”, landscape reserves “Dobrușa”, “Saharna”, “Codrii Tigheci”.

3. Ass. *Hieracio umbrosi-Quercetum petraeae*

Pînzaru, Cantemir, Manic et Popescu 2017, *J. Plant Dvelop*. **24**: 103-115

T y p u s: PÎNZARU et al. 2017, Tab. 1, rel. 7

Characteristic species: *Quercus petraea*, *Hieracium umbrosum*.

Chorology and site conditions. The association comprises rare, xero-mesophilous phytocoenoses of sessile oak with shade hawkweed (*Quercus petraea* with *Hieracium umbrosum*), occurring on the Central Moldavian Plateau – in Hîncești District (Mereșeni, Logănești, Lăpușna) and Ialoveni District (Văsieni, Cărbuna). They grow on high hills at 210-360 m a.s.l., on gently inclined slopes (7-25°) with predominantly north-western and south-western aspects, on sandy clays and yellow sands. Soil *p*H ranges from 4.5 to 6.0.

Conservation status. The phytocoenoses of this association are protected in the Logănești Nature Reserve of Medicinal Plant, plot 36, and in the "Hîncești Forest" Landscape Reserve, plot 41 [PÎNZARU & al. 2017].
References: PÎNZARU & al. 2017; PÎNZARU & CANTEMIR, 2021; POSTOLACHE, 2024.

Conclusions

The suballiance *Tilio tomentosae-Quercenion petraeae* (Pînzaru et Cantemir 2023) Pînzaru, Sfeclă et Cantemir, hoc loco comprises West-Pontic, xero-mesophilous to mesophilous, colline associations occurring at 138-367 m a.s.l., on yellow sands and sandy clays, as well as on clay-illuviated chernozems and grey soils; soil pH ranges from 4.5 to 6.0.

Within the Republic of Moldova, three associations are recognized: *Hieracio umbrosi-Quercetum petraeae* Pînzaru, Cantemir, Manic & Popescu 2017; *Carpino orientalis-Quercetum petraeae* Pînzaru, Sfeclă & Cantemir, ass. nova, hoc loco; and *Tilio tomentosae-Quercetum petraeae* Sârbu 1979, corr. hoc loco, the first two associations are rare, and we propose that they be included in the forthcoming *List of Rare Associations of the Republic of Moldova.*

The associations *Carpino orientalis-Quercetum petraeae* Pînzaru, Sfeclă & Cantemir, ass. nova, hoc loco; *Tilio tomentosae-Quercetum petraeae* Sârbu 1979, corr. Pînzaru, Sfeclă & Cantemir, hoc loco; and *Hieracio umbrosi-Quercetum petraeae* Pînzaru et al. 2017 are grouped within the suballiance *Tilio tomentosae-Quercenion petraeae* (Pînzaru et Cantemir 2023) Pînzaru, Sfeclă et Cantemir hoc loco, the alliance *Quercion petraeae* Issler 1931, the order Quercetalia pubescenti-petraeae Klika 1933, the class QUERCO-FAGETEA SYLVATICAE Br.-Bl. et Vlieger in Vlieger 1937.

In the southern Republic of Moldova, forest plantations should employ native, thermophilous and xerophilous species adapted to arid conditions, such as *Carpinus orientalis* Mill., *Quercus pubescens* Willd., *Quercus robur* subsp. *pedunculiflora* (K. Koch) Menitsky, *Fraxinus coriariifolia* Scheele, *Fraxinus ornus* L., *Tilia tomentosa* Moench, *Cerasus mahaleb* (L.) Mill., and *Cotinus coggygria* Scop.; black locust (*Robinia pseudoacacia* L.) should be avoided, as it is currently undergoing dieback.

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Table 1. Ass. *Carpino orientalis-Quercetum petraeae* Pînzaru, Sfeclă et Cantemir ass. nova

Relevé no.	1	2	3	*4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	K
Altitude(m)	211	210	210	210	210	215	216	216	215	212	205	239	204	187	215	236	223	171	152	150	168	210	233	230	201	
Aspect	-	NE	-	N	N	NV	S	S	SE	SE	V	S	NE	NE	N	-	NV	S	SV	NE	NV	NV	NE	V	S	
Slope (°)	-	5	-	20	10	10	5	5	10	5	25	5	5	5	5	-	25	35	25	10	15	10	10	15	15	
Tree layer coverage A1(%)	80	70	75	70	80	70	70	70	70	70	75	80	80	80	80	60	85	60	60	70	65	80	80	60	70	
Tree layer coverage A2(%)	30	35	45	50	10	50	50	45	30	45	50	20	10	10	40	50	50	70	50	60	50	60	35	70	30	
Shrub layer coverage (%)	35	30	50	40	40	45	40	35	40	35	30	15	10	5	2	10	10	10	5	-	3	5	5	35	65	
Spring herbaceous layer coverage (%)	85	85	80	95	80	70	100	100	100	85	60	70	40	20	100	100	40	45	100	100	90	80	90	75	10	
Summer herbaceous layer coverage (%)	80	60	65	80	75	60	55	50	30	35	70	80	60	10	20	20	80	90	45	10	35	55	75	40	45	
Surface of relevé (m²)	600	600	600	600	600	600	600	600	600	600	600	600	600	600	600	600	600	600	600	600	600	600	600	600	600	
Number of species	50	50	49	52	39	53	53	39	48	36	29	28	34	32	40	36	38	30	38	30	44	42	53	36	51	
Plots no.	58	1	7A	7	7	43	42	42	42	40	12	13	17	18	18	15	10	10	22M	22M	22M	22M	22S	44M	40	
Charact. species																										
Carpinus orientalis	1	+	1, 2	1, 2	+	+	+	+	+	1	3	1	+	3	1, 2	3	1	2	2	3	4	3	+	+	+	V
Tilio tomentosae-Quercenion petraeae																										
Allium siculum ssp. dioscoridis	1, 3	1	+	1	+	+	1, 2	-	r	-	r	-	1	-	1, 2	+	-	-	1, 2	2, 3	1, 2	1, 3	r	-	-	IV
Fritillaria montana	r	-	-	-	-	+	1	+	-	+	-	-	-	-	-	-	-	-	+	1	1, 2	+	-	+	-	III
Tulipa biebersteiniana var. biebersteiniana	+	+	+	1	-	1, 2	1, 2	1	+	1	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	III
Laser trilobum	-	-	r	+	r	+	r	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	II
Tilia tomentosa	-	+	+	+	2	+	-	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	r	r	II
Doronicum hungaricum	-	-	-	-	-	r	r	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I
Lathyrus aureus	-	-	-	r	r	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I
Vicia pisiformis	-	-	+	r	+	-	-	-	-	-	-	-	-	-	-	-	r	-	-	-	-	-	-	-	-	I
Quercion petraeae																										
Quercus petraea	4	3	4	4	3	3	4	3	3	4	3	4	2	3	3	2	3	2.3	1	2	3	3	2	3	4	V
Sorbus torminalis	r	+	+	+	+	+	+	-	-	-	-	-	-	-	-	+	+	r	-	-	r	+	r	+	-	III
Digitalis lanata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	I
Potentilla micrantha	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	r	-	I
Sorbus aucuparia	-	r	r		r	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	r	r	-	I

Quercetalia pubescenti-petraeae																										
Asparagus tenuifolius	+	+	r	r	+	r	r	+	r	-	-	r	r	-	-	r	r	-	-	-	r	-	r	+	r	IV
Glechoma hirsuta	1, 3	-	1	1	-	2	1	-	+	1	1	1	1	+	+	1	1, 3	1	-	-	1, 3	1, 2	-	+	1, 2	IV
Lathyrus niger	r	+	+	+	+	+	+	-	+	+	-	-	-	-	-	-	+	+	-	-	+	+	+	+	+	IV
Mercurialis ovata	-	+	+	1	+	+	+	+	r	+	-	+	-	-	-	+	+	r	+	-	+	r	+	+	-	IV
Aegonichon purpurocaeruleum	2	1, 2	1, 2	1, 2	1, 2	1	1	+	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	1, 2	1, 3	III
Chaerophyllum nodosum	1	+	-	+	-	+	1	-	-	-	-	-	-	-	+	+	-	-	+	1, 2	-	+	-	+	-	III
Euonymus verrucosus	1	1	1	+	-	2	1	1	-	2	+	+	-	r	-	-	+	r	-	-	-	-	-	-	1	III
Carex muricata	-	+	+	r	+	-	+	-	-	-	r	-	-	-	-	-	-	-	-	-	+	-	-	-	-	II
Cotinus coggygria	+	1	1, 2	1, 2	1, 3	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1, 2	II
Ligustrum vulgare	-	-	+	+	+	-	-	-	-	-	+	-	-	r	-	-	r	+	-	-	+	r	-	-	-	II
Tanacetum corymbosum	r	-	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	II
Symphytum tauricum	+	-	-	-	-	-	+	-	r	+	-	-	-	-	r	+	-	-	-	-	-	-	-	-	-	II
Arabis turrita	-	-	-	+	-	-	-	r	-	-	-	-	-	-	-	-	-	-	-	-	r	-	-	-	-	I
Iris graminea	-	-	-	-	-	r	r	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	r	-	I
Piptatherum virescens	-	-	-	-	r	-	-	-	-	-	-	-	-	r	-	-	-	-	-	-	+	-	-	-	1	I
Viola suavis	+	-	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I
Carpinion betuli																										
Stellaria holostea	2	1, 2	2	3	2	1, 2	2, 4	1	+	1	3	3	1	-	+	1, 2	+	-	1, 3	1, 2	+	2, 3	-	1	-	V
Carex brevicollis	+	+	1	+	1	1	+	-	+	+	-	r	-	-	r	r	+	+	+	+	1, 2	+	+	1, 3	-	V
Carpinus betulus	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	II
Corydalis marschalliana	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	1, 2	1, 2	+	1	-	-	II
Isopyrum thalictroides	-	-	-	-	-	-	-	1, 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I
Fagetalia																										
Anemonoides ranunculoides	1	1	1	1	1	1, 2	1	1	1	1	1	2	-	-	1	1, 2	+	+	+	1	1	+	+	+	-	V
Corydalis solida	2, 3	2	1	3	2	2	2	2, 3	1, 2	1, 2	3	3	1	+	1, 2	1, 2	1, 2	1	1, 2	2, 3	3, 4	3	3, 4	3	-	V
Scilla bifolia	+	+	+	1	+	1	+	+	+	+	+	-	1	1	+	1	+	+	+	+	+	+	+	+	-	V
Euphorbia amygdaloides	r	+	+	r	+	+	+	r	r	-	-	-	-	-	-	-	-	-	-	-	+	-	r	-	-	III
Gagea lutea	+	-	-	-	+	-	+	-	+	+	-	-	-	-	+	+	-	+	+	+	+	+	+	+	-	III
Pulmonaria officinalis	+	r	+	r	+	+	+	+	-	-	-	r	-	-	-	-	+	+	r	-	-	-	+	-	+	III
Acer platanoides	-	+	-	r	+	-	-	r	r	-	-	r	-	-	-	-	-	-	+	-	-	r	+	-	-	II
Allium ursinum	-	-	-	1	-	-	-	1, 2	1, 4	-	-	-	-	-	-	-	-	-	+	-	-	1	+	-	-	II
Bromus ramosus	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	+	+	-	-	II

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
Corydalis cava	-	+	-	2	1, 2	1, 2	-	+	+	-	-	+	-	-	1, 2	-	-	-	-	-	-	-	-	-	-	II
Euonymus europaeus	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	II
Ficaria verna	-	-	-	-	-	-	-	-	+	-	+	-	-	-	1, 3	-	-	-	1, 2	1, 2	+	1	1, 2	-	+	II
Hedera helix	-	1, 2	-	-	1	-	-	-	+	-	-	-	-	-	-	-	r	-	-	-	+	1	-	-	-	II
Myosotis sparsiflora	-	-	-	-	+	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	+	II
Scrophularia nodosa	r	-	-	r	-	-	r	-	-	r	-	-	-	-	r	r	-	-	-	-	-	-	-	-	-	II
Hordelymus europaeus	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	I
Ranunculus auricomus	-	+	r	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I
Ulmus glabra	-	-	-	-	-	-	-	-	-	-	-	-	r	-	+	-	-	-	-	-	+	-	-	-	-	I
Querco-Fagetea																										
Acer campestre	+	2	1	1	1	2	2	+	+	1	-	1	+	-	+	+	1	1	1	+	+	+	1	2, 3	1	V
Arum orientale	r	r	-	r	-	r	r	r	r	-	+	-	+	+	+	+	-	r	+	+	+	+	+	+	-	V
Fraxinus excelsior	1	1	1, 2	1	1	1	1	1	1	-	1	1	3	2	1, 2	2	1, 2	1	3	2	1	2	2	2	-	V
Geum urbanum	r	+	+	+	+	+	+	+	r	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	V
Melica uniflora	+	1, 2	2, 3	1	2	1	1	+	+	+	-	-	+	1	+	+	1, 3	+	+	+	+	+	1, 2	-	1	V
Polygonatum hirtum	2, 3	2	2	1, 2	1, 3	1, 3	+	1, 3	+	1, 2	2	+	+	+	+	1, 2	-	+	+	+	1	-	+	+	1	V
Acer tataricum	1	+	-	1	+	1	2	2	+	1	1	1	+	+	-	+	-	-	+	-	r	-	r	1	1	IV
Campanula rapunculoides	+	+	+	+	+	1, 2	1, 2	+	+	1, 2	+	-	-	-	-	+	1, 3	1	-	-	+	-	-	-	+	IV
Dactylis glomerata	-	-	-	+	+	+	+	+	-	-	+	+	+	+	+	-	-	r	+	+	+	+	+	-	-	IV
Poa nemoralis	1	1	-	1	1, 3	-	-	-	-	-	+	+	3	+	r	+	+	+	+	+	1	+	1, 2	-	+	IV
Brachypodium sylvaticum	-	-	-	-	-	-	+	+	r	-	+	+	+	-	+	-	-	-	-	-	+	-	+	+	+	III
Scutellaria altissima	-	1	+	+	1	+	+	-	-	-	+	-	+	+	-	-	+	-	-	-	+	-	+	-	-	III
Viola odorata	-	-	-	-	-	+	-	+	-	-	+	+	+	+	+	+	-	+	1, 2	+	+	+	+	-	+	III
Cerasus avium	-	r	-	r	r	r	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	r	+	+	+	II
Chaerophyllum temulum	-	-	+	-		-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	-	II
Lapsana communis	r	-	-	r	-	-	-	-	+	r	r	-	-	r	-	-	-	-	+	+	-	+	+	-	-	II
Ulmus minor	-	+	-	-	-	-	-	-	-	-	-	-	r	-	-	-	+	+	-	-	-	-	-	+	+	II
Convallaria majalis	-	-	-	1, 2	1, 2	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1	-	-	-	-	I
Cornus sanguinea	-	-	+	+	-	-	1	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	I
Galium intermedium	-	-	+	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	I
Sedum maximum	r	-	-	-	-	-	+	-	-	r	-	-	-	-	-	-	-	-	-	-	-	-	r	-	r	I
Crataego-Prunetea																										
Crataegus monogyna	+	+	1	1	1	1	1, 2	1	1	+	1	1	-	r	r	+	+	+	+	-	r	r	+	+	1	V
Viburnum lantana	+	+	+	+	+	+	+	1	1	+	+	+	r	-	r	r	+	-	+	-	+	-	+	+	+	V

***TILIO TOMENTOSAE-QUERCENION PETRAEAE* (PÎNZARU ET CANTEMIR 2023) …**

Cornus mas	1	r	+	1	1	1	-	-	+	-	-	-	-	-	-	-	+	1	-	-	-	-	-	-	3, 4	II
Prunus spinosa	+	-	-	+	-	+	1	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	II
Rhamnus cathartica	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	I
Rosa canina	-	-	-	-	-	+	-	-	r	-	-	-	-	-	-	+	-	-	-	-	-	-	r	-	+	I
Aliae																										
Galium aparine	+	+	+	+	+	+	1	-	1	+	+	1	1	+	+	+	+	+	1, 3	1, 2	-	1, 2	1, 2	1, 2	+	V
Veronica hederifolia	2	-	-	2	1, 2	2	1, 2	1, 2	1	2, 3	+	1	1	1	1, 2	1, 2	1	1	2, 3	1, 2	1	1, 2	1	1, 2	+	V
Anthriscus longirostris	-	+	-	+	-	1, 2	1, 2	1	+	-	-	-	2	+	4, 5	4, 5	+	-	1, 4	2, 3	1	1	2, 4	1, 2	-	IV
Lamium purpureum	2	+	1	2	1, 2	2	2	2, 3	1	1, 3	-	1	-	+	1	1, 2	+	-	-	-	-	-	-	-	+	IV
Alliaria petiolata	2, 3	1	+	+	+	+	1, 2	+	+	-	-	-	+	+	+	r	-	r	-	-	-	-	-	-	+	III
Fallopia dumetorum	+	r	-	-	+	-	-	-	-	-	-	-	+	+	-	r	+	-	-	-	-	+	+	+	+	III
Galium mollugo	-	-	r	r	r	-	-	-	r	r	-	-	-	-	-	-	-	-	-	-	-	-	r	-	-	II
Stellaria media	-	-	-	-	+	-	-	-	-	-	-	-	+	+	1.2	-	-	-	-	-	-	-	-	-	+	II
Ballota nigra var. ruderale	-	-	-	-	-	-	-	-	-	-	-	-	-	-	r	-	-	-	1	-	-	-	-	+	-	I
Chaerophyllum bulbosum	+	-	-	-	-	+	r	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I
Urtica dioica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	r	-	-	-	+	+	-	-	-	-	-	I
Vicia angustifolia	-	-	r	-	-	-	-	-	r	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	I

<u>Place and date of the relevés</u>: rel. 1-5, Cărbuna commune, Ialoveni district, 16.06.2019; rel. 6-10, Rezeni commune, Ialoveni district, 24.04.2021; rel. 11-12, Cărbuna commune, Ialoveni district, 20.04.2022; rel. 13-14, Cărbuna commune, Ialoveni district, 01.06.2022; rel. 15-16, Cărbuna commune, Ialoveni district, 04.05.2023; rel. 17-18, Zloți village, Cimișlia district, 12.06.22023; rel. 19-24, Zloți village, Cimișlia district, 20.07.2023, 29.03.2024; rel.25, Suruceni commune, Ialoveni district, 20.05.2024, 19.03.2025;

<u>Species recorded only in 1 or 2 surveys:</u> ***Quercetalia pubescenti-petraeae***: Carex montana (rel. nr. 25); Carex tomentosa (25); Cerasus mahaleb (6); Geranium divaricatum (15); Lactuca quercina (6,10); Quercus pubescens (13, 14); Rosa gallica (5, 14); Silene coronaria (7, 23); Silene nutans (16, 25), Sorbus domestica (6); Vincetoxicum hirundinaria (4, 25); ***Fagetalia sylvaticae***: Campanula trachelium (22); Cephalanthera damasonium (5); Granium robertianum (15, 23); Gagea minima (23); Lamium maculatum (14, 15); Mercurialis perennis ((1, 3); Stachys sylvatica 22); Tulipa biebersteiniana var. biebersteiniana (16); Viola mirabilis (4); ***Querco-Fagetea***: Epipactis helleborine (5); Lactuca muralis (1); Lilium martagon (9); Malus sylvestris (7); Polygonatum multiflorum (17); Quercus robur (22); ***Trifolio-Geranietea***: Astragalus glycyphyllos (23, 25); Clinopodium vulgare (25); Potentilla argentea (25); Trifolium alpestre (25); Veronica chamaedrys (25); ***Aliae***: Ajuga laxmannii (25); Ajuga reptans (25); Bromus tectorum (13), Rumex acetosa (23); Hypericum perforatum (23); Senecio vernalis (5, 23); Verbascum nigrum (25).

Table 2. Ass. *Tilio tomentosae-Quercetum petraeae* Sârbu 1979 corr. Pînzaru et al. h. l. subass. *typicum* Sârbu 1979

Relevé no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	K
Altitude (m)	270	282	138	326	195	222	220	219	203	300		270	275	300	305	314	334	367	
Aspect	-	W	SW	W	N	N	N	S	E	NW	NW	NE	NE	NW	NE	-	NE	V	
Slope (°)	-	5	25	10	25	15	10	5	5	15	10	5	5	15	15	-	10	15	
Tree layer coverage (%)	80	85	75	80	75	80	85	75	80	80	85	85	80	80	80	85	80	80	
Schrub layer coverage (%)	30	40	35	5	30	30	30	55	45	5	5	50	15	5	3	3	-	-	
Spring herbaceous layer coverage (%)	x	60	60	35	45	x	x	15	10	15	10	10	10	x	x	80	70	20	
Summer herbaceous layer coverage (%)	55	90	70	90	60	50	60	85	90	80	90	90	90	90	50	35	90	90	
Relevé surface (m²)	600	600	600	600	600	600	600	600	600	600	600	600	600	600	600	600	600	600	
Number of species	57	56	49	36	55	36	31	62	50	49	26	32	30	40	27	38	44	30	
Plots no.	37			37	48	16	17	12	13	12		30L	30K	33	35	51	43	38	
Characteristic species																			
Tilia tomentosa	2	3	1	1	1	1	1	2	2	+	1	2	2	3	+	1	2	1	V
Tilio tomentosae-Quercenion petraeae																			
Tulipa biebersteiniana var. biebersteiniana	-	+	1, 2	-	-	-	-	1, 2	2, 3	-	-	-	-	-	-	-	-	-	II
Allium siculum subsp. dioscoridis	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	1	-	I
Fritillaria montana	-	-	1, 2	-	-	-	-	+	1	-	-	-	-	-	-	-	-	-	I
Galanthus plicatus	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I
Lathyrus aureus	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	I
Vicia cassubica	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	I
Quercion petraeae																			
Quercus petraea	3	2	4	4	4	3	3	3	3	5	5	3	3	2	4	3	3	3	V
Sorbus torminalis	-	+	r	-	+	+	+	+	r	+	+	-	r	+	r	r	+	-	IV
Pulmonaria mollis	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I
Stachys officinalis	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	I
Quercetalia pubescenti- petraeae																			
Glechoma hirsuta	1	1	1, 2	1, 2	1	1	1	1	3	1	1	+	-	1	1	+	2	2	V
Euonymus verrucosus	+	1	1	+	-	+	+	1	+	+	-	+	+	+	+	-	+	-	IV
Lathyrus niger	+	+	-	+	+	+	-	+	-	-	-	+	+	-	+	-	+	-	III
Aegonychon purpuro-caeruleum	-	-	-	-	3	-	1	1, 2	1	1	-	-	1	1, 2	1	-	-	-	II
Lathyrus vernus	-	+	-	-	+	-	-	-	-	+	-	+	+	+	-	-	-	-	II

Ligustrum vulgare	-	-	1	-	-	1	1	+	+	-	-	-	-	-	-	-	-	-	II
Viola suavis	-	-	+	+	-	+	+	+	-	-	-	-	-	-	-	-	+	+	II
Campanula persicifolia	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-	I
Carex muricata	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-	-	-	-	I
Melica picta	-	-	-	-	-	+	-	1, 2	+	-	-	-	-	-	-	-	-	-	I
Mercurialis ovata	-	-	2	-	-	2	+	-	-	-	-	-	-	-	-	-	-	-	I
Carpinion																			
Carex brevicollis	-	+	1	1, 2	+	1	1	1	1	1	1	+	1	1	+	2	1, 2	2, 4	V
Carpinus betulus	+	+	+	+	+	1, 2	1, 2	+	+	+	+	-	-	+	1, 2	1, 2	1	+	V
Stellaria holostea	2	1	1, 2	1	+	1, 3	1, 3	2	1, 3	+	2	2, 4	1, 2	+	1, 2	1, 3	1,3	2	V
Tilia cordata	+	-	-	-	r	-	-	-	-	-	-	-	-	+	+	+	-	-	II
Corydalis marschalliana	-	1, 2	2	x	-	-	-	-	-	+	-	-	-	-	-	-	1, 2	-	I
Isopyrum thalictroides	-	-	2	x	-	-	-	-	-	-	-	-	-	-	-	+	-	-	I
Lathyrus venetus	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	I
Ranunculus auricomus	-	+	-	-	+	-	-	+	+	+	r	-	-	-	-	-	-	-	I
Fagetalia sylvaticae																			
Carex pilosa	1, 2	1	2	2, 4	3	1	1, 2	3, 4	4, 5	3, 4	3, 4	-	2, 4	3, 4	1, 3	-	1	1, 2	V
Acer platanoides	-	1	-	+	r	r	r	+	+	+	r	+	+	+	-	+	+	-	IV
Pulmonaria officinalis	+	+	+	-	+	-	-	+	+	+	r	+	+	+	r	-	+	-	IV
Hedera helix	-	+	-	-	-	1	1	1, 2	1, 2	+	1	-	-	1	1	-	-	+	III
Anemonoides ranunculoides	+	1	1, 2	x	-	x	x	1, 2	1, 2	+	-	+	+	-	-	2	+	x	III
Cardamine bulbifera	-	2	1	-	1	1	-	+	1, 2	+	+	-	-	+	-	1	-	-	III
Corydalis solida	1	1, 2	2	x	2	x	x	2, 3	-	1	-	r	+	-	-	2, 3	1, 2	x	III
Euphorbia amygdaloides	-	r	+	+	+	r	+	+	-	-	r	-	-	+	-	+	-	+	III
Gagea lutea	+	+	+	x	+	x	x	-	+	+	-	-	-	-	-	+	+	x	III
Lamium maculatum	+	+	1	-	-	-	-	r	+	+	-	-	+	-	-	-	+	1, 2	III
Scilla bifolia	+	1	+	x	+	x	x	+	1	+	-	-	+	-	-	1	+	x	III
Allium ursinum	-	1, 2	-	x	-	x	x	+	1	-	+	-	-	-	-	5	2, 3	x	II
Asarum europaeum	-	+	+	-	+	r	-	-	2	-	r	+	-	-	-	+	1	-	II
Carex digitata	-	+	-	-	+	+	-	+	+	+	-	-	-	-	+	-	-	-	II
Corydalis cava	1	-	-	x	1	x	x	1	2, 3	-	-	-	-	-	-	1, 2	-	x	II
Ficaria verna	+	2	1, 2	x	-	x	x	-	+	+	-	-	-	-	-	1, 2	-	x	II
Galium odoratum	-	1, 2	-	_	-	-	-	-	-	+	2	-	-	-	1	+	-	-	II
Mercurialis perennis	-	1, 2	-	+	-	-	-	1	2	-	-	+	-	+	-	3	1	-	II
Stachys sylvatica	+	-	-	+	+	-	-	-	-	-	-	-	-	+	-	-	+	+	II
Staphylea pinnata	-	1	-	+	-	-	-	+	+	+	-	-	+	-	-	-	-	-	II

Viola mirabilis	+	+	-	-	+	-	-	-	-	-	+	+	+	-	-	-	-	-	II
Viola reichenbachiana	+	+	-	-	+	-	-	-	+	-	-	-	-	-	-	+	-	+	II
Aegopodium podagraria	-	+	-	-	-	-	1	-	-	-	-	-	-	-	-	1, 2	-	+	I
Hordelymus europaeus	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	+	+	I
Scrophularia nodosa	-	-	-	+	-	-	-	+	r	-	-	-	-	-	-	-	+	-	I
Querco-Fagetea																			
Acer campestre	+	+	1	+	+	+	+	2	1	+	1	+	1, 2	+	+	+	+	1	V
Geum urbanum	+	+	+	+	+	+	+	+	+	+	r	+	+	+	+	+	+	+	V
Dactylis glomerata	+	+	-	+	+	+	+	+	+	+	-	+	-	+	+	-	+	+	IV
Fraxinus excelsior	-	1	1	+	+	+	-	+	+	-	r	1	1	+	-	+	+	1, 2	IV
Melica uniflora	+	+	-	1	+	-	-	+	-	+	+	+	+	1	1	-	1	1, 2	IV
Polygonatum hirtum	+	1	2	+	+	+	1, 2	-	1	+	1, 2	+	+	-	-	1	+	-	IV
Campanula rapunculoides	+	+	1	-	-	+	+	+	1, 2	+	-	-	+	-	-	+	-	-	III
Polygonatum multiflorum	-	-	1	-	+	+	-	+	+	+	-	-	-	+	+	+	-	-	III
Acer tataricum	+	-	+	-	-	+	+	+	+	-	-	+	+	-	-	-	-	-	II
Arum orientale	-	+	+	-	+	-	+	r	-	-	-	-	-	-	-	+	r	-	II
Cerasus avium	+	-	r	-	-	-	-	-	+	r	-	-	-	-	-	-	+	-	II
Chaerophyllum temulum	+	+	-	+	+	-	-	-	-	+	-	-	-	+	-	-	+	+	II
Convallaria majalis	1	-	2	-	-	+	1	2, 3	-	2, 3	-	-	-	-	1, 2	-	-	-	II
Euonymus europaeus	-	-	-	+	+	+	-	+	-	-	-	+	+	-	-	-	-	r	II
Galium intermedium	+	-	-	2	1	+	-	-	-	1	-	+	-	-	-	-	-	-	II
Poa nemoralis	1, 3	-	-	-	+	+	-	+	-	-	-	+	+	+	+	-	-	-	II
Scutellaria altissima	+	-	-	-	+	+	+	-	-	-	-	+	-	+	-	-	-	+	II
Viola odorata	+	+	-	-	+	-	-	+	+	-	-	+	+	+	-	-	-	-	II
Bromus ramosus	-	-	-	1	-	-	-	-	-	-	-	-	-	+	-	-	+	1, 2	I
Brachypodium sylvaticum	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	+	-	-	I
Lapsana communis	+	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	I
Ulmus minor	+	-	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	I
Cornus sanguinea	+	-	-	-	+	-	-	1	+	-	-	-	-	-	-	-	-	-	I
Geranium robertianum	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	I
Hypericum hirsutum	-	-	-	r	+	-	-	-	-	-	-	-	-	-	-	-	r	-	I
Lamium galeobdolon	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	1	-	-	I
Crataego-Prunetea																			
Viburnum lantana	+	+	1	-	+	+	+	+	1	+	+	+	+	+	+	-	-	-	IV

Crataegus monogyna	2	+	1	+	+	+	+	1	+	+	-	-	-	-	-	-	-	-	III
Cornus mas	-	-	+	-	+	r	-	-	+	+	-	+	-	+	1	r	-	-	III
Trifolio-Geranietea																			
Astragalus glycyphyllos	-	-	-	-	r	-	-	+	-	+	-	-	-	-	-	-	-	-	I
Veronica chamaedrys	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	I
Aliae																			
Alliaria petiolata	+	+	-	-	+	-	-	+	+	-	+	+	+	+	-	-	+	2	III
Fallopia dumetorum	+	+	-	-	+	-	-	+	-	-	-	-	-	+	-	-	+	-	II
Galium aparine	-	-	1	1	-	-	-	-	-	-	-	+	-	+	-	-	2, 5	2	II
Veronica hederifolia	+	+	1, 2	-	-	x	x	1	1, 2	+	-	-	-	-	-	1	-	1	II
Stellaria media	-	+	1	-	-	-	-	+	-	-	-	-	-	-	-	1	-	-	I
Anthriscus longirostris	-	+	1	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	I

Place and date of the relevés: rel. 1, Brînzeni commune, Edineț district, 24.07.1993; rel. 2, Boldurești commune, Nisporeni district, 08.06.1996; rel. 3, Capaclia commune, Cantemir district, 11.04.2009; rel. 4, Stejăreni village, Strășeni district, 25.04.2009; rel. 5, Saharna Nouă commune, Rezina district, 18.05.2009, 20.06.2009; rel. 6-7, Hănășeni village, Cantemir district, 03.07.2009; rel. 8-9, Dunbrava village, Chișinău municipality, 29.09.2017, 17.04.2018; rel 10, Logănești commune, Hîncești district, 24.04.2018; rel. 11, Bahmut commune, Călărași district, 23.04.2021; rel. 12-13, Olișcani commune, Șoldănești district, 14.07.2023, 31.03.2024; rel. 14-15, Ulmu commune, Ialoveni district, 19.07.2023; rel. 16, Căpriana commune, Strășeni district, 15.07.2024; rel. 17-18, Stejăreni village, Strășeni district, 28.07.2025.

Species recorded only in 1 or 2 surveys: ***Quercetalia pubescenti-petraeae***: Vincetoxicum hirundinaria (1, 14); Asparagus tenuifolius (3, 5); Cotinus coggygria (3); Rhamnus tinctoria (1); Silene noctiflora (9); Silene nutans (5); Symphytum tauricum (8); Vicia pisiformis (4, 18); ***Fagetalia sylvaticae***: Acer pseudoplatanus (11); Adoxa moschatellina (3); Ajuga reptans (1); Campanula trachelium (13); Epipactis purpurata (4); Melampyrum polonicum (1); Sanicula europaea (18); Scopolia carniolica (2); Ulmus glabra (2); ***Querco-Fagetea***: Lactuca muralis (2, 4); Lilium martagon (5); Malus sylvestris (2);Ornithogalum pyrenaicum (17); Primula veris (1); Quercus robur (1); Sedum maximum (10); ***Crataego-Prunetea***: Clematis vitalba (3); Prunus spinosa (1, 12); Rosa canina (5); ***Aliae***: Anthriscus sylvestrys (1); Chelidonium majus (2, 3); Erygeron annuus (4); Galeopsis tetrahit (1); Hypericum perforatum (18); Torilis japonica (4, 6); Urtica dioica (1).

Table 3. *Tilio tomentosae-Quercetum petraeae cotinetosum coggygriae* Sârbu 1979; *cornetosum mas* (Sârbu 1979) Pînzaru et al. stat. nov. h. l.

Subass.	*cotinetosum coggygriae*						*cornetosum maris*													
Relevé no.	19	20	21	22	23		24	25	26	27	28	29	30	31	32	33	34	35	36	
Altitude (m)	250	251	240	249	256		306	268	264	256	250	340	314	342	292	313			220	
Aspect	-	-	N	-	S		-	-	-	-	-	SW	SW	SW	SW	W	-	E	NW	
Slope (°)	-	-	40	-	20		-	-	-	-	-	30	25	10	10	10	-	20	10	
Tree layer coverage (%)	80	75	85	75	75	K	70	75	80	75	75	70	70	75	80	80	70	80	80	K
Schrub layer coverage %	80	80	70	70	80		60	50	50	60	70	75	65	60	50	50	50	55	60	
Spring herbaceous layer coverage (%)	-	-	30	30	10		20	80	80	80	50	60	90	x	x	30	35	60	20	
Summer herbaceous layer coverage (%)	30	35	30	35	25		40	40	35	75	60	70	80	65	90	90	90	80	70	
Relevé surface (m²)	600	600	600	600	600		600	600	600	600	600	600	600	600	600	600	600	600	600	
Number of species	45	31	28	33	26		37	44	34	50	36	42	41	34	28	55	42	65	35	
Plots no.	41	48							50			41	42	42	43	52	57	63		
Charact. species ass. species																				
Tilia tomentosa	1	+	3	3	+	V	1	1, 2	1, 2	1, 2	1	1	1, 2	1, 2	1, 2	1, 2	1	1	+	V
Dif. subass.																				
Cotinus coggygria	3, 5	2, 5	2, 4	2, 4	3, 4	V	+	-	-	-	-	-	-	-	-	-	-	-	-	I
Cornus mas	-	-	1	1	1	III	2	3	3	3, 4	3, 4	3, 4	3	3	2	2, 3	2, 3	2	2	V
Carpino orientalis-Tilenion tomentosae																				
Allium siculum subsp. dioscoridis	-	-	-	-	-	-	-	+	-	3	-	-	3	-	-	1	1	2	-	III
Tulipa biebersteiniana var. biebersteiniana	-	-	-	-	-	-	-	2	+	1	-	-	-	-	-	-	-	2	1	II
Lathyrus aureus	-	-	-	r	r	II	+	-	-	-	-	-	-	-	-	-	-	-	-	I
Quercion petraeae																				
Quercus petraea	4	4	2	4	4	V	4	2, 3	3	3	4	2	1, 2	1, 2	3	3	3	4	5	V
Sorbus aucuparia s.l.	r	r	-	-	+	III	+	-	-	-	-	-	-	-	-	-	-	-	-	I
Cruciata glabra	2	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stachys officinalis	+	-	-	-	-	I	-	-	-	-	-	-	-	-	-	+	-	-	-	I
Loranthus europaeus	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	I
Quercetalia pubescenti-petraeae																				
Glechoma hirsuta	1	1	-	+	2	IV	-	2	1, 2	1, 3	+	1	2, 3	1, 2	1, 2	1	1	1	1	V
Sorbus torminalis	1	1	-	+	2	IV	+	+	+	+	r	-	r	-	r	+	+	+	+	V
Euonymus verrucosus	1	-	+	1	-	III	2	+	+	1	1	-	+	-	-	-	-	+	1	III

Symphytum tauricum	-	-	-	r	-	I	-	-	1, 2	+	+	+	+	+	-	-	+	-	-	III
Viola suavis	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	-	III
Aegonychon purpurocaeruleum	1, 2	-	-	3	-	II	1	2	-	2	-	-	+	-	-	-	-	-	-	II
Asparagus tenuifolius	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	r	-	-	II
Lathyrus vernus	+	+	-	-	-	II	+	-	-	+	-	-	+	-	-	-	-	-	+	II
Carex muricata	+	1	-	-	+	III	-	+	-	+	-	-	-	-	-	+	-	-	-	II
Lathyrus niger	+	1	r	+	+	V	+	-	-	-	-	-	+	-	-	+	-	+	+	II
Melica picta	1, 2	-	-	-	-	I	2	-	-	+	-	-	-	-	-	1	-	-	-	II
Piptatherum virescens	-	-	-	+	+	II	-	1	-	1	+	-	-	-	-	+	-	-	-	II
Vincetoxicum hirundinaria	+	+	-	r	+	IV	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carpinion																				
Carex brevicollis	-	-	1	-	2	II	+	1, 3	+	1	+	+	+	1, 2	2	2, 3	-	+	-	V
Stellaria holostea	1	1, 2	r	-	+	IV	2	3	1, 2	2, 3	1, 2	1, 4	2	2, 4	-	1, 3	3, 4	3	+	V
Corydalis marschalliana	-	-	-	-	-	-	1	-	-	1, 2	-	-	1, 2	-	-	1, 3	+	2, 3	+	III
Carpinus betulus	-	-	+	-	+	II	-	+	-	-	-	-	-	-	+	+	-	+	+	II
Isopyrum thalictroides	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	2	3	3	-	II
Tilia cordata	+	-	-	-	1	II	-	-	-	-	-	-	-	-	-	+	+	+	-	II
Lathyrus venetus	-	-	+	-	-	I	-	-	-	-	-	-	-	-	-	-	-	+	-	I
Omphalodes scorpioides	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	I
Fagetalia sylvaticae																				
Anemonoides ranunculoides	-	-	r	+	-	II	1, 2	1, 2	1, 2	1, 2	1	+	+	+	1	+	+	1	+	V
Carex pilosa	-	-	+	1	1	III	2	-	+	3	2	+	+	1, 4	1, 3	1, 3	1	2, 3	1, 3	V
Corydalis solida	-	-	+	r	-	II	-	1	-	1, 2	1	1, 2	1, 2	1	2	1, 2	1	3	1	V
Gagea lutea	-	-	-	-	-	-	+	+	+	+	-	+	+	+	+	+	+	+	-	V
Scilla bifolia	-	-	1	1	-	II	1	1	1	1	1, 2	+	+	+	+	r	+	1	+	V
Hedera helix	-	-	+	1	-	II	1, 2	1, 3	1, 3	1, 2	+	+	-	+	-	1	+	1, 2	1, 2	IV
Lamium maculatum	-	-	+	-	-	I	-	+	-	+	-	+	1	+	+	2	1, 2	1	-	IV
Mercurialis perennis	-	-	-	+	-	I	2	+	1, 2	2	-	+	1	+	-	2	1	2, 3	-	IV
Allium ursinum	-	-	-	-	-	-	-	3	3, 4	+	+	-	1, 4	-	-	+	3	3	-	III
Euphorbia amygdaloides	-	-	-	-	-	-	-	r	-	+	-	+	-	r	-	+	+	+	-	III
Pulmonaria officinalis	+	+	r	+	-	IV	+	r	+	r	-	-	+	-	-	+	-	+	-	III
Acer platanoides	-	-	1	-	-	I	+	-	+	-	-	-	-	-	-	+	-	+	r	II

Asarum europaeum	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	1	+	1	-	II
Cardamine bulbifera	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	2	-	II
Corydalis cava	-	-	-	-	-	-	-	2, 3	1	-	1	-	-	-	-	-	-	-	-	II
Ficaria verna	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	2	3	-	II
Galium odoratum	-	-	+	-	-	I	2	-	-	-	-	-	-	-	-	+	-	1	+	II
Stachys sylvatica	-	-	-	-	-	-	-	-	-	-	-	r	-	-	-	+	+	-	-	I
Querco-Fagetea																				
Convallaria majalis	2	1	1, 2	1, 2	1, 2	V	2	2	1, 2	1, 2	1, 3	+	-	1, 2	-	1	2	2	+	V
Dactylis glomerata	-	-	-	+	-	I	-	+	+	+	+	+	+	+	+	+	+	+	+	V
Fraxinus excelsior	-	r	+	-	-	II	+	1	1	1	1	3	2, 3	2	1	1, 2	1	+	-	V
Geum urbanum	+	-	-	+	-	II	+	+	-	+	+	+	+	+	+	+	+	+	+	V
Acer campestre	1	1	-	-	1, 2	III	1	-	-	+	-	-	+	1	+	+	1	1	1	IV
Melica uniflora	-	-	+	+	+	III	-	+	+	-	+	+	2	+	1, 3	1, 3	+	+	-	IV
Brachypodium sylvaticum	+	-	-	-	-	I														
Bromus ramosus	-	-	-	-	-	-	-	+	r	-	-	+	+	+	+	1, 2	+	-	-	III
Chaerophyllum temulum	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	1	+	+	-	III
Polygonatum hirtum	-	1	-	-	-	I	-	+	+	1, 2	1	+	-	-	-	+	-	1	-	III
Scutellaria altissima	+	+	-	-	+	II	+	+	-	-	1	+	-	+	+	-	+	-	-	III
Viola odorata	+	-	-	+	-	II	-	+	+	+	-	-	-	-	-	+	-	+	+	III
Arum orientale	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	r	-	+	-	II
Campanula	-	-	-	-	-	-														
rapunculoides	+	-	2, 3	-	+	III	+	-	-	3	1	-	-	-	-	-	+	+	-	II
Poa nemoralis	2	1, 2	+	+	+	V	-	-	-	+	+	-	-	-	-	-	+	-	+	II
Polygonatum	-																			
multiflorum	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	+	r	II
Geranium robertianum	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	I
Lactuca muralis	-	-	-	+	-	I	-	-	-	-	+	-	-	-	+	+	-	-	-	I
Sedum maximum	+	+	-	-	r	III	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Crataego-Prunetea																				
Crataegus monogyna	1	+	-	-	-	II	1	-	-	-	1	+	-	+	-	+	-	+	+	III
Prunus spinosa	+	+	-	-	-	II	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rosa canina	+	+	-	-	-	II	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Viburnum lantana	-	+	+	1, 2	-	III	+	+	+	1	1, 2	+	+	+	-	-	-	+	+	III

Aliae																				
Galium aparine	-	-	+	1	+	III	-	1, 3	+	+	+	1, 2	1	1, 2	1	2, 5	1, 4	2, 3	+	V
Alliaria petiolata	-	-	r	+	-	II	-	+	-	+	+	2, 3	2	1, 3	1, 2	1, 2	-	+	+	IV
Veronica hederifolia	+	+	-	+	-	III	-	1	-	2, 3	2, 3	1	2	-	-	-	+	2	-	III
Anthriscus longirostris	-	-	-	-	-	-	-	-	-	-	+	3	2, 3	-	1, 2	-	-	-	+	II
Falopia dumetorum	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	+	-	-	II
Urtica dioica	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	+	-	I

Place and date of the relevés: subass. *cotinetosum coggygriae* - rel. 19-20, Mereșeni commune, Hîncești district, 05.08.2016; rel. 21-23, Malcoci commune, Ialoveni district, 19.03.2025, 02.06.2025; subass. *cornetosum mas* – rel. 24, Logănești commune, Hîncești district, 24.04.2018; rel. 25-26, Condrița commune, Chișinău municipality, 19.03.25, 02.06.2025; rel .27-28, Malcoci commune, Ialoveni district, 19.03.25, 02.06.2025; rel. 29-32, Stejăreni village, Strășeni district, 28.07.2025; rel. 33-35, Horodca village, Hîncești district, 28.07.2025.

Species recorded only in 1 or 2 surveys: subass. *cotinetosum coggygriae*: ***Quercetalia pubescenti-petraeae***: Clematis recta (19); Campanula persicifolia (22, 23); Tanacetum corymbosum (19), Veronica spuria (19); Vicia pisiformis (19); ***Fagetalia sylvaticae***: Carex digitata (21); Scrophularia nodosa (19); ***Querco-Fagetea***: Acer tataricum (20); Galium intermedium (19, 20); Pyrus pyraster (19); ***Trifolio-Geranietea***: Astragalus glycyphyllos (19, 20); Centaurea jacea (19); Clinopodium vulgare (19, 20); Trifolium alpestre (19); ***Aliae***: Hypericum perforatum (19, 220); Stellaria media (22); Torilis japonica (19); subass. *cornetosum maris*: ***Quercetalia pubescenti-petraeae***: Campanula persicifolia (33); Lactuca quercina (27); Ligustrum vulgare (26); ***Fagetalia sylvaticae***: Adoxa moschatellina (35); Aegopodium podagraria (35); Carex digitata (34); Lathraea squamaria (35); Milium effusum (31, 35); Ranunculus auricomus (35); Scrophularia nodosa (35); Staphylea pinnata (33, 34); Ulmus glabra (33); Viola mirabilis (34, 35); Viola reichenbachiana (35, 36); Viscum album (27); ***Querco-Fagetea sylvaticae***: Acer tataricum (35); Cephalanthera damasonium (35); Cornus sanguinea (24); Epipactis helleborine (26, 27);Euonymus europaeus (30, 31); Galium intermedium (24); Lapsana communis (36); Lilium martagon (27, 35); Moehringia trinervia (29, 35); Ornithogalum pyrenaicum (33); Quercus robur (30); Ulmus minor (31);***Trifolio Geranietea***: Astragalus glycyphyllos (27, 28); Veronica chamaedrys (27, 29); ***Aliae***: Chelidonium majus (29, 35); Erigeron annuus (27); Stellaria media (25, 27).

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Research Article

SHRUBS-DOMINATED VEGETATION IN NEAGRA BROŞTENILOR RIVER BASIN (ROMANIAN EASTERN CARPATHIANS)

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Abstract: The article presents a numerical analysis of shrubs-dominated vegetation in Neagra Broştenilor river basin (Romanian Eastern Carpathians). The study focuses on the floristic variability and syntaxonomic diversity of these vegetation types, on diagnostic species, and ecological preferences. Data classification, carried out using a hierarchical agglomerative clustering procedure, revealed seven well-defined plant communities, related to associations described in the literature based on their diagnostic species: *Digitali ambigue* - *Calamagrostietum arundinaceae* Sillinger 1933 subass. *spiraeetosum chamaedryfoliae* (Resm. et Csűrös 1966) Coldea 1991, *Rubetum idaei* Gams 1927, *Salici* - *Alnetum viridis* Colic et al. 1962, *Rhododendro myrtifolii* - *Pinetum mugo* Coldea 1991, *Rhododendro myrtifolii* - *Vaccinietum* Coldea et al. 1981, *Campanulo abietinae* - *Juniperetum nanae* Simon 1966 and *Empetro* - *Vaccinietum gaultherioidis* Br.-Bl. in Br.-Bl. et Jenny 1926 corr. Grabherr in Grabherr et Mucina 1993. All described syntaxa were characterized in terms of diagnostic, constant and dominant species, distribution, and aspects related to floristic and phytosociological composition.

Keywords: diversity, floristic composition, numerical classification, shrub vegetation, syntaxonomy.

Introduction

Classification and description of shrubs-dominated vegetation represent useful tools for their inventory and monitoring, as well for development of particularized management, conservation and restauration projects [BIURRUN & al. 2019; PEET & ROBERTS, 2013]. Also, vegetation is used to define habitat types, some of them considered priorities for conservation.

The Neagra Brostenilor river basin is located in the central area of the Bistrița Mountains (where crystalline geological formations predominate) and include a small area in the eastern slopes of the Călimani Mountains (of volcanic nature) and the Drăgoiasa – Glodu depression (with sedimentary geological formations). The river is 42 km long, and the hydrographic basin has an area of approximately 350 km^2 on the territory of Suceava County [Atlasul cadastrului apelor din R.S.R., 1972]. The maximum elevation is on the Căliman Izvor peak (2033 m), and the minimum at Broșteni (630 m), while the Drăgoiasa – Glodu depression is located at approximately 1000 m altitude. From a pedological perspective, this river basin overlaps the domain of mountain soils, with characteristic units and subunits [BARBU & al. 1984]. In addition, peaty soils are found in limited areas in some intra-montane depressions. The climate is specific to the mountain areas, characterized by average precipitation values of

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600-1100 (1200) mm/year (depending on altitude) and mean annual temperatures between 0 and 4 ºC.

The only protected area that partially overlaps the Neagra Broştenilor basin is the Călimani Mountains National Park. It is characterized by a remarkable diversity of both cormophyte species, cryptogam species and habitat types [SÂRBU, 2007], as 6230* Species-rich *Nardus* grasslands, on siliceous substrates in mountain areas, 4070* Bushes with *Pinus mugo* and *Rhododendron hirsutum* (*Mugo-Rhododendretum hirsuti*) or 9420 Alpine *Larix decidua* and/or *Pinus cembra* forests, etc.

The first published vegetation data from the study area resulted from investigations carried out by CSŰRÖS (1951), in the Călimani Mountains. The author reported four plant associations from the area of the Izvorul Călimanului and Călimanul Cerbului peaks, and characterized the subalpine belt distinguishing several plant associations (e.g. *Pinetum mughi carpaticum*), without making detailed specifications on their floristic composition. A few years later, POP (1960) reported the peat bog from Drăgoiasa, noting that flora is poor, including species adapted to the specific conditions of the peatbogs and presented few syntaxa dominated by Cyperaceae species, without phytosociological tables.

A particularly important contribution to the study of the flora and vegetation of cormophytes and bryophytes in the Neagra Broştenilor river basin was made by LUNGU (1971), who studied the peat bog located on the right side of the Cristişor stream, as part of her doctoral thesis. From this territory, with an area of only 14 ha, the author presented a wide range of herbaceous phytocoenoses characteristic of peat bogs and marshy meadows. The woody vegetation was represented by alder communities that follow the water courses and spruce forests on the slopes. SEGHEDIN (1986), in his doctoral thesis, approached the study of the flora and vegetation of the Bistrița Mountains, and from the total of 22 associations the author presented several phytocoenoses from the association *Rubo - Chamenerietum* Hadač 1969.

Data on the vegetation of the studied area were also published in various synthesis works [MITITELU & al. 1987; MITITELU & al. 1989; POPOVICI & al. 1996], presenting numerous syntaxa from various localities, but without description or phytosociological tables. As can be seen, the studies are numerous and well documented. However, it can be noted that they do not cover the entire area and the entire phytosociological diversity of the study area. Thus, this paper was focused on the floristic variability and syntaxonomical diversity of shrubs-dominated plant communities in Negra Broştenilor basin, on their diagnostic species, and on their ecological preferences.

Material and methods

The current analysis was based on a dataset consisting on 40 relevés. The methodological approach used to conduct the relevés was developed by Zürich-Monpellier School of vegetation study [BRAUN-BLANQUET, 1964]. They were classified using a hierarchical agglomerative clustering procedure (ß-flexible algorithm with ß = -0.25, Bray-Curtis dissimilarity and square root-transformed data of the mid-percentage values corresponding to the Braun-Blanquet cover-abundance scale). The classification procedure was carried out using the Gingko software within the VEGANA package [BOUXIN, 2005]. The optimum number of clusters was identified using the corrected Rand index and the mean Silhouette index.

Diagnostic species were identified based on their fidelity - the phi index [TICHÝ & CHYTRÝ, 2006], while the Fisher's exact test allowed to retain only species statistically significant associated to the clusters. Based on the diagnostic species, the clusters were assigned to the phytosociological associations described in literature [COLDEA, 1991; COLDEA, 2015; ŠIBÍK & al. 2010]. Nomenclature of plant species followed Euro+Med PlantBase (2006-) while classification to higher syntaxa mainly followed MUCINA & al. (2016).

Results and discussions

The cluster analysis (highlighted in the dendrogram) indicated seven interpretable clusters (Figure 1). Cluster one included perennial semi-natural vegetation on acidic soils while cluster two comprised red raspberry communities on nutrient-rich soils, all developed in forest margins and clearings or on disturbed forests soils. Cluster three included subalpine green alder scrubs on nutrient-rich soils, and cluster four consisted on subalpine silicicolous pine krummholz. Clusters five and six comprised subalpine, chionophilous, low juniper scrubs and acidophilous *Rhododendron myrtifolium* heaths, while cluster seven included subalpine dwarf *Vaccinium* sp. heaths in wind-exposed habitats, on silicicolous substratum. The floristic composition of all seven groups is well differentiated, and all are characterized by a significant number of diagnostic species (Table 1).

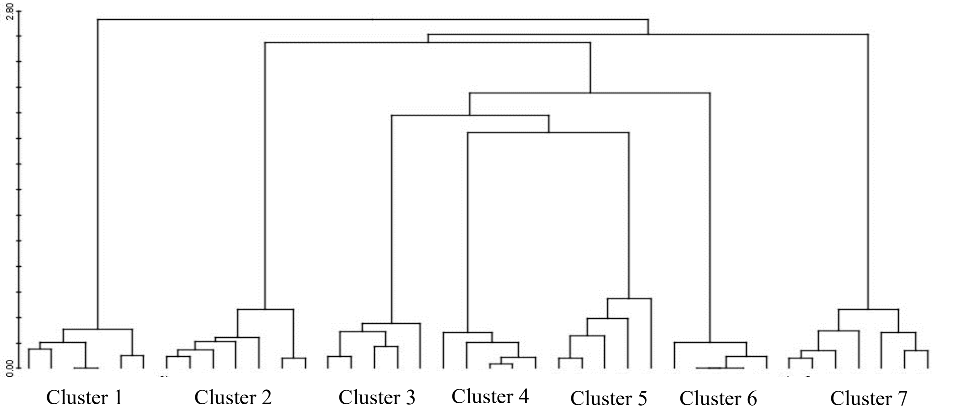

Figure 1. Dendrogram showing the results of the cluster analysis of the 40 relevés based on the Flexible β and Bray-Curtis distance.

Syntaxonomic scheme

EPILOBIETEA ANGUSTIFOLII R. Tx. et Preising ex von Rochow 1951
GALEOPSIO-SENECIONETALIA SYLVATICI Passarge 1981
Epilobion angustifolii Oberd. 1957
Ass. *Digitali ambigue - Calamagrostietum arundinaceae* Sillinger 1933 *spiraeetosum chamaedryfoliae* (Resm. et Csűrös 1966) Coldea 1991
SAMBUCETALIA RACEMOSAE Oberd. 1957
Sambuco-Salicion capreae Tx. et Neumann ex Oberd. 1957
Ass. *Rubetum idaei* Gams 1927
ROSO PENDULINAE-PINETEA MUGO Theurillat in Theurillat et al. 1995
JUNIPERO-PINETALIA MUGO Boşcaiu 1971
Pinion mugo Pawlowski et al. 1928

Ass. *Rhododendro myrtifolii - Pinetum mugo* Coldea 1991

BETULO CARPATICAE-ALNETEA VIRIDIS Rejmánek ex Boeuf, Theurillat, Willner, Mucina et Simler in Boeuf et al. 2014

ALNETALIA VIRIDIS Rübel ex Karner etWillner in Willner et Grabherr 2007

Alnion viridis Schnyder 1930

Ass. *Salici - Alnetum viridis* Colic et al. 1962

LOISELEURIO- ACCINIETEA Eggler ex Schubert 1960

RHODODENDRO FERRUGINEI-VACCINIETALIA Br.-Bl. in Br.-Bl. et Jenny 1926

Loiseleurio procumbentis-Vaccinion Br.-Bl. in Br.-Bl. et Jenny 1926

Ass. Empetro - Vaccinietum gaultherioidis Br.-Bl. in Br.-Bl. et Jenny 1926 corr. Grabherr in Grabherr et Mucina 1993

Rhododendrion myrtifolii de Foucault ex Theurillat et Mucina 2016

Ass. *Rhododendro myrtifolii - Vaccinietum* Coldea et al. 1981

VACCINIO MICROPHYLLI-JUNIPERETALIA NANAE Rivas-Mart. et Costa 1998

Juniperion nanae Br.-Bl. in Br.-Bl. et al. 1939

Ass. *Campanulo abietinae - Juniperetum nanae* Simon 1966

Cluster 1. Ass. *Digitali ambigue - Calamagrostietum arundinaceae* Sillinger 1933 subass. *spiraeetosum chamaedryfoliae* (Resm. et Csűrös 1966) Coldea 1991

Diagnostic species: *Ajuga reptans*, *Angelica sylvestris*, *Betula pendula*, *Cirsium oleraceum*, *Dactylis glomerata* subsp. *glomerata*, *Digitalis grandiflora*, *Dryopteris filix-mas*, *Fagus sylvatica* juv., *Gentiana asclepiadea*, *Leucanthemum vulgare* subsp. *vulgare*, *Luzula luzuloides*, *Picea abies*, *Scrophularia nodosa*, *Hylotelephium maximum*, *Silene nutans* subsp. *dubia*, *Solidago virgaurea* subsp. *virgaurea*, *Spiraea chamaedryfolia*, *Stachys sylvatica*, *Trifolium repens* subsp. *repens*, *Veronica urticifolia*.

Constant species: *Campanula abietina*, *Gnaphalium sylvaticum*, *Hypericum maculatum*, *Lonicera xylosteum*, *Oxalis acetosella*, *Rubus idaeus*, *Salix caprea*, *Sambucus racemosa*, *Sorbus aucuparia* subsp. *aucuparia*.

Dominant species: *Calamagrostis arundinacea*, *Spiraea chamaedryfolia*.

This subassociation includes shrub communities sporadically spread in Neagra Broştenilor river basin, on former forest cuttings, on the administrative territory of Neagra Broşteni, and Dârmoxa localities. They are installed on deforested slopes, with inclinations varying between 25 - 40º, with varied aspects, on neutral or slightly acidic, nutrient-rich soils. The tree layer has a low cover, up to 10-15%, and its composition more frequently includes *Picea abies*, *Sorbus aucuparia* subsp. *aucuparia*, and *Betula pendula*. The shrub layer, characterized by high cover (ranging between 35 - 60%) is dominated by the differential species - *Spiraea chamaedryfolia*, alongside which *Vaccinium myrtillus*, *Lonicera xylosteum*, *Sambucus racemosa*, *Salix caprea*, etc. also occur sporadically. The herbaceous layer is more diverse, has variable coverage, ranging between 35 and 65% and includes numerous species: *Epilobium angustifolium*, *Poa nemoralis*, *Gentiana asclepiadea*, *Euphorbia amygdaloides*, *Solidago virgaurea*, etc. Most of the species in floristic composition have Eurasian and European areal (70%). Also, in the floristic composition, there is highlighted the higher frequency of some diagnostic species of the alliance Epilobion angustifolii (e.g. *Epilobium angustifolium*, *Calamagrostis arundinacea*, *Luzula luzuloides*, etc.), the order Galeopsio-Senecionetalia sylvatici (*Stachys sylvatica*, *Cirsium oleraceum*, etc.) and the class Epilobietea angustifolii (*Senecio ovatus*, *Fragaria vesca*, *Gnaphalium sylvaticum*, etc.). Along with these, the investigated phytocoenoses also include species from deciduous or mixed forests of class Querco - Fagetea (*Dryopteris filix-mas*, *Poa nemoralis*, *Scrophularia nodosa*, *Fagus sylvatica* juv., etc.), from coniferous forests classified in class Vaccinio - Piceetea (*Oxalis acetosella*, *Campanula abietina* etc.), or from semi-natural communities of the class Galio - Urticetea (*Veronica urticifolia*, *Salvia glutinosa*, *Leucanthemum vulgare*, etc.).

Cluster 2. As. *Rubetum idaei* Gams 1927

Diagnostic species: *Acer pseudoplatanus*, *Agrostis capillaris*, *Cirsium erisithales*, *Cruciata glabra*, *Euphorbia amygdaloides*, *Festuca rubra*, *Galeopsis speciosa*, *Hypericum maculatum*, *Lonicera xylosteum*, *Luzula sylvatica*, *Mycelis muralis*, *Myosotis sylvatica*, *Polygonatum verticillatum*, *Ribes uva-crispa*, *Rubus idaeus*, *Salix caprea*, *Salvia glutinosa*, *Sambucus racemosa*, *Senecio ovatus*.

Constant species: *Digitalis grandiflora*, *Dryopteris filix-mas*, *Gentiana asclepiadea*, *Gnaphalium sylvaticum*, *Luzula luzuloides*, *Origanum vulgare*, *Oxalis acetosella*, *Picea abies*, *Scrophularia nodosa*, *Sorbus aucuparia*, *Spiraea chamaedryfolia*, *Stachys sylvatica*, *Veronica urticifolia*.

Dominant species: *Rubus idaeus*.

The association comprises shrub communities dominated by *Rubus idaeus*, developed on nutrient-rich soils. They frequently occur in the studied region, on deforested areas and in forest clearings, on slopes with inclinations varying between 15-45º, with various aspects. The shrub layer presents high cover, ranging between 65-85%, and is dominated by *Rubus idaeus*, alongside which *Sambucus racemosa*, *Viburnum opulus*, *Salix caprea*, *Ribes uva-crispa*, *Spiraea chamaedryfolia*, *Lonicera xylosteum* also occur sporadically, or rare juvenile specimens of *Picea abies* or *Sorbus aucuparia*. The more species-rich herbaceous layer presents variable cover, ranging between 10 and 40% and includes numerous species, as: *Fragaria vesca*, *Galeopsis speciosa*, *Poa nemoralis*, *Salvia glutinosa*, *Oxalis acetosella*, *Luzula luzuloides*, *Digitalis grandiflora*, *Cruciata glabra*, *Myosotis sylvatica*, *Impatiens noli-tangere*, etc. Floristic composition mainly includes species with Eurasiatic and European areal. Some species characteristics for the alliance Sambuco racemosae - Salicion capreae and the order Sambucetalia racemosae (*Sorbus aucuparia*, *Sambucus racemosa*, *Betula pendula*, *Urtica dioica*, etc.), as well as for the class Epilobietea angustifolii (*Galeopsis speciosa*, *Salix caprea*, *Corylus avellana*, *Senecio ovatus*, *Epilobium angustifolium*, *Gnaphalium sylvaticum*, *Eupatorium cannabinum*) present high constancy indices. The investigated plant communities also include species from class Querco - Fagetea (*Mycelis muralis*, *Euphorbia amygdaloides*, *Acer pseudoplatanus*, *Dryopteris filix-mas*, etc.), from coniferous forests classified in the Vaccinio - Piceetea class (*Picea abies*, *Calamagrostis arundinacea*, *Abies alba* juv., *Campanula abietina*, etc.), and from the tall-herb, semi-natural perennial vegetation on disturbed forest edges in Trifolio - Geranietea (*Gentiana asclepiadea*) or Galio - Urticetea classes (*Veronica urticifolia*, *Geranium robertianum*, etc.).

Cluster 3. As. *Salici - Alnetum viridis* Colic et al. 1962

Diagnostic species: *Aconitum degenii*, *Alnus viridis*, *Athyrium distentifolium*, *Cicerbita alpina*, *Cystopteris montana*, *Deschampsia cespitosa*, *Lonicera nigra*, *Ranunculus montanus* subsp. *pseudomontanus*, *Salix silesiaca*, *Veronica officinalis*.

Constant species: *Deschampsia flexuosa*, *Epilobium angustifolium*, *Gnaphalium sylvaticum*, *Luzula luzuloides*, *L. sylvatica*, *Lycopodium selago*, *Picea abies*, *Salix caprea*, *Sorbus aucuparia*, *Vaccinium myrtillus*, *Vaccinium vitis-idaea*, *Veratrum album*.

Dominant species: *Alnus viridis*.

The association *Salici* - *Alnetum viridis* comprises phytocoenoses dominated by *Alnus viridis*, sporadically occurring in the subalpine belt (along streams), on the eastern slopes of the Călimani Mountains where it occupies steep and wet slopes. The shrub layer is compact, has a cover between 70 - 80% and is built up by *Alnus viridis*, and individuals of *Salix silesiaca*, *Picea abies*, *Sorbus aucuparia*, *Spiraea chamaedryfolia*, etc. The herbaceous layer presents low cover (5 - 15%) and reduced diversity, and includes several species such as: *Deschampsia cespitosa*, *Calamagrostis villosa*, *Veratrum album*, *Valeriana tripteris*, *Epilobium angustifolium*, etc. In the floristic composition, including a mix of circumpolar, Central European and Eurasian species, higher frequencies present some species characteristic of the alliance Alnion viridis (*Salix silesiaca*, *Lonicera nigra*, etc.), order Alnetalia viridis and class Betulo carpaticae - Alnetea viridis (*Cicerbita alpina*, *Athyrium distentifolium*, etc.). Because the phytocoenoses of the association come into contact with junipers communities or coniferous forests, the floristic composition also includes a series of species from the Vaccinio - Piceetea class (e.g. *Picea abies*, *Deschampsia flexuosa*, *Campanula abietina*, *Homogyne alpina*, etc.).

Cluster 4. As. *Rhododendro myrtifolii – Pinetum mugo* Coldea 1991

Diagnostic species: *Calamagrostis villosa*, *Pinus mugo*.

Constant species: *Deschampsia flexuosa*, *Juniperus communis* subsp. *nana*, *Luzula luzuloides*, *Rhododendron myrtifolium*, *Sorbus aucuparia*, *Vaccinium myrtillus*, *Vaccinium vitis-idaea*.

Dominant species: *Pinus mugo*.

The communities with *Pinus mugo* were identified on the eastern slopes of the Căliman Izvor and Călimanul Cerbului peaks, where it forms a continuous band at 1800 – 1900 (1950) m altitude. The association is installed on slopes with different inclinations (between 5 and 20º or on flat lands), on acidic soils, poor in nutrients. They are included in priority habitat type 4070* *Pinus mugo* and *Rhododendron hirsutum* (*Mugo* - *Rhododendretum hirsuti*) thickets, with the mention that *R. hirsutum* is replaced in the Carpathians by the vicariant species *R. myrtifolium*.

The shrub layer, characterized by high cover (ranging between 80 and 95%), is dominated by *Pinus mugo*, and include also individuals of *Juniperus communis* subsp. *nana* or specimens of *Picea abies* or *Sorbus aucuparia* (reduced to shrub size). The sub-shrub layer is generally well represented, presenting covers of up to 25%, its composition more frequently including: *Vaccinium myrtillus*, *Rhododendron myrtifolium*, *Vaccinium vitis-idaea* and *Vaccinium gaultherioides*. The herbaceous layer is the most diverse, presenting variable cover (ranging between 20 and 55%) and is formed, besides the diagnostic species *Calamagrostis villosa*, by some other species such as: *Luzula luzuloides*, *Campanula abietina*, *Homogyne alpina*, *Deschampsia flexuosa*, *Hypericum richeri* ssp. *grisebachii* etc. In the floristic composition, high frequency presents the diagnostic species of alliance Pinion mugo and of the orders Junipero - Pinetalia mugo (*Juniperus communis* subsp. *nana*, *Rhododendron myrtifolium*) and Piceetalia excelsae (*Luzula luzuloides*, *Deschampsia flexuosa*), and especially of the class Roso pendulinae-Pinetea mugo (*Homogyne alpina*, *Lycopodium selago*, *Sorbus aucuparia*, etc.). Besides these, the investigated phytocoenoses also harbor species from the (sub)alpine dwarf-shrubs communities within class Loiseleurio - Vaccinietea (*Vaccinium gaultherioides*, *Vaccinium vitis-idaea*), or species infiltrated from the alpine meadows from class Juncetea trifidi (*Hieracium alpinum*, *Juncus trifidus*, *Antennaria dioica*, *Potentilla aurea* subsp. *chrysocraspeda*, etc.).

The *Pinus mugo* with *Rhododendron myrtifolium* communities are integrated in the Natura 2000 habitat - 4070* [Bushes with *Pinus mugo* and *Rhododendron hirsutum* (*Mugo-Rhododendretum hirsuti*), considered priority for conservation [Habitats Directive 92/43/EEC, 1992]. Among the main threats to the *Pinus mugo* communities there are: cutting, fires and tourism [SĂRĂȚEANU & al. 2022]. These high-altitude habitats are also considered particularly vulnerable to climate change [ŠVAJDA & al. 2011]. However, in Carpathians Mountains this habitat type was assessed as presenting an ecologically satisfactory status [BARANČOK & al. 2014] because it is sufficiently large in terms of area and distribution and presents a significant capacity of resilience to natural or human-induced disturbances. Also, in Romanian Carpathians a number of 24 Natura 2000 sites were designated to conserve these subalpine habitats [SĂRĂȚEANU & al. 2022] while in European Red List of Habitats (2016), this habitat type was assessed as Least Concern.

Cluster 5. As. *Rhododendro myrtifolii - Vaccinietum* Coldea et al. 1981

Diagnostic species: *Juniperus communis* subsp. *nana*, *Ligusticum mutellina*, *Rhododendron myrtifolium*, *Thymus alpestris*, *Vaccinium gaultherioides*.

Constant species: *Antennaria dioica*, *Campanula abietina*, *Campanula alpina*, *Deschampsia flexuosa*, *Hieracium alpinum*, *Homogyne alpina*, *Juncus trifidus*, *Juniperus communis* subsp. *nana*, *Potentilla aurea* subsp. *chrysocraspeda*, *Vaccinium gaultherioides*, *Vaccinium myrtillus*, *Vaccinium vitis-idaea*.

Dominant species: *Rhododendron myrtifolium*.

Association *Rhododendro myrtifolii* - *Vaccinietum* was identified at high altitudes, on the slopes of Căliman Izvor and Călimanul Cerbului peaks. It includes dwarf shrubs communities dominated by *Rhododendron myrtifolium* and various *Vaccinium* species, developed on moderately humid, acidic and nutrient-poor soils.

The floristic composition is characterized by a reduced number of species, more than half of these presenting circumpolar and alpine distribution. The shrub layer has a cover of 65 - 85% and includes few other dwarf-shrub species such as *Vaccinium myrtillus*, *Juniperus communis* subsp. *nana*, *Pinus mugo*, etc. The herbaceous layer is more consistent and includes several species: *Campanula alpina*, *Festuca supina*, *Nardus stricta*, *Luzula sudetica*, *Veratrum album*, etc. In the studied communities, higher frequencies were highlighted for diagnostic species of alliances Rhododendrion myrtifolii and Loiseleurio - Vaccinion, for the order Rhododendro - Vaccinietalia (*Ligusticum mutellina*, *Primula minima*, *Vaccinium gaultherioides*, etc.) and the class Loiseleurio - Vaccinietea (*Vaccinium myrtillus*, *Vaccinium vitis-idaea*, *Juniperus communis* subsp. *nana*, etc.). The association also comprises species from the vegetation of alpine meadows from class Juncetea trifidi (*Pulsatilla alba*, *Potentilla aurea* subsp. *chrysocraspeda*, *Juncus trifidus*, *Carex atrata*, etc.), from primary subalpine meadows on acidic soils classified in class Nardetea strictae (*Antennaria dioica*, *Nardus stricta*), or infiltrated from the communities of Vaccinio - Piceetea class (*Campanula abietina*, *Lycopodium selago*, *Deschampsia flexuosa*, *Luzula luzuloides*, *Pinus mugo*, etc.).

Cluster 6. As. *Campanulo abietinae - Juniperetum nanae* Simon 1966

Diagnostic species: *Campanula rotundifolia* subsp. *polymorpha*, *Hypochaeris uniflora*, *Juniperus communis* subsp. *nana*, *Vaccinium gaultherioides*, *Vaccinium vitis-idaea*.

Constant species: *Antennaria dioica*, *Anthoxanthum odoratum*, *Campanula abietina*, *Deschampsia flexuosa*, *Homogyne alpina*, *Juncus trifidus*, *Ligusticum mutellina*, *Luzula luzuloides*, *Picea abies* juv., *Rhododendron myrtifolium*, *Vaccinium myrtillus*.

Dominant species: *Juniperus communis* subsp. *nana*.

Phytocoenoses within *Campanulo abietinae - Juniperetum nanae* were identified in the subalpine belt of the Călimani Mountains, at high altitudes, including compact heaths dominated by *Juniperus communis* subsp. *nana*, occurring on moderately inclined slopes, moderately moist, acidic and nutrient-poor soils.

The floristic composition is species-poor, comprising a significant proportion of circumpolar, alpine and Carpathian - Balkan elements. The shrub layer is compact, with a cover of 80 - 95% and includes shrubs species such as: *Rhododendron myrtifolium*, *Vaccinium myrtillus*, *Pinus mugo*, *Vaccinium vitis-idaea*, etc. The herbaceous layer presents low cover (5 - 20%), and includes several species, such as: *Antennaria dioica*, *Homogyne alpina*, *Festuca supina*, *Nardus stricta*, *Luzula sudetica*, *Anthoxanthum odoratum*, *Pilosella aurantiaca* s.l. In floristic composition high frequencies present the diagnostic species of the alliances Juniperion nanae and Rhododendro - Vaccinion and order Vaccinio microphylli-Juniperetalia nanae (*Calamagrostis villosa*, *Vaccinium gaultherioides*), as well as for class Loiseleurio-Vaccinietea (*Vaccinium myrtillus*, *Vaccinium vitis-idaea*, etc.). In addition, the association also harbors species from the vegetation of alpine meadows classified in class Juncetea trifidi (*Juncus trifidus*, *Hypochaeris uniflora*, *Pulsatilla alba* subsp. *alba*, *Hieracium alpinum* s.l., etc.), or from the forests and dwarf pine stands within class Roso pendulinae - Pinetea mugo (*Deschampsia flexuosa*, *Lycopodium selago*, *Pinus mugo*, etc.).

Cluster 7. Ass. *Empetro - Vaccinietum gaultherioidis* Br.-Bl. in Br.-Bl. et Jenny 1926 corr. Grabherr in Grabherr et Mucina 1993

Diagnostic species: *Campanula alpina*, *Cetraria islandica*, *Empetrum hermaphroditum*, *Juncus trifidus*, *Thamnolia vermicularis*, *Vaccinium gaultherioides*.

Constant species: *Antennaria dioica*, *Deschampsia flexuosa*, *Hieracium alpinum*, *Juniperus communis* subsp. *nana*, *Ligusticum mutellina*, *Rhododendron myrtifolium*, *Vaccinium vitis-idaea*.

Dominant species: *Vaccinium gaultherioides*.

This association groups phytocoenoses dominated by nanophanerophyte species (*Vaccinium gaultherioides*) in the subalpine belt of Călimanul Cerbului and Căliman Izvor mountains, at high altitudes, installed on acidic and nutrient-poor soils. The floristic composition is characterized by a reduced number of species. The vegetation cover (including the lichen layer) varies between 75 - 95% and includes, besides the dominant species: *Vaccinium vitis-idaea*, *Juniperus communis* subsp. *nana*, *Festuca supina*, *Luzula sudetica*, etc. Most of the component species present circumpolar and alpine distribution (~ 70%). High frequencies were highlighted for some diagnostic species of the alliance Loiseleurio-Vaccinion, the order Rhododendro - Vaccinietalia and the class Loiseleurio - Vaccinietea (*Primula minima*, *Cetraria islandica*, *Vaccinium myrtillus*, *Thamnolia vermicularis*, etc.). The floristic composition includes also species from the vegetation of alpine meadows of the class Juncetea trifidi (*Juncus trifidus*, *Hieracium alpinum*, *Campanula alpina*, etc.).

Table 1. Synoptic table with fidelity (diagnostic species were considered only those with fidelity Phi coefficient value > 30 - multiplied by 100). The associations (groups) are: 1 - Ass. *Digitali ambigue - Calamagrostietum arundinaceae* subass. *spiraeetosum chamaedryfoliae*, 2 - Ass. *Rubetum idaei*, 3 - Ass. *Salici - Alnetum viridis*, 4 - Ass. *Rhododendro myrtifolii - Pinetum mugo*, 5 - Ass. *Rhododendro myrtifolii - Vaccinietum*, 6 - Ass. *Campanulo abietinae - Juniperetum*, 7 - Ass. *Empetro - Vaccinietum gaultherioidis*.

Group no.	**1**	**2**	**3**	**4**	**5**	**6**	**7**
No. of relevés	**6**	**7**	**5**	**5**	**5**	**5**	**7**
Spiraea chamaedryfolia	81.3	---	---	---	---	---	---
Cirsium oleraceum	79.3	---	---	---	---	---	---
Angelica sylvestris	79.3	---	---	---	---	---	---
Digitalis grandiflora	76.7	---	---	---	---	---	---
Gentiana asclepiadea	70	---	---	---	---	---	---
Betula pendula juv.	68	---	---	---	---	---	---
Trifolium repens subsp. *repens*	67.8	---	---	---	---	---	---
Hylotelephium maximum	67.8	---	---	---	---	---	---
Stachys sylvatica	63.6	---	---	---	---	---	---
Veronica urticifolia	59.4	---	---	---	---	---	---
Fagus sylvatica juv.	57.9	---	---	---	---	---	---
Scrophularia nodosa	57.9	---	---	---	---	---	---
Dryopteris filix-mas	57.9	---	---	---	---	---	---
Dactylis glomerata subsp. *glomerata*	54.6	---	---	---	---	---	---
Silene nutans subsp. *dubia*	54.6	---	---	---	---	---	---
Ajuga reptans	54.6	---	---	---	---	---	---
Solidago virgaurea subsp. *virgaurea*	54.6	---	---	---	---	---	---
Leucanthemum vulgare subsp. *vulgare*	54.6	---	---	---	---	---	---
Picea abies	45	---	---	---	---	---	---
Luzula luzuloides	32.4	---	---	---	---	---	---
Cicerbita muralis	---	91.7	---	---	---	---	---
Galeopsis speciosa	---	82.5	---	---	---	---	---
Rubus idaeus	---	78.9	---	---	---	---	---
Sambucus racemosa	---	77.8	---	---	---	---	---
Ribes uva-crispa	---	62.4	---	---	---	---	---
Viburnum opulus	---	62.4	---	---	---	---	---
Lonicera xylosteum	---	58.9	---	---	---	---	---
Acer pseudoplatanus juv.	---	54.3	---	---	---	---	---
Euphorbia amygdaloides	---	54.3	---	---	---	---	---
Polygonatum verticillatum	---	50.4	---	---	---	---	---

Myosotis sylvatica	---	50.4	---	---	---	---	---
Cruciata glabra	---	50.4	---	---	---	---	---
Festuca rubra	---	50.4	---	---	---	---	---
Agrostis capillaris	---	50.4	---	---	---	---	---
Cirsium erisithales	---	50.4	---	---	---	---	---
Hypericum maculatum	---	48	---	---	---	---	---
Salix caprea	---	47.5	---	---	---	---	---
Luzula sylvatica	---	39.6	---	---	---	---	---
Alnus viridis	---	---	100	---	---	---	---
Salix silesiaca	---	---	87.9	---	---	---	---
Cicerbita alpina	---	---	74.9	---	---	---	---
Athyrium distentifolium	---	---	74.9	---	---	---	---
Lonicera nigra	---	---	60.1	---	---	---	---
Aconitum degenii	---	---	60.1	---	---	---	---
Veronica officinalis	---	---	49.4	---	---	---	---
Ranunculus montanus subsp. *pseudomontanus*	---	---	46.1	---	---	---	---
Cystopteris montana	---	---	46.1	---	---	---	---
Deschampsia cespitosa	---	---	44.5	---	---	---	---
Pinus mugo	---	---	---	75.8	---	---	---
Ligusticum mutellina	---	---	---	---	61.8	---	---
Rhododendron myrtifolium	---	---	---	---	51.3	---	---
Thymus alpestris	---	---	---	---	46.1	---	---
Melampyrum saxosum	---	---	---	---	46.1	---	---
Juniperus communis subsp. *nana*	---	---	---	---	---	46.5	---
Hypochaeris uniflora	---	---	---	---	---	46.1	---
Campanula rotundifolia subsp. *polymorpha*	---	---	---	---	---	46.1	---
Vaccinium vitis-idaea	---	---	---	---	---	42.5	---
Thamnolia vermicularis	---	---	---	---	---	---	100
Cetraria islandica	---	---	---	---	---	---	100
Empetrum hermaphroditum	---	---	---	---	---	---	90.2
Vaccinium gaultherioides	---	---	---	---	---	---	54
Juncus trifidus	---	---	---	---	---	---	38
Campanula alpina	---	---	---	---	---	---	34.5
Poa nemoralis	68.8	55.5	---	---	---	---	---
Fragaria vesca	68.8	55.5	---	---	---	---	---
Calamagrostis arundinacea	67.4	41	---	---	---	---	---

Salvia glutinosa	53	42.8	---	---	---	---	---
Senecio ovatus	53	42.8	---	---	---	---	---
Epilobium angustifolium	39.3	43.8	---	---	---	---	---
Calamagrostis villosa	---	---	37.7	55	---	---	---
Homogyne alpina	---	---	37.3	37.3	---	---	---
Pulsatilla alba subsp. *alba*	---	---	---	---	60.1	---	47.5
Luzula sudetica	---	---	---	---	47.9	---	44.7
Festuca supina	---	---	---	---	42.9	42.9	42.9

Conclusions

The shrubs-dominated vegetation constitutes an important component in the landscape of investigated area. According to above presented research, seven well-delimited shrub communities (at the association level) were distinguished and contribute to the understanding of this vegetation type in Neagra Broştenilor river basin. They were classified into seven phytosociological alliances, five orders and four vegetation classes. Three of the associations belong to Natura 2000 habitats: 4060 Alpine and Boreal heaths (*Empetro - Vaccinietum gaultherioidis*, *Rhododendro myrtifolii - Vaccinietum Coldea*, and *Campanulo abietinae - Juniperetum nanae*), and one to habitat 4080 Sub-Arctic Salix ssp. scrub (*Salici - Alnetum viridis*). The *Pinus mugo* with *Rhododendron myrtifolium* communities are integrated in the Natura 2000 habitat - 4070* Bushes with *Pinus mugo* and *Rhododendron hirsutum* (*Mugo-Rhododendretum* hirsuti), considered priority for conservation. Although *Pinus mugo* communities are threatened by a series of human-related activities or climate change, in Carpathians Mountains this habitat type was assessed as presenting an ecologically satisfactory status, and it is protected in numerous Natura 2000 sites.

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Research Article

AGRONOMIC PERFORMANCE AND COMPETITION INDICES IN FONIO-MAIZE INTER-CROP UNDER RAIN-FED CONDITIONS

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Abstract: The investigation was to analyze for yield characters and competition indices in fonio-maize intercrop. NCRIACH2 fonio and DMR-SR maize were deployed, inter-cropping arrangement was in eleven proportions, having 10 rows of planting patterns. Higher land productivity and greater resource efficiency through increased yield and crop diversification were generally observed. 1F:1M combination recorded 32% advantage in land productivity, while 3F:1M has 1.55, showing significant land-use efficiency when fonio constitutes 75% of the system. FM 100:100 combination system indicated that maize and fonio can complement each other. 1F:3M system resulted in reduced fonio performance due to maize's dominance. Relative Crowding coefficient values confirm that maize has higher competitive pressure on fonio than vice versa. 1F:2M and 2F:2M systems could potentially balance for crop yield while optimizing space, nutrients, and water usage. Farmers in rain-fed areas are encouraged to adopt inter-cropping systems, particularly those with a balanced ratio of fonio and maize.

Keywords: combination system, competition indices, fonio, intercrop, maize, performance, resources.

Introduction

Fonio crop is often identified amongst small millet biodiversity. The ancient cereal is native to savanna zone of West Africa where CRUZ (2004) dates the cultivation back to 5,000 BC. Fonio, mostly called Acha in Nigeria belongs to the family Poacea, it is a C_4 annual and free-tillering herbaceous plant [ADOUKONOU-SAGBADJA, 2010] and may grow to the heights of 150 cm having a potential yield of 1.3 t/ha. The crop demonstrates high tolerance to most biotic and abiotic stresses. It is often grown in uneven marginal lands that are shallow and poor in fertility with low moisture retention capacity. In low rainfall arid regions where the crop is mostly grown, small holder farmers rely mostly on rainfed conditions for cultivation. KWON-NDUNG & al. (1998) opined that there are over 300 species in the genera, however, ISONG & al. (2022) identified two species (*Digitaria exilis* (Kippist) Stapf and *D. iburua* Stapf) as cultivated in Nigeria, mostly in the Northern regions of the country. Improved crop management practices are scarcely adopted by the farmers due to socio-economic constraints. While Nigeria and Guinea are leading in production and hectares of cultivation, the crop is highly challenged by strong competition from maize and other commodity crops in the country.

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Maize (*Zea mays* L.) in the other hand is an essential cereal crop in Nigeria being a better option to mitigate food shortage and a prominent source of carbohydrates, proteins, vitamins and minerals competing favorably with other starchy crops like rice and yams [OLANIYAN, 2015]. Sufficient supply of macro nutrients like nitrogen, phosphorus and potassium are essential for optimum growth and yield of maize while long-term cultivation impacts on soil organic matter and nutrient availability in the soils [MALO & al. 2005]. The suitability of maize to diverse environments is unmatched by any other crop. SHAW (1988) however noted that maize is grown from 58°N to 40°S, from below sea level to altitudes higher than 3000 m, and in areas with 250 mm to above 5000 mm of rainfall per year.

Farmers in their quest to improve adaptability, productivity and sustainability, have practiced different cropping systems [HAUGGARD-NIELSON & JENSEN, 2001]. WILLEY & OSIRU (1972) observed that primary focus was on monocropping. The exigency of climate change, population increases, industrialization and food preferences have placed demand on production of more food and feed, using less resource, time and water while maximizing the available nutrients. Therefore, for better resource use efficiency and moving towards agricultural sustainability, BEETS (1977) and DARIUSH & al. (2006) agreed that inter-cropping systems of agricultural production can be effective and sustaining. SANCHEZ (1976) had earlier define inter-cropping as a method of growing two or more crops in the same field at the same time. It is the practice of growing more than one crop simultaneously in alternating rows of the same plot [BEETS, 1990]. However, authors like BLADE (1992) and TSUBO & al. (2005) noted the gradual recognition of the importance of inter-cropping system due to its role in subsistence food production especially in developing countries. Other prominent potential of this kind of cropping system include improvement in utilization of growth resources by the inter-cropped species [BANIK & al. 2006], control of weeds, insect/diseases [SMITH & McSORLEY, 2000], control of soil erosion and conservation [JABBAR & al. 2009; MATUSSO & al. 2012], lodging resistance and total yield increment. MEAD & WILLEY (2008) however summarized that Inter-cropping is often used to increase the overall farm productivity per unit area. The system sometimes result in either positive or negative interactions among the component crops. GHOSH (2004) observed that where there is positive interaction, the component crops facilitate each other to achieve maximum productivity, while negative interaction results in yield reduction of the less competitive crop in the interaction. Interactions reported on inter-cropping system between cereals and legumes are widely available, however interactions in all cereals inter-cropping system is scarcely exploited.

Several authors [WILLEY & RAO, 1980; ESMAEILI & al. 2011; GHOSH, 2004; NDAKIDEMI & DAKORA, 2007; NYOKI & NDAKIDEMI, 2016], had itemized the use of some indices and methods to estimate the interaction among the component species. Land equivalent ratio (LER), the relative land area under sole crops that is required to produce same yields as in an in inter-cropping system is regarded as the measure of land use efficiency of the plants. HALL (1974) proposed relative crowding coefficient (RCC or K) as a measure of a crop producing more or less than expected in an inter-crop system. It is a measure of relative dominance of a crop over the other. A value of 1, less than 1 or greater than 1 signifies equal yield, less yield or greater yield than the expected yield respectively. While competitive ratio (CR) represents the ratio of individual land equivalent ratios of two crops, considering the proportion initially sown, Agrgressivity (A) measures the value by which the relative yield increase of one crop is greater than that of the other crop. The Actual Yield Loss (AYL) indices is based on yield per plant, BANIK & al. (2000) is of the opinion that it presents a more accurate information about the competition than other indices. Inter-cropping Advantage (IA) and

Monetary Advantage Indices are formulated to provide information on the economic advantage of the inter-cropping systems. Therefore, this investigation thus seek to study the performance of yield and other characters, and to analyze for the different competition indices present in fonio-maize inter-cropping systems under rain-fed conditions

Material and methods

Location and environmental condition of the experimental site

The Inter-cropping experiment was conducted at National Cereals Research Institute experimental fields, located at Badeggi, about 10 kilometers from Bida in Niger State, Nigeria. National Cereals Research Institute is at Latitude 9°45'N and Longitude. 6°07'E in the Southern Guinea Savannah, at an elevation of 98 m a.s.l. The experiment took place during the growing season (June-November) of 2022 and 2023, the climatic and edaphic conditions were typical of the Southern Guinea savanna.

Experimental material, design and crop management

The experimental materials used include a Fonio variety NCRIACH2, sourced from the National Cereals Research Institute (NCRI) gene bank and a Maize variety DMR-SR from National Centre for Genetic Resources and Biotechnology (NACGRAB) collections. Randomized Complete Block Design (RCBD) was employed and replicated three (3) times. The plot size of 3 m x 5 m was used, planting distance of 30 cm and 50 cm was adopted for both sole crop and inter-crop for fonio, and the plant density of 80,666 plants per hectare was maintained. Maize was planted at a spacing of 25 cm x 100 cm both in sole crop and inter-crop. However, in both crops, pure culture was inserted to serve as control treatment. The inter-cropping arrangements were in eleven (11) arrangements, formulated as indicated in Table 1, having 10 proportions of different crop combinations representing planting ratios of fonio to maize, FNMZ, 1F:1M, 2F:1M, 2F:2M, 1F:2M, 1F:3M, 3F:1M, 3F:2M and 2F:3M. Manual weeding was adopted, two times weeding at 30 DAP and at 60 DAP.

Observation, data collection and statistical analysis

Observation and data collection on test crop (fonio) were on days to 50% flowering (DFF), number of tillers per plant (NTPP), number of panicles per plant (NPPP), plant height (PH), spike length (SL), panicle length (PL), number of spikes per panicle (NSPP), days to maturity (DM), weight of panicle (PW) and grain yield (GY) expressed in kilogram per hectare. Also. observation and data collection on maize plants were on plant height (PH), number of ears per plant (NEP), number of rows per ear (NRE), number of grains per row (NGR), number of grains per ear (NGE), number of grains per plant (NGP), one thousand grain weigh (1000GW) and grain yield (GY). The Grain Yield of fonio and that of maize were as well presented in tons per hectare, the value of each crop per hectare was determined based on the prevailing market price of the two crops. Statistical Tool for Agricultural Research (STAR) version 2.0.1 (2014) was used for the analysis of yield and the yield characters in both the test crop and component crop. *Per se* performance of the crops at different planting combination systems for each of the traits were determined, analysis of variance was carried out and means were separated using Duncan Multiple Range Test (DMRT).

Competition indices and monetary advantage determination

Grain Yield of fonio and maize were as well presented in tons per hectare, the value for each of the crop per hectare was determined as follows:

$$V_f = Y_f \ x\ P_f$$

where, V_f = *Value of fonio*; Y_f =*Yield of fonio in tons per hectare*; P_f = *Prevailing market price of fonio during the time of harvest.*

$$V_m = Y_m \ x\ P_m$$

where, V_m = *Value of Maize*; Y_m =*Yield of maize in tons per hectare*; P_m = *Prevailing market price of maize during the time of harvest.*

The measure for mixed stand advantage was expressed in Land Equivalent Ratio (LER), which is an inter-cropping efficiency over mono-cropping in the use of environmental resources. This was obtained by dividing the inter-cropped yield by mono-crop yield for each crop in inter-cropping system. The total LER is by adding the partial LERs together. According to WILLEY (1979), when the value of LER is greater than one, more sole crop's land is necessary for a given yield value. But when it is less than one, the indication is that inter-cropping is negatively affecting the growth and yield of component crops in mixtures. The value of LERs were worked out following the formula of DHIMA & al. (2007) as follows:

$$L = L_1 + L_2 = L_{i1} / L_{s1} + L_{i2} / L_{s2}$$

where, L_1= *LER of Fonio*; L_2 = *LER of Maize*; Y_{i1} = *Yield of Fonio in intercrop*; Y_{i2} = *Yield of Maize in intercrop*; Y_{s1} = *Yield of Fonio in sole cropping*; Y_{s2} = *Yield of Maize in sole cropping.*

The relative dominance of a crop over the other measured by the Relative Crowding Coefficient (K) was calculated following the formula by DE WIT (1960). DHIMA & al. (2007) also hinted that when the value is 1, it means there is no yield advantage in the system. However, when the K value is greater than or less than 1, the yield advantage is recorded or there is yield disadvantage respectively:

$$K = (K_{fonio} \ x\ K_{maize})$$

$$K_{fonio} = Y_{fm} \ x\ R_{mf} / [(Y_f - Y_{fm}) \ x\ R_{fm}]$$

$$K_{maize} = Y_{mf} \ x\ R_{fm} / [(Y_m - Y_{mf}) \ x\ R_{mf}]$$

Where, K_{fonio} = *Relative Crowding Coefficient of Fonio in the Intertaction*; K_{maize} = *Relative Crowding Coefficient of Maize in the interaction*; Y_{fm} = *Yield of fonio in the interaction*; Y_{mf} = *Yield of maize in the interaction*; R_{fm} = *Ratio of fonio in the interaction*; R_{mf} = *Ration of maize in the interaction*; Y_f = *Yield of sole fonio*; Y_m = *Yield of sole maize*.

ESMAEILI & al. (2011) expressed the Competitive Ratio (CR) as the ratio of individual LERs of two crops, taking into consideration the proportion of the species initially planted. Also, the index was determined according to the method of DHIMA & al. (2007) as follows:

$$CR_{fonio} = (LER_{fonio} / LER_{maize})(R_{mf}/R_{fm})$$

$$CRmaize = (LER_{maize} / LER_{fonio})(R_{fm}/R_{mf})$$

where, CR_{fonio}= *Competitive Ratio of Fonio in the interaction*; CR_{maize} = *Competitive Ratio of maize in the interaction*; LER_{fonio} = *Land Equivalent Ratio of fonio in the interaction*; LER_{maize} = *Land Equivalent Ratio of maize in the interaction*; R_{fm} = *Ratio of fonio in the interaction*; R_{mf} = *Ratio of maize in the interaction*

Another index was the Aggressivity (AGG or A). This is a measure of how much the relative yield in one crop is greater than that for another crop in an inter-cropping system according to McGILCHRIST (1965). Aggressivity value of 0 denotes equal competition in both crops. A positive or negative value of Aggressivity inferred that the crop is dominant or being dominated respectively. The following formular were adopted to determine aggressivity as follows:

$$A_{fonio} = (Y_{fm} / Y_f \text{ x } R_{fm}) - (Y_{mf} / Y_m \text{ x } R_{mf})$$

$$A_{maize} = (Y_{mf} / Y_m \text{ x } R_{mf}) - (Y_{fm} / Y_f \text{ x } R_{fm})$$

where, *A_{fonio} = Aggressivity of Fonio in the interaction*; *A_{maize} = Aggressivity of Maize in the Interaction.*

Actual Yield Loss (AYL) index was also formulated. This index is based on yield per hectare, the proportionate loss or gain of yield in inter-crop compared to the respective sole species. AYL factors in yield per plant and the real proportion of the component species planted with that of the sole stand. The actual Yield Loss can be either a positive or negative in value, which signifies advantage or disadvantage inter-cropping relationship. The following formular were used to determine Actual Yield loss for fonio and maize in the inter-cropping interaction as demonstrated by KHONDE & al. (2018):

$$AYL = AYLf_{onio} + AYL_{maize}$$

$$AYL_{fonio} = [(Y_{fm} / R_{fm}) / (Y_f / R_f)] - 1$$

$$AYL_{maize} = [(Ymf / R_{mf}) / (Y_m / R_m)] - 1$$

where, *AYL_{fonio} = Actual yield loss of fonio in the interaction*; *AYL_{maize} = Actual yield loss of maize in the interaction.*

Two indices that provided information on the economic advantage of the inter-cropping systems were as well considered. One is Inter-cropping Advantage (IA), formulated according to the method of BANIK & al. (2000) as follows:

$$IA_{fonio} = AYL_{fonio} \times P_{fonio}$$

$$IA_{maize} = AYL_{maize} \times P_{maize}$$

where, *IA_{fonio} = Intercropping Advantage for fonio*; *IA_{maize} = Intercropping Advantage for Maize*; *P_{fonio} = Current commercial Price of fonio* (*₦800 per Kg*); *P_{maize} = Current commercial Price of Maize* (*₦300per Kg*).

Another economic Advantage index is the Monetary Advantage Index (MAI), which according to GHOSH (2004), the higher the MAI value the more profitable is the cropping system. This index was formulated as follows:

$$MAI = [(\textit{value of combined intercrops}) \text{ x } (LER - 1)] / LER$$

Results and discussions

The data for environmental and soil conditions of the experimental location for 2022 and 2023 growing seasons are presented in Figures 1, 2, 3a, 3b and 3c. While Figure 1 is the rainfall pattern during the growing seasons at Badeggi, Figure 2 shows the temperature variation and Figure 3a, 3b and 3c present the soil physical properties, soil chemical properties and soil exchangeable ions for the experimental plots. Patterns of inter-cropping arrangements are in Table 1. *Per se* performance of fonio and maize parameters as affected by the inter-cropping system are in Tables 2 and 3 respectively. Also, competition indices for sole stands and fonio-maize inter-crop in 2022 and 2023 seasons are presented in Table 4.

The planting combination 2F:1M and 3F:2M representing 67% fonio, 33% maize and 60% fonio, 40% maize respectively were the first to reach 50% flowering. The increase in days to flowering in 1F:3M suggests competition of maize with fonio for limited resources thereby causing flowering in fonio to delay. According to GHOSH & al. (2006), the delay could affect the overall productivity of fonio because the growing period is shortened. However, the 1F:2M and 2F:3M systems shows closer flowering times to sole fonio (107 days), indicating that reproductive timing in fonio is not severely hindered by the presence of maize. Planting combination 2F:1M recorded highest number of tillers per plant (27.80) and longest panicles (25.20). Inter-cropping systems having higher or equal fonio proportions with maize induce greater vegetative growth. This is in alignment with the findings of WILLEY (1979), suggesting that inter-cropping can enhance plant density and increased tillering ability. Meanwhile, authors like BIABANI & al. (2008), SADEGHPOUR & JAHANZAD (2012) suggested an advantage of inter-cropping systems in final yield as dependent on spatial arrangements of participating species. The robust number of panicles observed in 3F:1M system is an indication of a balanced competition for resources, indicating a beneficial combination for reproductive growth of fonio, corroborating the findings of JACKSON (1980). Increase in spikes and panicles in the FM 100:100 system may also suggest that maize is more dominant, leading to enhanced growth for both crops, but limiting potentials in fonio. Tallest fonio plants (167.93 and 150.40) were found in FM 100:100 and 50:50 combinations respectively. The inter-cropping systems that combine both crops at relatively equal proportions also appear to mature earlier. inter-cropping systems where maize proportion is high also appear to out-competes fonio. However, the 1F:3M system shows that when fonio is at a low proportion, it struggles to grow to its potential height confirming the discussion by WEINER (1990). Similarly, FM 100:100 combination seems to enhance the panicle weight of fonio, demonstrating better growth conditions for both crops in a balanced inter-cropping system. It also suggests synergistic effects between maize and fonio but in combination systems with relatively equal ratios.

Fonio in FM 100:100 system performance was as well good, having positive effects on number of panicles and number of spikes due to equal space allocation as suggested by SHAH & al. (2021). Also, in this system, both crops mature earlier, suggesting a positive interaction, in agreement with OFORI & STERN (1987) that in inter-cropping, earlier maturation due to complementary growth are likely to occur.

Planting pattern of 3 rows fonio and 1 row maize recorded highest number of panicles per plant and gave the highest grain yield of 555.47 kg/ha behind sole fonio crop of 637.90 kg/ha. Sole fonio yielded the most grain, which could indicate that fonio grows best without competition from maize. However, in FM 100:100 inter-cropping systems, fonio yields relatively high, suggesting positive interaction. The highest yield in sole fonio may suggest that fonio performs best in a competition-free environment [NWAMINI & al. 2020]. However, in fonio maize inter-crop, GHOSH & al. (2006) suggested a higher resource-use efficiency at optimal crop mixture.

Table 3 presents a maize height range of 176.80 cm in 2F:3M and 210.33 cm in 2F:1M combination systems, while the sole maize was 185.80 cm. A lower value of plant height was noted in 2F:3M than in sole crop, providing an opportunity for fonio to out-compete maize for resources. The result is in agreement with FU & al. (2023) that taller maize plants will intercept more light in inter-cropping than in monocropping. Sole maize recorded a significantly lower value of 1.74 ears/plant while the highest were in 1F:2M and 1F:3M (2.16 and 2.45ears/plant respectively), suggesting a better resource and reproductive outcomes [MEAD & WILLEY, 2008]. Higher number of ears in intercropping is therefore an indication of a positive interaction, meanwhile OFORI & STERN (1987) had hinted that some systems can enhance reproductive output between maize and fonio. The average number of rows per ear was 14.24, indicating that maize was seriously affected by the inter-crop, in which LI & al. (2020) viewed as due to stress from competition with a resource-demanding companion. Increase in the number of grains per row and number of grains per ear in 2F:2M combination system may lead to more efficient resource utilization because of a complementary effects. NWITE & al. (2017) made similar observation, where significant increase in grain set of maize in a maize legume inter-crop than in sole crop. The system with 75% fonio and 25% maize had significantly more grains per plant, suggesting improved access to resources [HASANVAND & al., 2019]. Meanwhile, reduction in 1000 grain weight and grain yield in some systems confirm that maize was stressed due to competition, which could reduce kernel size, seed weight and subsequent grain yield as reported by WILLEY (1979) and GHOSH & al. (2006). Exception on 3F:1M and 2F:1M combination systems, which shows relatively good yields of 3.11 t/ha and 3.10 t/ha respectively, suggesting that combining with fonio helps to optimize land use efficiency. The 1F:2M, 2F:1M, and 3F:1M inter-cropping systems are promising results for improved resource utilization. 2F:2M and 3F:2M systems allow for more efficient space and nutrient sharing, which could be useful in areas with limited arable land.

In Table 4, maize yields are generally lower in inter-crop than in sole maize system. FM (100:100) has a land equivalent ratio of 1.70, indicating a land-use advantage of 70%. 1F:1M has land equivalent ratio of 1.32, implying a 32% advantage in land productivity, while 3F:1M has 1.55, showing significant land-use efficiency when fonio constitutes 75% of the system. GHOSH & al. (2006) proposed that when Land Equivalent ratio is above 1, the inter-cropping can make more efficient use of land resources than monocropping. Relative crowding coefficient recorded below 1 values for fonio but closer to 1 for maize, showing that maize often has a higher competitive pressure on fonio than vice versa. In 3F:1M, Relative crowding coefficient were 0.57 and 2.22 for fonio and maize respectively. This is a reflection of stronger competition for resources by maize, signifying that fonio is a relatively low-competition crop but can be grown alongside other crops without significant yield loss. However, MEAD & WILLEY (2008) also recommended low competitive crop like fonio as suitable companion crop due to their ability to improve soil health, prevent erosion, and increase biodiversity. In combination where 33% is fonio, maize recorded higher aggressivity score of 2.29 and fonio recorded 0.465, suggesting stronger competition from maize. But in FM (100:100) combination system, fonio displays a higher aggressivity (3.71) than maize, suggesting that fonio is more adapted to the condition of the system. While maize tends to have a higher competitive advantage in most systems, adaptability assists fonio to thrive even under reduced resource availability [GHOSH & al. 2006]. Consequently, fonio had significant yield loss of -11.82 in FM 100:100 combination system, resulting in a slightly reduced yield advantage. In 2F:2M, the inter-cropping advantage is more balanced, resulting in a positive (+0.94) value in fonio. WILLEY (1979) noted that yield losses in intercrops are typical, but often compensated by total higher yields per unit of land. However, BROOKER (2015) also highlighted that Inter-cropping advantage (IA) is usually evident in drought-prone ecologies having unstable weather conditions where it provides food diversification and better resource utilization. 1F:1M and 2F:2M inter-cropping systems showed considerable monetary advantages due to higher fonio yields with extra revenue. Meanwhile, FM (100:100) combination shows a negative monetary advantage index, suggesting that farmers in that location may not benefit financially in adopting the system.

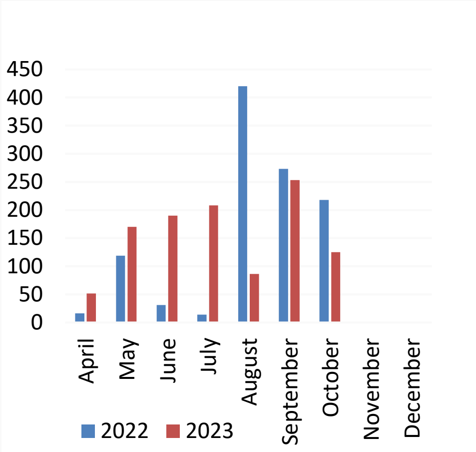

Figure 1. Rainfall pattern during the growing season at Badeggi

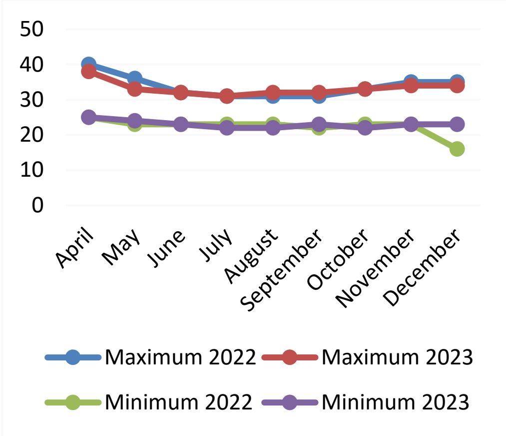

Figure 2. Temperature variation during the growing season at Badeggi

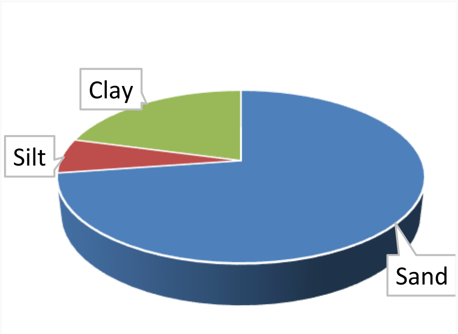

Figure 3a. Soil Physical property composition of experimental location in 2022 and 2023

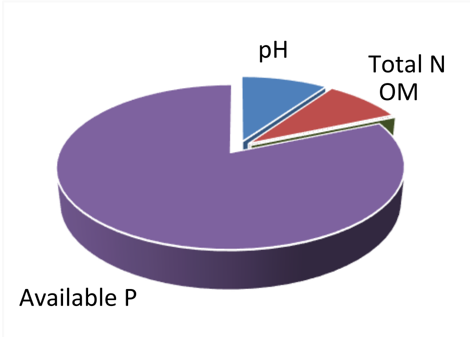

Figure 3b. Soil Chemical property composition of experimental location in 2022 and 2023

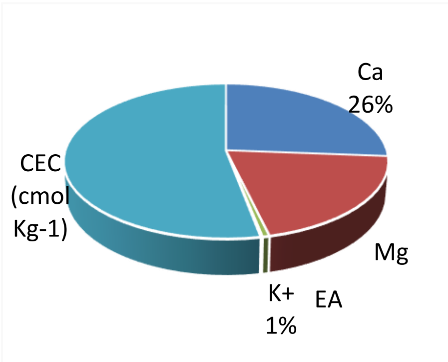

Figure 3c. Soil Exchangeable ions of experimental location in 2022 and 2023

Table 1. Intercropping arrangements in ten rows under rain-fed condition

Mix-Proportion (%)	Sole fonio	Sole Maize	Both crops grown mixed	1 alternate rows of fonio and maize	2 alternate rows of fonio, 1 of maize	2 alternate rows of fonio and maize	1 alternate rows of fonio, 2 of maize	1 alternate row of fonio, 3 of maize	3 alternate rows of fonio, 1 of maize	3 alternate rows of fonio, 2 of maize	2 alternate rows of fonio, 3 of maize
	100	100	100:100	50:50	67:33	50:50	33:67	25:75	75:25	60:40	40:60
Rows	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11
1	ΔΔΔΔ	ψψψψ	ΔψΔψ	ΔΔΔΔΔ	ΔΔΔΔΔ	ΔΔΔΔΔ	ΔΔΔΔΔ	ΔΔΔΔΔ	ΔΔΔΔΔ	ΔΔΔΔΔ	ΔΔΔΔΔ
2	ΔΔΔΔ	ψψψψ	ψΔψΔ	ψψψψ	ΔΔΔΔΔ	ΔΔΔΔΔ	ψψψψ	ψψψψ	ΔΔΔΔΔ	ΔΔΔΔΔ	ΔΔΔΔΔ
3	ΔΔΔΔ	ψψψψ	ΔψΔψ	ΔΔΔΔΔ	ψψψψ	ψψψψ	ψψψψ	ψψψψ	ΔΔΔΔΔ	ΔΔΔΔΔ	ψψψψ
4	ΔΔΔΔ	ψψψψ	ψΔψΔ	ψψψψ	ΔΔΔΔΔ	ψψψψ	ΔΔΔΔΔ	ψψψψ	ψψψψ	ψψψψ	ψψψψ
5	ΔΔΔΔ	ψψψψ	ΔψΔψ	ΔΔΔΔΔ	ΔΔΔΔΔ	ΔΔΔΔΔ	ψψψψ	ΔΔΔΔΔ	ΔΔΔΔΔ	ψψψψ	ψψψψ
6	ΔΔΔΔ	ψψψψ	ψΔψΔ	ψψψψ	ψψψψ	ΔΔΔΔΔ	ψψψψ	ψψψψ	ΔΔΔΔΔ	ΔΔΔΔΔ	ΔΔΔΔΔ
7	ΔΔΔΔ	ψψψψ	ΔψΔψ	ΔΔΔΔΔ	ΔΔΔΔΔ	ψψψψ	ΔΔΔΔΔ	ψψψψ	ΔΔΔΔΔ	ΔΔΔΔΔ	ΔΔΔΔΔ
8	ΔΔΔΔ	ψψψψ	ψΔψΔ	ψψψψ	ΔΔΔΔΔ	ψψψψ	ψψψψ	ψψψψ	ψψψψ	ΔΔΔΔΔ	ψψψψ
9	ΔΔΔΔ	ψψψψ	ΔψΔψ	ΔΔΔΔΔ	ψψψψ	ΔΔΔΔΔ	ψψψψ	ΔΔΔΔΔ	ΔΔΔΔΔ	ψψψψ	ψψψψ
10	ΔΔΔΔ	ψψψψ	ψΔψΔ	ψψψψ		ΔΔΔΔΔ	ΔΔΔΔΔ		ΔΔΔΔΔ	ψψψψ	ψψψψ

Table 2. Performance of Fonio Parameters as affected by inter-cropping system

Planting pattern	Ratio (%)	DFF	NTPP	NPPP	PH (cm)	SL(cm)	PL (cm)
Sole Fonio	100	107.34^{bc}	22.00^{ef}	14.40^{de}	127.53^{cd}	14.00^{cd}	22.07^{d}
FM	100:100	109.67^{b}	26.67^{ab}	15.20^{d}	167.93^{a}	16.67^{a}	25.13^{a}
1F:1M	50:50	100.33^{cd}	23.27^{de}	20.60^{ab}	150.40^{b}	14.47^{bc}	23.67^{bc}
2F:1M	67:33	98.33^{d}	27.80^{a}	19.13^{bc}	120.20^{de}	15.07^{bc}	25.20^{a}
2F:2M	50:50	101.67^{bcd}	26.87^{ab}	20.53^{ab}	129.07^{c}	$12.80e^{f}$	21.53^{de}
1F:2M	33:67	100.67^{cd}	19.07^{g}	14.20^{de}	116.73^{ef}	12.00^{f}	20.40^{ef}
1F:3M	25:75	119.33^{a}	26.67^{ab}	13.00^{e}	109.47^{f}	10.40^{g}	20.20^{f}
3F:1M	75:25	120.33^{a}	24.67^{cd}	21.73^{a}	126.60^{cd}	12.13^{ef}	23.40^{c}
3F:2M	60:40	98.33^{d}	20.87^{fg}	18.33^{c}	113.07^{ef}	15.60^{ab}	24.87^{ab}
2F:3M	40:60	98.70^{cd}	25.27^{bc}	19.53^{bc}	147.47^{b}	13.27^{de}	23.40^{c}
Mean		105.47	24.32	17.67	130.85	13.64	22.99
Max		120.33	27.8	21.73	167.93	16.67	25.2
Min		98.33	19.07	13.00	109.47	10.4	20.2
SE		2.51	0.51	0.42	2.31	0.32	0.37
CV%		2.91	2.58	2.92	2.16	2.86	1.95
LSD5%		8.98	1.83	1.51	8.27	1.14	1.31

Table 2. (contd.)

Planting pattern	Ratio (%)	NSPP	DM	PW(g)	GY (kg/ha) 2022	2023	Mean
Sole Fonio	100	9.13[a]	130.67[e]	250.45[b]	613.26[a]	662.54[a]	637.90[a]
FM	100:100	8.73[a]	150.67[a]	212.42[c]	481.49[d]	521.98[bc]	501.735[cd]
1F:1M	50:50	7.13[c]	145.33[ab]	274.90[a]	305.84[f]	328.76[e]	317.30[f]
2F:1M	67:33	7.73[b]	136.00[cde]	185.52[d]	403.86[e]	503.12[cd]	453.49[e]
2F:2M	50:50	6.20[d]	138.33b[cde]	204.87[c]	518.55[c]	509.38[cd]	513.965[c]
1F:2M	33:67	7.40[bc]	143.67[abc]	216.86[c]	488.27[d]	474.53[d]	481.4d[e]
1F:3M	25:75	5.60[e]	138.67[bcd]	152.78[e]	323.78[f]	308.07[e]	315.925[f]
3F:1M	75:25	8.73[a]	133.00[de]	204.34[c]	551.27[b]	559.67[b]	555.47[b]
3F:2M	60:40	7.73[b]	133.67[de]	261.08[ab]	509.56[cd]	502.65[cd]	506.105[cd]
2F:3M	40:60	7.13[c]	135.67[de]	172.68[d]	502.67[cd]	521.67[bc]	512.17[c]
Mean		7.55	138.57	213.59	469.85	489.24	479.55
Max		9.13	150.67	274.9	613.26	662.54	637.90
Min		5.6	130.67	152.78	305.84	308.07	315.93
SE		0.13	2.15	4.41	7.93	10.74	8.43
CV%		2.03	1.90	2.53	2.07	2.69	2.15
LSD5%		0.45	7.72	15.82	28.42	38.49	30.22

DFF= Days to 50% flowering, NTPP = Number of tillers per plant, NPPP = Number of panicle per plant, PH = Plant height (cm), SP = Spike Length (cm), PL = Panicle Length (cm), NSPP = Number of Spikes per plant, DM = Days to maturity, GY = Grain yield (kg/ha). Local check – popular farmers' variety

Table 3. Performance of Maize Parameters as affected by inter-cropping system

Planting pattern	Ratio (%)	PH	NEP	NRE	NGR	NGE
Sole Maize	100	185.80[c]	1.74[f]	13.76[cd]	40.76[b]	726.51[abcd]
ACMZ	100:100	188.53[bc]	1.66[f]	16.56[a]	48.45[a]	622.87[f]
1F:1M	50:50	185.33[c]	2.05b[cd]	13.07[d]	49.32[a]	644.61[ef]
2F:1M	67:33	210.33[a]	2.02[cd]	13.86[cd]	48.34[a]	699.99[cd]
2F:2M	50:50	209.00[a]	1.98[de]	14.34[bc]	50.65[a]	766.32[a]
1F:2M	33:67	201.73[a]	2.16[bc]	13.56[cd]	50.05[a]	728.68[abc]
1F:3M	25:75	204.60[a]	1.82[ef]	15.04[b]	47.87[a]	749.97[ab]
3F:1M	75:25	200.13[ab]	2.45[a]	14.24[bc]	49.87[a]	760.15[ab]
3F:2M	60:40	184.53[c]	1.98[de]	13.77[cd]	48.80[a]	711.98[bcd]
2F:3M	40:60	176.80[c]	2.21[b]	14.21[bc]	47.76[a]	678.67[de]
Mean		194.68	2.01	14.24	48.19	708.98
Max		210.33	2.45	16.56	50.65	766.32
Min		176.80	1.66	13.07	40.76	622.87
SE		3.46	0.049	0.248	1.14	13.78
CV%		2.18	3.02	2.14	2.9	2.38
LSD5%		12.41	0.177	0.89	4.086	49.42

Table 3. (contd.)

Planting pattern	Ratio (%)	NGP	1000GW (g)	GY(t/ha) 2022	2023	Mean
Sole Maize	100	1264.13^{f}	315.44^{c}	4.62^{a}	4.62^{a}	4.62^{a}
ACMZ	100:100	1033.96^{g}	247.38^{e}	4.21^{b}	4.18^{b}	4.20^{b}
1F:1M	50:50	1321.45^{ef}	380.26^{b}	3.74^{c}	3.76^{cd}	3.75^{c}
2F:1M	67:33	1413.98^{cde}	305.87^{cd}	3.00^{f}	3.20^{e}	3.10^{e}
2F:2M	50:50	1517.31^{bc}	488.67^{a}	3.03^{ef}	3.00^{e}	3.02^{e}
1F:2M	33:67	1573.95^{b}	279.78^{d}	3.48^{d}	3.98^{bc}	3.73^{c}
1F:3M	25:75	1364.95^{def}	300.65^{cd}	3.66^{cd}	3.64^{d}	3.65^{c}
3F:1M	75:25	1862.37^{a}	308.67^{c}	3.00^{f}	3.21^{e}	3.11^{e}
3F:2M	60:40	1409.72^{cde}	302.76^{cd}	2.74^{g}	2.63^{f}	2.69^{f}
2F:3M	40:60	1499.86^{bcd}	290.56^{cd}	3.24^{e}	3.55^{d}	3.40^{d}
Mean		1426.17	322.0	3.47	3.58	3.53
Max		1862.37	488.67	4.62	4.62	4.62
Min		1033.96	247.38	2.74	2.63	2.69
SE		38.30	7.33	0.063	0.095	0.064
CV%		3.29	2.79	2.21	3.24	2.23
LSD5%		137.32	26.29	0.23	0.34	0.23

N/B: F = Fonio; M = Maize; NRE: number of rows per ear: NGR: number of grains per row; NEP: number of ears per plant; NGE: number of grains per ear; NGP: Number of Grains per plant; HSW: hundred grain weight; GY: grain yield

Table 4. Competition Indices in Sole stand and Inter-cropping System

Planting pattern	mix (%)	GY (ton/ha)			Value (N/ha)			LER		
		Fonio	Maize	Total	Fonio	maize	total	Fonio	Maize	LER
Sole Fonio	100	0.64		0.64	513		513			
Sole maize	100		4.62	4.62		1386	1386			
FM	100:100	0.5	4.20	4.70	404	1257	1661	0.79	0.91	1.70
1F:1M	50:50	0.32	3.75	4.07	258	1126	1384	0.51	0.81	1.32
2F:1M	67:33	0.45	3.10	3.55	370	940	1310	0.71	0.67	1.38
2F:2M	50:50	0.52	3.02	3.53	411	903	1314	0.81	0.66	1.47
1F:2M	33:67	0.48	3.73	4.21	382	1144	1526	0.76	0.81	1.56
1F:3M	25:75	0.31	3.65	3.96	246	1094	1340	0.49	0.79	1.29
3F:1M	75:25	0.56	3.11	3.66	445	942	1387	0.88	0.68	1.55
3F:2M	60:40	0.505	2.69	3.19	403	800	1203	0.8	0.58	1.38
2F:3M	40:60	0.51	3.40	3.91	410	1034	1444	0.8	0.74	1.54

Table 4. (contd.)

Planting pattern	mix (%)	RCC			CR		AGG	
		Fonio	Maize	RCC	Fonio	Maize	Fonio	Maize
Sole Fonio	100	1.0		1.00				
Sole maize	100		1.0	1.00				
FM	100:100	3.71	9.89	36.62	0.87	1.15	-11.82	11.545
1F:1M	50:50	1.02	4.31	4.39	0.625	1.605	-15.655	15.11
2F:1M	67:33	1.24	4.17	5.28	0.375	2.23	22.755	-22.795
2F:2M	50:50	4.59	1.88	8.65	1.24	0.81	9.955	-9.94
1F:2M	33:67	6.66	2.28	13.88	2.29	0.465	-23.175	1.87
1F:3M	25:75	2.91	1.26	3.65	2.1	0.52	-46.06	45.655
3F:1M	75:25	2.47	6.19	14.89	0.57	2.22	50.71	-51.205
3F:2M	60:40	2.74	2.09	5.81	0.97	1.275	26.205	-24.83
2F:3M	40:60	6.20	1.89	11.36	1.74	0.52	-9.655	9.235

Table 4. (contd.)

Planting pattern	mix (%)	AYL			IA			MAI
		Fonio	Maize	Total	Fonio	Maize	Total	
Sole Fonio	100							
Sole maize	100							
FM	100:100	-0.21	-0.09	-0.31	-126	-18.5	-144.5	683.94
1F:1M	50:50	0.02	0.63	0.64	12	125	137	330.21
2F:1M	67:33	-0.10	1.03	1.08	-60	206.9	146.9	375.60
2F:2M	50:50	0.63	0.30	0.94	378	60.9	438.9	407.91
1F:2M	33:67	1.30	0.21	1.51	780	41	821	549.96
1F:3M	25:75	0.96	0.06	1.02	576	11.1	587.1	287.60
3F:1M	75:25	0.17	1.69	1.86	99	337.9	436.9	492.16
3F:2M	60:40	0.33	0.45	0.78	195	90	285	320.94
2F:3M	40:60	1.01	0.17	1.18	606	33.4	639.4	506.35

N/B: GY = Grain yield (kg/ha), LER= Land equivalent ratio, RCC = Relative crowding coefficient (K), CR = competitive ratio, AGG = Aggressivity, AYL = Actual yield loss, IA = inter-cropping advantage, MAI = Monetary Advantage Index

Conclusions

Inter-cropping systems involving fonio and maize generally provide higher land productivity, greater resource efficiency, and economic benefits through increased yield and crop diversification. Although fonio performs best in a sole cropping system, FM 100:100 system seems particularly beneficial for both crops, suggesting that maize and fonio can complement each other when planted in equal proportions. Furthermore, the 1F:3M combination system, with a lower proportion of fonio, results in reduced performance of fonio due to domination from maize. Systems with a higher proportion of maize tends to delay flowering, reduce plant height, and negatively affects yield of fonio. Relative crowding coefficient values were below 1 for fonio but closer to 1 for maize, showing that maize often have higher competitive pressure on fonio than vice versa. Selecting early maturing fonio varieties can reduce the competition with maize and allow for better resource allocation. However, adaptability assists fonio to thrive even under reduced resource availability. Systems like 1F:2M and 2F:2M, constituting about 50-75% maize, should be promoted to farmers in regions where water scarcity is a concern. These systems could potentially balance the crop yield while optimizing space, nutrition and water usage. Farmers in rainfed areas are encouraged to adopt inter-cropping systems, particularly those with a balanced ratio of fonio and maize, which help ensure food security and increased resilience to climate variability. Research on inter-cropping has shown that optimal crop mixture often leads to higher resource use efficiency compared to monoculture. However, further research regarding combination systems with positive monetary advantage index should be extended to other fonio growing ecologies, with particular focus on improved adoption and economic impacts of the intercropping systems.

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Research Article

YIELD PARAMETERS OF OKRA (*ABELMOSCHUS ESCULENTUS*) PLANT AND NUTRITIONAL CONTENTS OF THE HARVESTED OKRA FRUITS AS INFLUENCED BY THE APPLICATION OF PLANT EXTRACTS AND SYNTHETIC INSECTICIDES

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Abstract: At Ladoke Akintola University of Technology, Teaching and Research Farm, Ogbomoso, we conducted this experiment during the major planting seasons of 2019 and 2020 to determine the effects of *Petiveria alliacea* and *Azadirachta indica* extracts as botanical insecticides and synthetic insecticides (cypermethrin and dichlorvos) on the yield parameters, proximate, phytochemical, and mineral contents of okra. We included untreated plants in the experiment for comparison. We arranged the treatments in a randomized complete block design, replicating each treatment three times. We collected data on the plant height, leaf area, and yield, proximate, phytochemical, and mineral contents of the harvested okra fruits. The results revealed that cypermethrin and *P. alliacea* extracts had a higher okra plant height (36.5 cm), but all the treatments had the same effects on leaf area. However, tested plant extracts and synthetic insecticides produced the same okra fruit yield. Harvested okra fruits from plants treated with dichlorvos had the highest proximate contents, followed by *P. alliacea* and *A. indica* extracts. Meanwhile, okra fruits from plants treated with *P. alliacea* extracts had the highest phytochemical contents (5.2 mg/100 g), but okra fruits from *A. indica* and *P. alliacea* had the highest mineral contents (234.1 to 231.5 mg/100 g). Therefore, we can use the two tested plant extracts as stimulants for okra plant growth and nutritional enhancers.

Keywords: *Azadirachta indica*, Okra, *Petiveria alliacea*, phytochemical compounds, proximate contents.

Introduction

Okra (*Abelmoschus esculentus* (L.) Moench) belongs to the family Malvaceae, and it is believed to have originated from Ethiopia in Africa [SATHISH & ESWAR, 2013]. It is now widely cultivated throughout the tropics and sub-tropics, as well as in the warmer parts of the temperate regions of the world [DURAZZO & al. 2019; ISLAM & al. 2019]. It is a nutritious vegetable (lady's finger) that is an important source of carbohydrate, protein, vitamins A, B, and C, calcium, potassium, dietary fibers, and minerals, and hence plays a vital role in the human diet [RASHWAN, 2011; GEMEDE & al. 2015; DANTAS, 2021]. Young, immature fruits can be consumed in many different forms, i.e., raw, steamed, boiled, or fried [FARINDE & al. 2007; WANKHADE & al. 2013].

Numerous phytophagous insects, diseases, and mites can attack okra during its development, leading to declines in yield quality and quantity [EKOJA, 2012; KUMAR & al. 2014]. The common strategy being employed by farmers for insect pest management is the use of synthetic insecticides; this can be attributed to their quick response in the control of field insect pests [ALAO & al. 2011; MUSA & al. 2013; ALAO & al. 2018]. Researchers have shown that most insects develop resistance to synthetic insecticides after prolonged use [SHABANA & al. 2017]. Not only that, the availability of most of these chemicals is a critical need, and

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environmental hazards resulting from their use are serious concerns [SANDE & al. 2011; BABARINDE & al. 2018]. The literature also states that the negative effects of insecticides on non-target organisms pose a serious threat to organism biodiversity [SANDE & al. 2011]. Most of these synthetic chemicals cause chronic ailments in humans as a result of exposure to or consumption of pesticide-contaminated crops [DAMALAS & KOUTROUBAS, 2015; KUMARI & al. 2014]. Insecticide residue in agricultural products, particularly vegetables, is a growing concern for producers, traders, and consumers in many parts of the world. There is pressure to minimize the use of synthetic insecticides. One of the efforts is to develop botanical insecticides as a novel alternative. People have described botanical insecticides as environmentally friendly and less toxic to humans [ISMAN, 2014; SHABANA & al. 2017]. Most botanical insecticides are less toxic, more quickly biodegradable, and do not damage the soil, water supply, or wildlife [SOKOVIĆ & al. 2010]. Many studies have examined the nutritional value of okra in the past [OGUNGBENLE & OMAEJALILE, 2010; GEMEDE & al. 2016; ROMDHANE & al. 2020], but few have examined how the addition of natural and man-made insecticides alters the nutritional value.

The Neem tree, also called the Indian lilac [SCHMUTTERER, 1990], is a fast-growing, evergreen plant that has amazing antifeedant properties. It can stop insects from wanting to eat at concentrations as low as 1 part per million [ISMAN & al. 1991]. Literature has it that the neem tree has more than 200 allelochemicals with different notable insecticidal properties [KOUL & WAHAB, 2004]. Further, the functional ingredients of neem exhibit therapeutic significance, as neem oil, bark, leaves, and their purified biochemicals are documented to have anticancer [PAUL & al. 2011] and antimicrobial [RAUT & al. 2014] properties. *Petiveria alliacea* (Phytolaccaceae) is an herbaceous plant with a typical height of about 1 m. This plant has inhibitory effects against *Trypanosoma cruzi*, insecticidal activity, acaricide activity, and antimicrobial activity against some strains [BENEVIDES & al. 2001; RUFFA & al. 2002; DUARTE & LOPES, 2005; WEBSTER & al. 2008; GUEDES & al. 2009].

There is no atom of doubt that the management practices of cultivating crops on the field might have impacts on the morphological and biochemical contents of the target crops. According to literature, applied synthetic insecticides and selected plant extracts influence watermelon's fatty acid compounds [ALAO & al. 2018]. Therefore, we conducted this experiment to examine how synthetic insecticides, *A. indica*, and *P. alliacea* affect the yield parameters and nutritional contents of okra.

Material and methods

Study site

The Ladoke Akintola University of Technology (LAUTECH) Teaching and Research Farm in Ogbomoso, Oyo State, hosted the field experiment in the cropping seasons of 2019 and 2020. This region is at longitude 4˚3′E and latitude 10˚5′N. We can describe the region as humid tropical falls in the Southern Guinea Savannah of Nigeria.

Land preparation and management

After choosing the site, we ploughed and harrowed the land to eliminate the roots of existing plants and weeds from the plot. We cleared the debris and then harrowed the land to create a suitable tilt for planting. We demarcated and arranged fifteen (15) plots in a randomized complete block design. Each plot had three plant rows. The plot's size was 3 m x 3 m, with 0.5 m spacing between the block plots and 1 m spacing between the replicates. There were five treatments, namely: Cypermethrin, Nano *P. alliacea* (leaves), Nano *P. alliacea* (root), aqueous

P. alliacea (leaves), *P. alliacea* (root), and control. We replicated each of these treatments three times and planted the okra variety (NH47-4) on each plot with three planting rows. We manually weeded with a hoe at two-week intervals.

Preparation of plant extracts

For this formulation, we used the roots of *P. alliacea* and the leaves of *A. indica*, which we air-dried separately for two weeks to prevent photodecomposition of the plant's chemical active compounds. We separately crushed the dried plant parts with a mortar and pestle into a powdered form, measuring out 700 g and mixing it with inert materials such as 20 g of black soap, 80 g of salt, 100 g of sulfur, and 100 g of camphor. We put this mixture into a 10-liter container with 3000 ml of water and vigorously stirred it with a stick, allowing it to stay overnight. We used muslin cloth for filtering, and stored the collected filtrates separately in 5-liter plastic kegs for future use.

Treatment application

We measured out 1000 ml from the stock solutions and determined the 20% v/v ratio. We further diluted each of the botanical insecticides with 800 ml of water, and separately mixed 1 ml of the two tested synthetic insecticides (cypermethrin) with 1000 ml of water. Three weeks after planting, we started applying the treatments early in the morning to prevent photodecomposition of the extracts, using a hand-held sprayer to prevent drifting. We applied the foliar treatment at 7-day intervals and conducted three weekly observations.

Analysis of proximate contents of harvested leaves

Sample preparation

We sorted the okra fruits, washed them, trimmed off the stumps with a stainless steel knife, and then cut the consumable parts longitudinally into portions of equal size. The samples were air-dried at room temperature. We ground the dried material into a fine powder, packed it into airtight polyethylene plastic bottles, and stored it in the desiccator until we needed it for analysis. We analyzed the dry samples for their proximate composition and minerals (Ca, Mg, Fe, and K). Determinations were carried out in duplicate. We express the mineral contents as mg per one hundred (100) g of dry weight (mg/100 g DW), while we express the proximate contents as a percentage. The Crop and Environmental Protection Laboratory at Ladoke Akintola University of Technology, Ogbomoso conducted this analysis.

Proximate analysis

We determined moisture, ash, crude fat, and crude fiber using the official methods of the association of official analytical chemists (AOAC, 2000), and used the micro-kjeldahl method to determine nitrogen. We then multiplied the nitrogen percentage by 6.25 to convert it to crude protein. Carbohydrate was determined by difference. The result was expressed in percentages.

Mineral analysis

The minerals in the harvested *Abelmoschus* leaves were analyzed from the solution obtained when 2.0 g of the samples were digested with concentrated nitric acid and concentrated perchloric acid in ratios of 5:3. The mixtures were placed in a water bath for three hours at 80 °C. The resultant solution was cooled, filtered into a 100-ml standard flask, and made to mark with distilled water [ASAOLU, 1995]. An atomic absorption sphectrophotometer (Buck Scientific Model 200A) was used. The result was calculated in mg/100 g.

Data collection and analysis

Data were collected on plant height, number of leaves, leaf area, and yield, which were calculated by weighing the harvested leaves and converted to t/ha. Data collected were analyzed using Analysis of Variance (AVONA), and significant means were separated with a Duncan multiple range test at a 5% probability level.

Results

Effects of insecticides on yield parameters

Table 1 revealed that insecticides had an effect on the yield parameters. Although cypermethrin-treated plants had the highest plant height (36.5 cm), they had the same significant height as plants treated with *P. alliacea* extracts. In the plots treated with *A. indica* extracts, the plant height was comparable to that of dichlorvos (35.4 cm). The untreated plants had the lowest plant height (33.4 cm). Plant extracts and synthetic insecticide-treated plants did not significantly differ in fruit yield, while untreated plants produced the least amount (452.4 kg/ha).

Table 1. Effects of insecticides on yield parameters

Insecticides	Yield Parameters		
	Leaf Area (cm)	Plant Height (cm)	Yield
Cypermethrin	90.5^{a}	36.5^{a}	581.6^{a}
Dichlorvos	80.3^{a}	35.4^{ab}	576.3^{a}
Petiveria alliacea	90.2^{a}	36.5^{a}	582.8^{a}
Azadirachta indica	83.9^{a}	35.8^{ab}	598.1^{a}
Control	83.6^{a}	33.4^{b}	452.4^{b}

*Means with the same superscripts are not significantly the same at 5% probability

Effects of insecticides on proximate contents of okra

The results presented in Table 2 show the effects of insecticides on harvested okra's proximate contents. Harvested okra fruits from plants treated with *A. indica* had the highest protein content (21.9%), while plants treated with *P. alliacea* extracts had the same protein content (20.12%) as untreated harvested okra fruits. The lowest protein content (20.13%) was discovered in the okra plants treated with Cypermethrin. The fiber contents (7.1%) observed from okra fruits treated with Cypermethrin were significantly the same as those of harvested fruits from untreated okra fruits. The harvested okra fruits from plants treated with *A. indica* extracts had higher fiber contents (5.3%), compared to the okra fruits from plants treated with *P. alliacea* (5.2%).

The level of moisture contents in harvested okra fruits from okra plants treated with dichlorvos had the significantly highest moisture contents (12.1%), while okra fruits from untreated plants had the least moisture contents (7.1%). Between the two tested plant extracts, harvested okra fruits from plants treated with *A. indica* extracts had lower moisture contents (9.15%) than the okra fruits from plants treated with *P. alliacea*. The highest carbohydrate content (63.9%) was discovered in the okra fruits from plants treated with dichlorvos, followed by the harvested okra fruits treated with *P. alliacea* extracts. Meanwhile, okra fruits from untreated plants had the fewest carbohydrate contents (61.1%).

The highest fat content (5.30%) was discovered in okra fruits harvested from plants treated with *P. alliacea* extracts, followed by okra fruits from plants sprayed with *A. indica* extracts (4.0%). Both tested synthetic insecticides decreased the fat contents of the harvested okra fruits when compared with the fat contents observed in the harvested okra fruits from untreated okra plants.

Okra fruits from plants treated with dichlorvos had the highest ash contents (8.0%), whereas the least ash contents (6.40%) were detected in the okra fruits treated with *P. alliacea* extracts. Harvested okra fruits from plants treated with *A. indica* extracts had higher ash contents (7.50%) than those from plants treated with cypermethrin.

Table 2. Effects of insecticides on proximate contents of okra

Insecticides	Proximate contents (%)					
	Proteins	**Fiber**	**Moisture**	**Carbohydrate**	**Fat**	**Ash**
Cypermethrin	20.12^{d}	7.1^{a}	8.20^{d}	61.58^{c}	3.06^{c}	7.06^{d}
Dichlorvos	20.14^{b}	5.2^{c}	12.1^{a}	63.86^{a}	2.80^{d}	8.00^{a}
Petiveria alliacea	20.13^{c}	5.2^{c}	9.15^{c}	62.97^{b}	5.30^{a}	6.40^{e}
Azadirachta indica	21.87^{a}	5.3^{b}	10.1^{b}	61.33^{d}	4.00^{b}	7.50^{c}
Control	20.13^{c}	7.1^{a}	7.4^{e}	61.07^{e}	4.10^{b}	7.60^{b}

*Means with the same superscripts are not significantly the same at 5% probability

Effects of insecticides on phytochemical contents of okra

Table 3 shows the effects of insecticides on the phytochemical contents of the harvested okra fruits. The amount of flavonoids found in okra fruits that came from plants that had been treated with the two tested plant extracts was significantly higher than that found in okra fruits that came from plants that had been treated with cypermethrin. The same was true for okra fruits that came from plants that had not been treated. However, plants treated with dichlorvos had the highest flavonoid content (1.80%).

The harvested okra fruits from plants treated with *P. alliacea* had the highest alkaloid content, followed by those from plants treated with cypermethrin. The tested plant extracts statistically improved the alkaloid contents of harvested okra fruits when compared with harvested fruits from untreated plants.

Plants treated with *P. alliacea* extracts and untreated plants produced okra fruits with the highest saponin contents (1.51%). On the other hand, more saponin (1.44%) was found in okra fruits that had been grown on plants treated with *A. indica* than in okra fruits that had been grown on plants treated with the two synthetic insecticides that were tested.

The amount of antioxidants in okra fruits treated with the two plant extracts was significantly higher than that in cypermethrin-treated plants. However, the tested plant extracts did not improve the antioxidant contents when compared with the quantity of antioxidants in the harvested okra fruits from untreated plants.

Table 3. Effects of insecticides on phytochemical contents of okra

Insecticides	Phytochemical content (mg/100 g)			
	Flavonoid	**Alkaloid**	**Saponin**	**Antioxidant**
Cypermethrin	1.22^{a}	1.35^{b}	1.15^{c}	2.03^{e}
Dichlorvos	1.80^{a}	1.20^{c}	1.09^{d}	2.37^{a}
Petiveria alliacea	1.67^{b}	2.00^{a}	1.51^{a}	2.14^{d}
Azadirachta indica	1.45^{c}	1.11^{d}	1.44^{b}	2.22^{c}
Control	1.26^{d}	1.08^{e}	1.51^{a}	2.26^{b}

*Means with the same superscripts are not significantly the same at 5% probability

Effects of insecticides on mineral contents of okra

As shown in Table 4, the level of potassium content in the harvested okra fruits was higher than other determined minerals. Harvested okra fruits from plants treated with *A. indica* extracts had the highest potassium contents (223.0 mg/100 g), followed by the okra fruits from plants treated with *P. alliacea* extracts, while the least potassium content (179.0 mg/100 g) was detected in the okra fruits from plants treated with dichlorvos.

The calcium content observed in the okra fruits from the plants treated with plant extracts was comparably low to that of harvested fruits from untreated plants. The two synthetic insecticides performed better than the plant extracts with respect to calcium contents.

The harvested okra fruits from plants treated with dichlorvos and *A. indica* extracts had the highest sodium content (1.79 and 1.87%, respectively). The sodium content in the okra fruits from plants treated with *P. alliacea* extracts was significantly the same as that of untreated okra fruits.

Table 4. Effects of insecticides on mineral contents of okra

Insecticides	Mineral contents (mg/100 g)		
	Potassium (K)	Calcium (Ca)	Sodium (Na)
Cypermethrin	216.0^{c}	12.90^{c}	1.47^{b}
Dichlorvors	179.0^{e}	13.31^{b}	1.79^{a}
Petiveria alliacea	218.0^{b}	12.41^{d}	1.06^{c}
Azadirachta indica	223.0^{a}	9.21^{e}	1.87^{a}
Control	186.0^{d}	13.60^{a}	1.08^{c}

*Means with the same superscripts are not significantly the same at 5% probability

Discussion

Researchers have described the use of plant extracts as insecticides as an environmentally friendly approach to protecting crops and our environment from hazardous synthetic pesticides. Therefore, we conducted this experiment to evaluate two plant extracts (*A. indica* and *P. alliacea*) as growth stimulants for okra plants, and their effects on the proximate, phytochemical, and mineral contents.

The data clearly demonstrates that the two plant extracts had a positive impact on the height of the okra plant, compared to the untreated plants, which had the lowest height. This implies that we can use *P. alliacea* and *A. indica* as growth stimulants. This concurs with earlier research by MOYIN-JESU (2010), who reported that neem extracts sprayed on maize plants had higher maize biomass and plant height. Also, application of neem leaf extracts resulted in high vegetative growth of Cavendish bananas [YI & al. 2021]. However, the applied insecticides did not significantly affect the leaf area of okra plants, while the okra yield from plants treated with synthetic insecticides (cypermethrin and dichlorvos) was comparable to that of the tested plant extracts. This revealed that the plant extracts induced fruiting as synthetic insecticides.

The observed protein content in the harvested okra fruits ranged from 20.13 to 20.87%, which was slightly higher than the reported protein by ADETUYI & al. (2011). This is an indication that the tested plant extracts improved the protein contents of the harvested fruits. However, okra plant-treated *A. indica* extracts had the highest protein contents (21.9%), which suggests that plant extracts improved the protein contents of okra fruits. The applied treatments significantly contribute to the okra pods' increased protein content, surpassing the standard plant source protein requirement of 12% [EFFIONG & al. 2009; ALI, 2010].

ADETUYI & al. (2011) reported that the fiber content of harvested okra varieties ranged from 10.8 to 11.7%. This was higher than the observed fiber contents of the harvested okra. Additionally, the plants treated with plant extracts did not exhibit any negative effects on the observed leaf area, which was comparable to the results of plants treated with synthetic insecticides. Meanwhile, plant extract-treated plants had lower fiber contents compared to cypermethrin-treated and untreated plants. However, the obtained fiber content is relatively higher than that of leafy vegetables, particularly *Amarathus hybridus* [ADETUYI & al. 2011]. This highlights the fact that consuming okra fruits treated with *P. alliacea* and *A. indica* extracts will increase digestibility and absorption processes in the large intestine, thereby preventing constipation [OGUNGBENLE & OMOSOLA, 2015].

The fat contents of harvested okra fruits ranged from 2.80 to 5.30%. Nevertheless, okra plants treated with *P. alliacea* extracts had the highest fat contents, followed by *A. indica* extracts. This shows that the plant extracts significantly improved the fat contents of the harvested okra fruits. The observed ash content from harvested okra fruits, which ranged from 6.40 to 8%, was comparable to the reported ash contents by EKWUMEMGBO & al. (2014). However, the tested plant extracts had higher ash contents than the two synthetic insecticides. This suggests that the two tested plant extracts can be sources for increasing the ash contents of okra fruits.

According to EKWUMEMGBO & al. (2014), the carbohydrate contents of okra fruits ranged from 37.6 to 52.3, whereas the carbohydrate content of harvested okra fruits was considerably higher. This is an indication that the applied treatments improved the carbohydrate contents of the harvested okra, but there was variation in the carbohydrate contents with respect to the treatments. For instance, okra fruits from plants treated with dichlorvos had the highest carbohydrate contents, followed by okra fruits from plants treated with *P. alliacea* extracts.

The collected data suggested that the applied insecticides had an effect on the mineral contents of the harvested okra fruits. The determination of mineral contents revealed that potassium content was the highest. Potassium contents ranged from 179.0 to 223 mg/100 g, and okra fruits harvested from plants treated with *A. indica* and *P. alliacea* extracts had higher potassium contents (223.0 and 218.0, respectively). This means that the two plant extracts made the potassium content of cultivated okra fruits higher compared to okra fruits that were picked from plants that were not treated and plants that were treated with synthetic insecticides. Researchers have reported that potassium improves iron utilization [ELINGE & al. 2012], controls hypertension, and prevents excessive potassium excretion through body fluid [ARINATHAN & al. 2003]. Meanwhile, the observed calcium content in okra fruits from plants treated with plant extracts was significantly lower than the okra fruits from plants treated with synthetic insecticides, including the untreated okra fruits. This implies that the tested plant extracts had negative effects on the calcium contents of okra fruits. The amount of sodium found in okra fruit-treated plants with *A. indica* extracts was higher than in other treated and untreated plants. The only exception was the okra fruits from dichlorvos that had the same amount of sodium. Okra fruits from plants treated with *P. alliacea* extracts had the same sodium contents as okra fruits from untreated plants. This implies that the plant extracts had no negative effects on the sodium contents of the harvested okra fruits. Literature has it that both microelements and macroelements are very essential in human nutrition, and okra is the major source due to its high mineral composition [HABTEMARIAM, 2019].

With respect to the treatments, the quantities of the phytochemical contents of the harvested okra pods varied. Harvested okra fruits from plants treated with Dirchlorvos had the highest flavonoid contents, followed by the okra fruits from plants sprayed with tested plant extracts. Plants treated with *P. alliacea* had significantly higher alkaloid contents than other applied treatments and untreated plants. However, okra fruits from plants sprayed with plant extracts had the highest saponin contents. This suggests that the plant extracts improved the phytochemical contents of the harvested okra fruits. The okra fruits from plants treated with the tested plant extracts had a higher antioxidant content than those from plants treated with cypermethrin, but a significantly lower amount than those from untreated plants. Flavonoid, alkaloid, and antioxidant contents are natural chemopreventive agents in traditional medicines for humans [LIAO & YIN, 2000].

Plants treated with *P. alliacea* and *A. indica* extracts yielded okra comparable to the two synthetic insecticides tested, while untreated plants yielded the least (452.4 kg/ha). This indicates that field management of okra plants can effectively utilize the tested plant extracts. This experiment clearly shows that the two plant extracts improved the yield parameters, as did synthetic insecticides. This concurs with earlier research work.

Conclusions

This experiment clearly demonstrated that the two tested plant extract formulations acted as growth stimulants and sources of increasing the nutritional contents of the harvested okra fruits. Synthetic insecticides were bad for the environment, and okra fruits that had been treated with cypermethrin and dichlorvos were not as healthy as okra fruits that had been treated with plant extracts. This implies that the use of tested plant extracts will serve as a means of protecting our environment from hazardous chemicals like synthetic insecticides. We should conduct further tests on other crops to determine the effectiveness of these two plant extracts as growth stimulants and nutritional enhancers.

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Research Article

INVESTIGATING THE INTERACTOME OF *ARABIDOPSIS THALIANA* MITOGEN-ACTIVATED PROTEIN KINASES: AN INTEGRATIVE APPROACH USING MULTIPLE SOURCES OF EVIDENCE

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Abstract: *Arabidopsis thaliana* mitogen-activated protein kinase (MPK) signaling network plays a role in various cellular processes. This study integrated protein-protein interaction, genetic interaction, and co-expression data from the STRING, BioGRID, and ATTED-II databases to provide a comprehensive dataset of interactions within the network. The key MPK network components from this set were identified and subjected to functional enrichment analysis, which revealed their involvement in diverse biological processes and pathways. This integrative approach, combining multiple sources of evidence, provides a comprehensive approach for understanding *Arabidopsis thaliana* MPK signaling network. The findings demonstrate the complex regulatory mechanisms that play a role in plant stress responses and development.

Keywords: gene co-expression, genetic interaction, multi-omics, protein-protein interaction, signaling network.

Introduction

Mitogen-activated protein kinase (MAPK) cascades are highly conserved signal transduction pathways that play crucial roles in regulating plant growth, development, and stress responses [MOHANTA & al. 2015; POPESCU & al. 2008; XING & FOROUD, 2021]. These signaling pathways consist of three main tiers of protein kinases: MPK kinase kinases (MAPKKKs), MPK kinases (MKKs), and MPKs, which are activated through sequential phosphorylation events in response to various extracellular and intracellular signals [LEE & al. 2008; POPESCU & al. 2008]. Traditional genetic and biochemical methods have identified MAPKKK/MKK/MPK signaling modules with overlapping roles in controlling cell division, development, hormone signaling and synthesis, and response to abiotic stress and pathogens [POPESCU & al. 2008].

With multiple members at each of the three levels of this kinase cascade, not all combinations occur. First, genetic evidence does not support the mix-and-match formation of MAPK cascades in plants; second, different kinases are assembled into distinct modules by scaffold proteins; third, other mechanisms function for the specificity of each modules or signaling pathway (e.g. spatiotemporal separation of pathways) [XING & FOROUD, 2021]. With the development of various omics analysis tools, the integration of multi-omics data has become an important component of systems biology, allowing for a comprehensive understanding of the molecular mechanisms underlying plant biology [KATAM & al. 2022; XING & FOROUD, 2021]. This integrated approach provides insights into complex biological

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processes, such as plant responses to environmental stimuli, interactions with other organisms, and the identification of potential traits for crop improvement [MEENA & al. 2017]. The integration of omics datasets has led to the discovery of different plant stress response mechanisms and has improved our understanding of plant biological processes [CRAMER & al. 2011; ZHOU & al. 2022].

To address the current research gaps and advance our understanding of MPK signaling in plants, in this study, we constructed a dataset by integrating various types of omics data, including protein-protein interactions (PPI), genetic interactions, and gene co-expression, to explore the MPK signaling network in *Arabidopsis thaliana* and to identify novel signaling pathways that may play important roles in plant stress responses.

Material and methods

Retrieval of protein-protein interaction data

The STRING Database

Protein-protein interaction data were obtained from the STRING database (version 12.0) using its application programming interface (API). STRING is an extensive resource that integrates both known and predicted protein-protein interactions, including physical interactions and functional associations. The database collects and scores evidence from various sources, such as automated text mining, experiments, computational predictions, and systematic transfers of interaction evidence across organisms. STRING assesses and transfers this information to less-well-studied organisms using hierarchical orthology [SZKLARCZYK & al. 2011, 2019, 2023].

Data collection and processing

For each of the 20 MPKs of *Arabidopsis thaliana*, we conducted a query in STRING, specifying the species using the NCBI Taxonomic ID 3702. The network type parameter was set to the ‘Functional Network’, which represents the ‘Full STRING Network’ and consists of both physical and functional associations. The minimum required score was set at a medium confidence score of 0.4. The maximum number of interactions to retrieve for each MPK was set at 500 to obtain a wide range of interactions. Following the retrieval of individual files, the data were consolidated into a single comprehensive file to simplify integration with subsequent genetic interaction and gene co-expression data. This consolidation process involved merging individual files and ensuring that the data were properly formatted and compatible for further analysis.

Retrieval of genetic interaction data

The BioGRID database

Genetic interactions for the MPKs were obtained from BioGRID (version 4.4.232) using the API. BioGRID is a database that curates protein and genetic interactions in various species, including *Arabidopsis thaliana*, based on primary experimental evidence from scientific literature. This ensures the high quality and reliability of the interactions in the database. BioGRID consists of both low-throughput studies and high-throughput datasets [OUGHTRED & al. 2018, 2020; STARK & al. 2006], and allows for a comprehensive overview of genetic interactions.

Data collection and processing

Each MPK was individually queried using BioGRID, with the 'NCBI Taxonomic ID' set to 3702 for *Arabidopsis thaliana*. The obtained data were then filtered to include only genetic interactions, as our focus was on understanding the genetic relationships between MPKs and other genes. The individual files were subsequently combined into a comprehensive file, retaining certain columns and eliminating others based on their relevance to the analysis. This process involved carefully selecting columns that contained essential information for our study and removing any irrelevant data. By doing so, we streamlined the dataset and prepared it for integration with the protein-protein interaction and gene co-expression data.

Retrieval of gene co-expression data

The ATTED-II database

To obtain gene co-expression data for *Arabidopsis thaliana* MPKs, we accessed the ATTED-II database (version 11.1) using the API. ATTED-II is a comprehensive resource that integrates RNA sequencing and microarray data from *Arabidopsis thaliana* and other plant species to predict gene functions and interactions. This resource identifies genes with similar expression profiles, suggesting functional relationships or joint roles in cellular processes [OBAYASHI & al. 2018, 2022].

The ATTED-II database utilizes principal component analysis (PCA) and ensemble calculations for sample balancing, which improves the quality of the co-expression data. Each gene pair in the database is assigned a metric called the 'Logit Score', which serves as an indicator of the strength of their co-expression relationship [OBAYASHI & al. 2018, 2022]. A higher Logit Score suggests a stronger co-expression relationship between the gene pair, implying a closer functional association. Conversely, a lower Logit Score indicates a weaker co-expression relationship, suggesting a less significant functional association.

Data collection and processing

The query parameters were set to retrieve co-expression relationships based on the "Ath-u.c3-0" platform. Additionally, we limited the number of co-expressed genes retrieved for each MPK to 200. After retrieving the co-expression data from ATTED-II, we processed the data to prepare it for integration with the protein-protein interaction and genetic interaction data. This processing step involved formatting the data, ensuring consistency in gene identifiers, and removing duplicate or irrelevant entries. By cleaning and organizing the co-expression data, we ensured its compatibility with other datasets and facilitated the subsequent integration and analysis steps.

Integration and ranking of data

Integration of data

To gain a comprehensive understanding of the potential interactors of MPKs in *Arabidopsis thaliana*, we integrated PPI data from the STRING database, genetic interaction data from the BioGRID database, and gene co-expression data from the ATTED-II database to construct a comprehensive dataset. Each of these data sources provided a specific type of evidence supporting the potential interactions between MPKs and predicted interactors. The integration process involved combining the three datasets based on common gene identifiers. By merging these datasets, we created a unified resource that captures the diverse types of interactions and relationships between MPKs and other genes in *Arabidopsis thaliana*. This integrated dataset served as the foundation for our subsequent analysis and prioritization of potential MPK interactors.

Ranking and prioritization strategy

To prioritize the relevant MPK interactions in the dataset, we used a ranking strategy that considered the presence of multiple sources of evidence supporting each interaction. This method allowed us to assign a higher degree of confidence to interactions supported by multiple sources. The ranking and prioritization strategies implemented are as follows:

Rank 1: Interactions supported by all three sources of evidence (PPIs from STRING, genetic interactions from BioGRID, and gene co-expression from ATTED-II) were ranked highest. These interactions were considered the most reliable, as they were consistently observed across different sources of evidence.

Rank 2: Interactions supported by two of the three sources of evidence were ranked lower than those supported by all three; however, they were still higher than the interactions supported by only one source of evidence.

Rank 3: Interactions supported by only a single source of evidence were ranked the lowest among all interactions. Although these interactions were considered potentially relevant, they were assigned a lower confidence level than those supported by multiple sources of evidence.

Selection of key MPK network components

After ranking the interactions, we further refined the dataset by selecting the interaction pairs with at least two sources of evidence. This criterion ensured that the selected interactions had a higher level of confidence and were supported by multiple experimental or computational approaches.

To focus on the most critical components of the MPK network, we further narrowed down the dataset to include only key interactions. This selection process involved considering factors such as the biological relevance of the interacting partners, their known functions, and their potential roles in MPK signaling pathways. By concentrating on these key components, we aimed to identify the most essential and influential interactions within the MPK network.

Following the selection of these key interactions, a literature search was conducted to determine whether they were already known or predicted. The results of this literature search were categorized into two groups: known interactions supported by experimental evidence and predicted interactions that had not been experimentally validated. This categorization provided valuable insights into the current state of knowledge regarding MPK interactions and highlighted potential novel interactions that warrant further investigation.

Fundamentals of overrepresentation analysis

For the functional genomics analysis of the selected MPK interactions, we used an overrepresentation-based enrichment approach. Overrepresentation Analysis (ORA) is a statistical method used to determine if a predefined set of genes is represented more than expected by chance within a specific category of genes, such as those belonging to a particular Gene Ontology (GO) term [ASHBURNER & al. 2000]. ORA typically involves the use of a hypergeometric test or similar statistical methods to calculate the probability that the observed overlap between the query gene set and GO category occurs by random chance [ASHBURNER & al. 2000].

Functional and pathway enrichment analysis

g:Profiler (g:GOSt) functional enrichment web server

The functional and pathway enrichment analysis for the predicted MPK interaction genes was performed using g:Profiler (version e111_eg58_p18_30541362), a bioinformatics tool specifically designed for this purpose. g:Profiler allows for the identification of biological pathways that are significantly represented in a given list of genes or proteins [KOLBERG & al. 2023].

Using g:Profiler involves several steps, starting with the input of a gene list derived from either computational or experimental data. The algorithm then determines statistically enriched pathways by comparing the input list to known biological pathways and assessing the overrepresentation of pathways in the gene list more than would be expected by chance. The results can be visualized and interpreted to gain insights into the functional relationships underlying the query genes [KOLBERG & al. 2023].

Statistical settings and data sources

The statistical domain scope was set to all annotated genes, and the significance method selected was g:SCS with a threshold of 0.05, which is the default method for multiple testing correction in g:Profiler. This helps with minimizing false-positive findings and ensuring reliable results [KOLBERG & al. 2023]. The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases were selected as the data sources. For the GO data, the GO Molecular Function (GO:MF), GO Biological Process (GO:BP), and GO Cellular Component (GO:CC) categories were selected. Additionally, electronic GO annotations were included in the enrichment analysis.

Visualization and analysis

To visually represent the distribution of enriched terms for each category, a Manhattan plot was generated using g:Profiler. The Manhattan plot was used to provide a comprehensive overview of the enrichment results, with each point representing a specific term and its corresponding adjusted p-value. The x-axis represents the functional categories (GO, KEGG, and WikiPathways (WP)), while the y-axis represents the negative logarithm of the adjusted p-value. This plot was used to assess the overall enrichment results and identify the most significant functional categories and pathways related to the MPK interactome.

Considering that all input genes were components of the MPK network, it was anticipated that several widely recognized broad enrichments would emerge. However, to gain novel insights into the potential roles and mechanisms of the MPK interactome, it was essential to identify unexpectedly significantly enriched terms, as these unanticipated enrichments might suggest previously uncharacterized functions or regulatory pathways.

To better visualize and interpret these unexpected enrichments, a binary enrichment heatmap was generated using the ggplot2 package in R [WICKHAM, 2016]. The heatmap displays the presence or absence of significantly enriched terms across different functional categories (GO terms, KEGG pathways, and WikiPathways) for each input gene. The binary representation allows for a clear and concise visualization of the enrichment patterns, facilitating the identification of shared and unique functional associations among the MPK-related genes.

Results

Integrated dataset of MPK interactions

The integration of protein-protein interaction data from STRING, genetic interaction data from BioGRID, and gene co-expression data from ATTED-II resulted in a comprehensive dataset containing potential interactors of *Arabidopsis thaliana* MPKs. The final dataset contains the Number of Sources (num_sources), Source Databases (source_db), STRING Score (string_score), ATTED Logit Score (atted_ls), and BioGRID Genetic Evidence Type (biogrid_evidence) for each interaction. For interactions where a specific field was not applicable, it was marked as "N/A". The dataset can be found in the supplementary materials [https://doi.org/10.5281/zenodo.11113250].

Selected MPK network components

The criteria for selecting the MPK network interactions for further analysis were based on the requirement of having a minimum of two sources of evidence and interactors being upstream of MPKs. These selected interactions include the following: MKK2-MPK4, MKK1-MPK1, MKK1-MPK2, MKK2-MPK2, MKK4-MPK3, MKK9-MPK3, MEKK1-MPK3, MKK1-MPK4, MKK2-MPK6, MKK5-MPK6, YDA-MPK9, MKK1-MPK11, MKK6-MPK13, and MEKK1-MPK17. Table 1 presents the selected interactions, displaying the STRING score, ATTED logit score, and BioGRID genetic evidence type for each interaction.

Table 1. Selected subset of upstream MPK interactions with multiple sources of evidence

MPK	Interactor	Number of Sources	Source	STRING Score	ATTED Logit Score	BioGRID Evidence Type
MPK4	MKK2	3	STRING, BioGRID, ATTED	0.993	4.9821	synthetic rescue
MPK1	MKK1	2	STRING, ATTED	0.693	3.344	n/a
MPK2	MKK1	2	STRING, ATTED	0.700	3.5388	n/a
MPK2	MKK2	2	STRING, ATTED	0.659	5.1195	n/a
MPK3	MKK4	2	STRING, ATTED	0.990	4.6728	n/a
MPK3	MKK9	2	STRING, ATTED	0.970	4.4093	n/a
MPK3	MEKK1	2	STRING, ATTED	0.705	3.6991	n/a
MPK4	MKK1	2	STRING, ATTED	0.996	3.8939	n/a
MPK6	MKK2	2	STRING, BioGRID	0.992	N/A	synthetic rescue
MPK6	MKK5	2	STRING, ATTED	0.991	3.8137	n/a
MPK9	YDA	2	STRING, ATTED	0.406	2.9775	n/a
MPK11	MKK1	2	STRING, ATTED	0.88	4.7415	n/a
MPK13	MKK6	2	STRING, ATTED	0.942	4.9706	n/a
MPK17	MEKK1	2	STRING, ATTED	0.459	3.0577	n/a

Experimental evidence and computational predictions

A literature search was conducted to categorize the selected interactions as either known interactions with supporting experimental evidence or computationally predicted interactions lacking experimental validation. The interactions that are known and established through experimental evidence are as follows: MKK2-MPK4, MKK2-MPK2, MKK4-MPK3, MKK9-MPK3, MKK1-MPK4, MKK2-MPK6, MKK5-MPK6, and MKK1-MPK11. These known interactions are presented in Table 2, which includes their associated functions according to the literature.

Table 2. Experimentally validated upstream interactions within the selected subset

MPK	Interactor	Functions	References
MPK4	MKK2	cold and salt stress signaling, represses cell death and immune response	KONG & al. (2012) and TEIGE & al. (2004)
MPK2	MKK2	positive regulation of red light-induced stomatal opening	LI & al. (2023)
MPK3	MKK4	Agrobacterium-triggered immunity, Agrobacterium-mediated transformation	LIU & al. (2021)
MPK3	MKK9	enhances phosphate acquisition, ethylene and camalexin biosynthesis, enhances salt stress sensitivity	LEI & al. (2014) XU & al. (2008)
MPK4	MKK1	negatively regulates immunity	KONG & al. (2012)
MPK6	MKK2	cold and salt stress signaling	TEIGE & al. (2004)
MPK6	MKK5	ABA regulation of primary root growth, stomatal response	LI & al. (2017)
MPK11	MKK1	n/a	LEE & al. (2008)

The predicted interactions, which lack experimental evidence but were identified through computational methods, are as follows: MKK1-MPK1, MKK1-MPK2, MEKK1-MPK3, YDA-MPK9, MKK6-MPK13, and MEKK1-MPK17. These computationally predicted interactions are presented in Table 3, including their associated scores from STRING and ATTED-II.

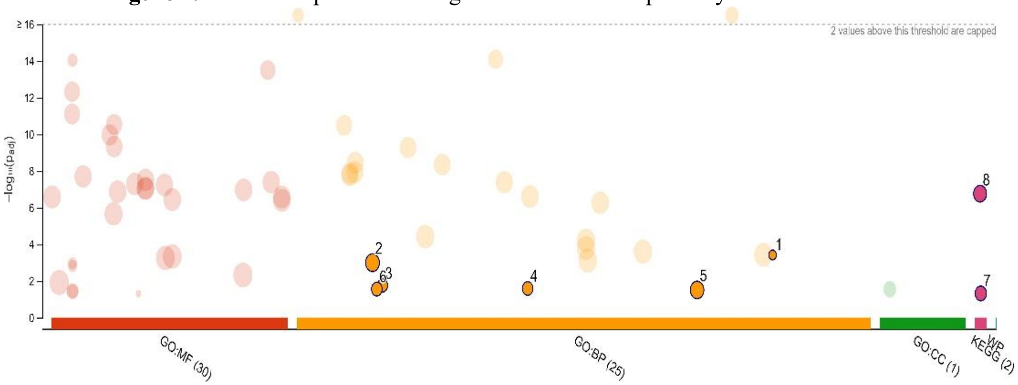
Table 3. Computationally predicted interactions within the selected subset

MPK	Interactor	Sources	STRING Score	ATTED Logit Score
MPK1	MKK1	STRING, ATTED	0.693	3.344
MPK2	MKK1	STRING, ATTED	0.700	3.5388
MPK3	MEKK1	STRING, ATTED	0.705	3.6991
MPK9	YDA	STRING, ATTED	0.406	2.9775
MPK13	MKK6	STRING, ATTED	0.942	4.9706
MPK17	MEKK1	STRING, ATTED	0.459	3.0577

Pathway enrichment analysis

The functional and pathway enrichment analysis of the predicted MPK interaction genes revealed a number of significantly enriched categories, consisting of GO terms and KEGG pathways. The Manhattan plot displayed in Figure 1 illustrates the distribution of the enrichment results.

Figure 1. Manhattan plot illustrating the functional and pathway enrichment results



Among the significantly enriched terms identified, many were anticipated based on prior knowledge. To focus the analysis on the most relevant and intriguing findings, terms that are particularly noteworthy were carefully selected. These selected terms of interest, along with their associated genes and adjusted p-values, are presented in Table 4. The dataset is saved as a .txt file and can be accessed at https://doi.org/10.5281/zenodo.11113422.

Table 4. Selected enriched terms, adjusted p-values, and the associated genes

Term Description	Adjusted P-Value	Genes
Response to L-glutamate	3.85×10^{-4}	MPK3, MEKK1
Response to wounding	1.01×10^{-3}	MPK2, MPK3, MKK1, MEKK1
Inflorescence development	1.76×10^{-2}	MPK3, YDA
Response to amino acid	2.59×10^{-2}	MPK3, MEKK1
Pollen-pistil interaction	2.78×10^{-2}	MPK3, MKK1
Post-embryonic plant morphogenesis	3.12×10^{-2}	MPK13, YDA, MKK6
Efferocytosis	4.79×10^{-2}	MPK13, MKK6

Discussion

Significance of the integrated MPK interaction dataset

The integration of multiple sources of evidence, such as protein-protein interactions, genetic interactions, and gene co-expression data, is crucial for exploring potential interactions within the *Arabidopsis thaliana* MPK signaling network. This approach enhances the reliability and accuracy of predictions by providing a comprehensive understanding of the complex nature of cellular processes [WU & al. 2010]. By integrating diverse biological and computational sources of evidence, we can mitigate the limitations of individual data sources, such as high noise levels in high-throughput PPI data and inherent variability in gene expression profiles [SRISWASDI & JENSEN, 2012; TU & al. 2006].

The integrated dataset offers several advantages over single-data-source approaches. For instance, gene expression data can refine the topology of PPI networks by removing less relevant interactions, thereby simplifying the interactome for improved biological coherence [SRISWASDI & JENSEN, 2012]. Additionally, the condition-specific nature of PPIs suggests that integrating data on transcriptional regulation and gene expression can provide insights into the dynamic behavior of protein complexes under different environmental conditions [LUO & al. 2010]. The integration of multiple sources of evidence compensates for the weaknesses of individual data types and provides a more robust framework for the prediction and analysis of PPIs and genetic interactions, leading to improved prediction coverage and accuracy [LI & al. 2012; WU & al. 2010]. There are several potential applications of this dataset. By providing a comprehensive view of the *Arabidopsis thaliana* MPK signaling network, this dataset can help address current research gaps, such as the characterization of uncharacterized MPKs and understanding of MAPKKK gene functions in plant stress responses. The integrated dataset can serve as a valuable resource for researchers investigating the roles of MPKs in various cellular processes and can guide future experimental studies aimed at validating predicted interactions and uncovering novel functional relationships within the MPK signaling network.

Characteristics of the selected MPK network components

The selection of key MPK network components from the integrated dataset was based on the presence of multiple sources of evidence supporting each interaction. Interactions supported by all three sources of evidence (PPIs from STRING, genetic interactions from BioGRID, and gene co-expression from ATTED-II) were ranked highest, followed by those supported by two sources, and finally those supported by a single source. This ranking strategy allowed us to prioritize the interactions based on the strength and consistency of the evidence supporting them, focusing on the most promising and reliable interactions for further investigation and validation.

The confidence scores associated with PPIs in the STRING database served as quantitative indicators of the strength of evidence for each predicted interaction. Higher scores suggest a greater likelihood of true biological significance, allowing for the prioritization of interactions for further investigation [SZKLARCZYK & al. 2011, 2019, 2023]. Similarly, the logit score used in the ATTED-II database to measure gene co-expression provides a reliable assessment of the strength of co-expression between genes, with higher scores indicating stronger co-expression relationships [OBAYASHI & al. 2018, 2022]. The combined co-expression platform, which integrates both microarray and RNA-Seq data, has been shown to have a higher function score than individual platforms, indicating better predictive performance [OBAYASHI & al. 2018, 2022].

Genetic interaction data from the BioGRID database was limited for *Arabidopsis thaliana* MPKs, with only two interactions (MKK2-MPK4 and MKK2-MPK6) identified in a previous study [TEIGE & al. 2004]. These interactions were categorized as “Synthetic Rescue”, referring to the rescue of lethality or growth defects in a strain mutated/deleted for one gene by the mutation/deletion of another gene [OUGHTRED & al. 2018, 2020; STARK, 2006]. The limited availability of genetic interaction data for MPKs in *Arabidopsis thaliana* highlights the need for further experimental studies to uncover additional genetic relationships within the MPK signaling network.

The distribution of evidence types among the selected MPK network components has important implications for the reliability and biological relevance of the predicted interactions. Interactions supported by multiple sources of evidence are more likely to represent true functional relationships, as they have been consistently observed across different experimental and computational approaches. By focusing on these high confidence interactions, we can identify the most essential and influential components of the MPK signaling network and guide future research efforts towards understanding their roles in plant stress responses and development.

Experimentally validated interactions and biological roles

In this exploratory study, we identified several experimentally validated interactions among selected interactions in *Arabidopsis thaliana*. These interactions are supported by various experimental approaches that provide valuable insights into their functional roles and biological significance.

(1) MKK2-mediated interactions and their role in cold and salt stress signaling, immunity, and red light-induced stomatal opening

The interactions mediated by MKK2 are important for the regulation of stress signaling, immunity, and stomatal opening. Specifically, MKK2 activates MPK4, a process essential for plants to respond to cold and salt stress, as well as the negative regulation of plant immunity, which helps suppress unnecessary cell death and immune responses under normal conditions [KONG & al. 2012; TEIGE & al. 2004].

The MKK2-MPK2 interaction plays an important role in red light-induced stomatal opening, with MKK2 phosphorylating MPK2 in guard cells to trigger stomatal opening [LI & al. 2023]. Additionally, the interaction of MKK2 with MPK6 contributes to the adaptation of plants to cold and salt stress [TEIGE & al. 2004], highlighting the diverse roles of MKK2 in various physiological processes.

(2) MKK4 and MKK9-mediated interactions and their role in agrobacterium-triggered immunity, phosphate acquisition, and stress responses

The MKK4-MPK3 interaction is important for regulating plant immunity and transforming responses to Agrobacterium infection, inducing defense-responsive gene expression [LIU & al. 2021]. The MKK9-MPK3 interaction enhances phosphate acquisition by regulating the transcription of phosphate-responsive genes and activates MPK3, influencing ethylene and camalexin biosynthesis as well as salt stress responses [LEI & al. 2014; XU & al. 2008].

(3) MKK1 and MKK5-mediated interactions and their role in suppressing immunity and ABA-regulated root growth and stomatal responses

The MKK1-MPK4 interaction is a key element of the MPK signaling cascade that suppresses immune responses to prevent autoimmunity [KONG & al. 2012]. Additionally, the MKK5-MPK6 interaction, as part of the AIK1-MKK5-MPK6 cascade, plays a critical role in ABA regulation of root growth and stomatal responses, and is involved in regulating stomatal development, ethylene signaling, nitric oxide production, and hydrogen peroxide responses during plant growth and development [LI & al. 2017].

(4) Limited biological significance of the MKK1-MPK11 interaction

Although the physical interaction between MKK1 and MPK11 has been determined using yeast two-hybrid screening, the biological function of this interaction appears to be limited. *In vitro* phosphorylation assays showed very weak activity of MKK1 with MPK11 as a substrate

[LEE & al. 2008], suggesting that the functional significance of this interaction may be less prominent than that of other validated interactions.

Indications of biological roles from computational prediction

(1) MPK signaling in response to nutrient sensing

Our computational predictions have identified significant interactions within the MPK signaling pathways, particularly involving MPK3 and MEKK1 in the response to L-glutamate and amino acids. These results suggest a potential link between MPK signaling and the sensing or metabolism of these molecules. L-glutamate serves as a metabolic signaling molecule in plants, potentially activating the MEKK1-MPK3 signaling to influence nutrient sensing and signaling mechanisms compounds [FORDE & LEA, 2007; KAN & al. 2017; MCCOY & al. 2020].

(2) MPK pathways in wound response

The involvement of MPK2, MPK3, MKK1, and MEKK1 in response to wounding highlights a robust activation of MPK cascades following physical damage. This finding aligns with previous research, suggesting that MPK signaling is crucial for initiating hormonal signaling and transcriptional reprogramming during defense responses and tissue repair. [SÖZEN & al. 2020].

(3) Developmental roles of MPK cascades

The MPK signaling network appears to play an important role in various developmental processes. The association of MPK3 with YDA, and the involvement of the YDA-MPK9 module, might be related to their regulatory roles in cellular patterning and differentiation within floral tissues [ZHANG & ZHANG, 2022]. Our analysis also indicates a role for MPK3 and MKK1 in pollen-pistil interactions, which are important for successful reproduction [LI & al. 2013]. This interaction may influence pollen tube growth and guidance, highlighting the integral role of MPK signaling in facilitating communication between pollen and pistil tissues.

(4) Novel roles in plant cell death

While the direct link between plant MAPKs and efferocytosis (the process of engulfing and clearing apoptotic cells) isn't extensively studied, MAPKs are known to be involved in related processes like programmed cell death and autophagy [ZHANG & ZHANG, 2022], both of which can be linked to efferocytosis. Interestingly, in this work, the involvement of MPK13 and MKK6 in "efferocytosis" (Table 4) opens new avenues for research into the clearance of apoptotic cells, a relatively unexplored area in plant biology. This finding suggests a potential regulatory role for these kinases in managing plant cell death and survival.

Conclusions

The mitogen-activated protein kinase signaling network plays a pivotal role in regulating plant stress responses and development. Our work has employed a systematic and integrative approach to exploring the complex interactions within the *Arabidopsis thaliana* MPK network. Through the integration of protein-protein interactions, genetic interactions, and gene co-expression data, a comprehensive dataset of potential MPK interactors was constructed and analyzed. This integrative strategy enhanced the reliability of predictions by leveraging multiple lines of evidence, compensating for the limitations of individual data sources.

The functional genomics analysis of the predicted MPK interactors revealed novel potential roles for MPK cascades in nutrient sensing, wound response, developmental processes, reproductive biology, and plant cell death regulation. These findings contribute to our understanding of the intricate regulatory mechanisms underlying the MPK signaling network, and establish a foundation for future experimental validation, mutagenesis studies, and the development of strategies to enhance plant stress tolerance.

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Research Article

PREDICTION OF 3D STRUCTURE OF MITOGEN-ACTIVATED PROTEIN KINASE KINASE 2 USING ALPHAFOLD 2 AND SIMULATION OF ITS INTERACTION WITH MITOGEN-ACTIVATED PROTEIN KINASE 6 IN *ARABIDOPSIS THALIANA*

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Abstract: *Arabidopsis thaliana* mitogen-activated protein kinase (MPK or MAPK) signaling network plays significant roles in various cellular processes. The three-dimensional structure of mitogen-activated protein kinase kinase 2 (MKK2), an upstream kinase in the MAP kinase cascade, was predicted using AlphaFold 2, and protein-protein docking simulations were performed between MPK6 and MKK2. The docking analysis identified important residues mediating their interaction. This structural prediction and protein docking analysis provide a further understanding at protein structure level.

Keywords: HADDOCK, interface visualization, kinase cascade, protein docking, stress.

Introduction

A mitogen-activated protein kinase cascade consists of three main tiers of protein kinases: MPK kinase kinases (MAPKKKs), MPK kinases (MKKs), and MPKs, which are activated through sequential phosphorylation events in response to various extracellular and intracellular signals [LEE & al. 2008; POPESCU & al. 2009]. In *Arabidopsis thaliana*, there are 60 MAPKKKs, 10 MKKs and 20 MPKs, thus leaving a multitude of possibilities to form cascades, even when bearing in mind that not all combinations occur [XING & FOROUD, 2021]. Recent advances in protein structure prediction, particularly with the emergence of AlphaFold 2, have revolutionized the field of computational biology and protein research. AlphaFold 2, a deep learning-based approach, has demonstrated remarkable success in accurately predicting protein structures [PAKHRIN & al. 2021]. The accuracy of AlphaFold 2 has been reported to be close to that of experimental determination techniques, signifying a significant leap in the reliability of predicted protein structures [WANG & al. 2022]. AlphaFold 3 is a major advance over AlphaFold 2 and it broadens the scope from protein-only structures to multi-molecule complexes [ABRAMSON & al. 2024]. Ever since its publication, AlphaFold 2 began to pave the way for the convergence of structural bioinformatics and artificial intelligence, with ongoing efforts to establish standardized models for protein structure prediction [SZELOGOWSKI, 2023].

AlphaFold 2 is a highly accurate deep learning algorithm developed to predict the three-dimensional (3D) structure of proteins from their amino acid sequences [JUMPER & al. 2021; MA & al. 2022). This algorithm has demonstrated remarkable accuracy in predicting protein structures, as evidenced by its success in the 14th Community Wide Experiment on the Critical Assessment of Techniques for Protein Structure Prediction (CASP14) [TAKEI & ISHIDA, 2022]. The success of AlphaFold 2 in predicting the 3D structures of single protein

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chains has raised questions about its future role in the field of protein structure prediction [KWON & al. 2021]. One of the key advantages of AlphaFold 2 is its ability to predict the 3D structures of proteins even when their native structures are unknown. This is achieved through the use of structure-based prediction methods, such as homology modeling or the application of its deep learning capabilities [PAK & IVANKOV, 2022]. The neural network-based method of AlphaFold 2 has allowed for the prediction of 3D structures for a significant portion of the human proteome, making these predicted structures publicly available [ZWECKSTETTER, 2021]. In our study here, we used the amino acid sequence of *Arabidopsis thaliana* MKK2 as a query in ColabFold, an extension of AlphaFold 2, to generate the top five ranked structural models.

The MKK2-MPK6 interaction plays an important role in plant responses to cold and salt stress signaling TEIGE & al. (2004). In this study, we also explored the molecular basis of this interaction through *in silico* docking analysis using HADDOCK. This software enables us to model the protein-protein interface between MKK2 and MPK6, providing insight into how specific residues mediate their interaction. By understanding these molecular contacts, we aim to shed light on the structural underpinnings of the MKK2-MPK6 signaling mechanism.

Material and methods

MKK2 structure prediction and visualization by ColabFold

ColabFold is an extension of AlphaFold 3 that focuses on predicting protein complexes, offering both accuracy and speed in its predictions [CHANG & al. 2024]. By combining fast homology search with AlphaFold 2 and RoseTTAFold, ColabFold can efficiently predict large protein complexes [JUSSUPOW & KAILA, 2023]. This implementation significantly reduces computation time, making it possible to predict protein-peptide complexes within a few minutes, depending on the size of the system [CHANG & al. 2024]. There have been various applications of ColabFold such as modeling protein structures and predicting changes in protein structure associated with genetic effects on traits and disorders [EINSON & al. 2022; HARIO & al. 2024]. Additionally, ColabFold has been used to build complexes with specific peptides, demonstrating its versatility in various protein modeling tasks [SALIMINASAB & al. 2023; ZLOBIN & al. 2023].

In this study, we used the amino acid sequence of *Arabidopsis thaliana* MKK2 as a query in ColabFold, an extension of AlphaFold 2, to generate the top five ranked structural models. The top four models were subsequently selected for further analysis. For the identification of domains and functional sites, we used ScanProSite (https://prosite.expasy.org/scanprosite) and InterProScan (https://www.ebi.ac.uk/interpro/about/interproscan). We used ChimeraX for the visualization and analysis of the protein models [PETTERSEN & al. 2021]. Additionally, Root Mean Square Deviation (RMSD) values were calculated between different models to assess their structural divergence.

MPK6 structure retrieval from Protein Data Bank

The experimental 3D structure of MPK6 was originally determined through x-ray diffraction as reported by PUTARJUNAN & al. (2019) and was also retrieved from Protein Data Bank (PDB, https://www.rcsb.org).

MKK2-MPK6 docking analysis with HADDOCK

HADDOCK (High Ambiguity Driven protein-protein DOCKing) is a computational tool widely used for modeling protein-protein interactions. It specializes in utilizing experimental data such as NMR, mutagenesis, or bioinformatics predictions to drive the docking process by integrating biochemical and biophysical information. The strength of HADDOCK lies in its ability to handle ambiguous interaction restraints, allowing it to predict complexes with high accuracy even when precise details of the interaction are unknown [DOMINGUEZ & al. 2003]. This makes it particularly useful in the study of complex biological mechanisms and the design of therapeutic molecules.

Visualization and analysis

For visualization and analysis of the docking simulations, PyMOL (https://www.pymol.org) was used to examine the interaction interfaces This included a detailed investigation of bond types and interacting residues across each of the four simulations conducted.

Results and discussions

AlphaFold 2 was accessed through the ColabFold interface, where the amino acid sequence of MKK2 was inputted. This resulted in five top-ranking models, with the top four selected for further analysis. Various visualizations of these models are presented in Figure 1. The analysis of the structural models revealed significant variations among the top four MKK2 models as indicated by Root Mean Square Deviation (RMSD) values. The RMSD between the first and second models was 6.470 Å, between the first and third models was 5.264 Å, and between the first and fourth models was 5.699 Å. Comparatively, the RMSD values between the second and third models, and between the second and fourth models were higher, at 8.112 Å and 7.624 Å, respectively, while the smallest deviation was observed between the third and fourth models at 3.565 Å. In regard to the structural variance among the MKK2 models, the RMSD values suggest that although there is a notable consistency between some models, significant disparities exist, especially between models two and three.

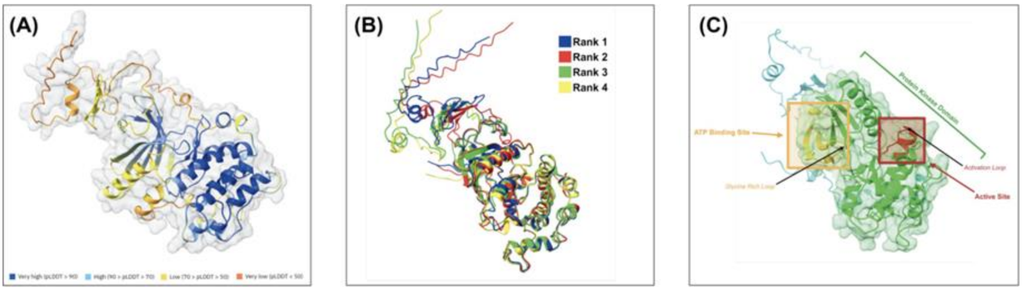

Figure 1. (A) Top-ranked MKK2 model colour-coded by predicted Local Distance Difference Test (plDDT) scores. (B) Superimposed structures of the MKK2 models, ranks 1-4. (C) Top-ranked MKK2 model with key functional sites labeled, identified using ScanProSite and InterProScan.

Functional site analysis predicted the presence of a protein kinase domain spanning residues 70 to 330, an ATP binding site across residues 76 to 99, and an active site between residues 188 to 200. These predictions highlight the critical regions potentially involved in the catalytic function and substrate interactions of the kinase. The analysis also reinforces the importance of specific residues in kinase activity and ATP binding, crucial for the functional integrity for the protein. These structural insights are foundational for subsequent analysis on where these four models are docked with MPK6. We then performed a docking study, which aimed to analyze the interaction dynamics and the molecular mechanisms underlying the MKK2-MPK6 signaling pathway.

The outcomes of the docking simulations between MKK2 and MPK6 are shown in Figure 2, highlighting the specific residues involved from both proteins. The docking simulation results are summarized in Table 1.

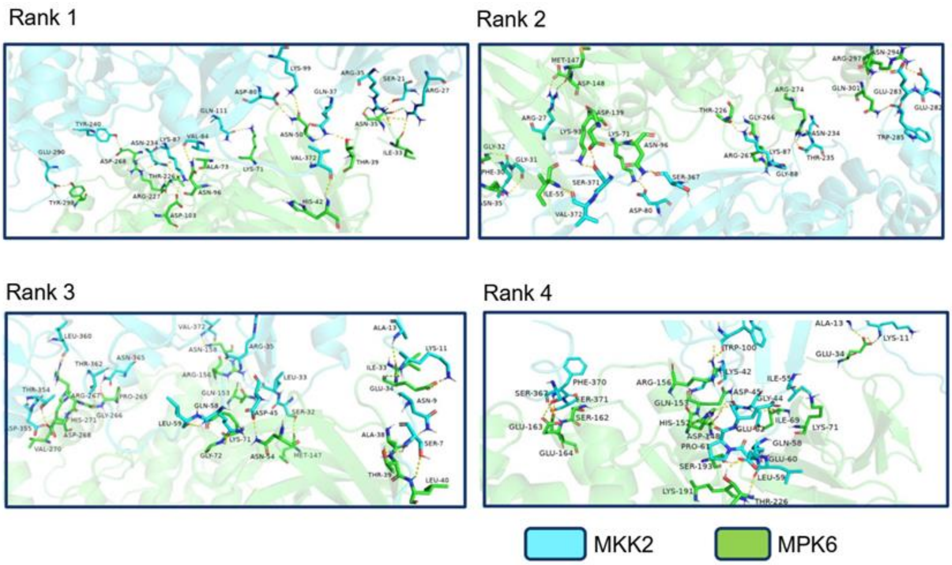

Figure 2. 3D Visualization of the MKK2-MPK6 interface for each MKK2 model being docked with MPK6, indicating the involved residues from both proteins.

Table 1. Summary of MKK2-MPK6 docking simulation results

	Binding Energy	Buried Surface Area	Desolvation Energy
#1	-475.02	3802.2	-7.71484
#2	-338.541	3796.74	-7.85969
#3	-445.666	3740.65	7.90779
#4	-318.102	4005.41	-14.3504

Discussion

In this study, we explored the protein structure of MKK2 and the molecular basis of MKK2-MPK6 interaction. In regard to the structural variance among the MKK2 models, the RMSD values suggest that although there is a notable consistency between some models, significant disparities exist, especially between models two and three.

The functional site analysis reinforces the importance of specific residues in kinase activity and ATP binding, crucial for the functional integrity of proteins. These structural insights are foundational for subsequent analysis on where these four models are docked with MPK6. This docking study should further illustrate the interaction dynamics and the molecular mechanisms underlying the MKK2-MPK6 signaling pathway.

The MKK2-MPK6 docking analysis reveals the critical residues and binding interfaces involved in this essential signaling pathway. The interaction sites identified suggest that these proteins form a stable complex that likely ensures efficient and accurate signal transmission. This reinforces the hypothesis that MKK2-MPK6 binding is a significant step in the activation of downstream stress response genes.

The structural insights gained from this analysis could be instrumental in developing strategies for improving plant resilience. Understanding the specific residues involved in the MKK2-MPK6 interaction can help in the design of targeted mutations or small molecules that could enhance or disrupt this complex. This could be particularly useful for engineering plants with increased resistance to harsh environmental conditions.

Our current work has indicated the importance of integrating computational and experimental approaches in unraveling the complexities of biological systems. Despite the modeling approach, our docking analysis is limited by the static nature of *in silico* models. *In vivo* or *in vitro* studies are necessary to confirm the interaction dynamics and the role of specific residues identified in this study. Future research should focus on validating these findings through mutagenesis or protein interaction assays, as well as expanding the analysis to other related signaling pathways to uncover broader regulatory networks.

Note: Our data are valid even when AlphaFold 3 [ABRAMSON & al. 2024] was introduced after the completion of our work due to improvement in specific areas of molecular interactions.

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Research Article

GC-MS SCREENING OF ACTIVE BIOMOLECULES FROM *ERYTHRINA VARIEGATA* L.

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Abstract: Plant-derived natural substances have been utilized to treat different health conditions in individuals. Phytochemicals are metabolites produced by plants as secondary compounds. They are naturally occurring chemical compounds in plants that are biologically active. In the current study, the phytochemical components found in butanolic extracts of the leaves, stems, and roots of *Erythrina variegata* were determined through Gas Chromatography and Mass Spectroscopy (GC-MS) analysis to evaluate their biological activity. GC-MS analysis of butanolic extracts from leaves, stems, and roots identified seven, five, and seven bioactive compounds, respectively. The key prevalent bioactive compounds found in the butanolic extracts of the leaf, stem, and root include Butane, 1,1-dibutoxy with peak areas of 54.89 %, 66.62 %, and 64.59 %, respectively, while the butanolic extract of the root shows the highest peak area for Butanoic acid, butyl ester at 10. 14%. The research reveals the existence of different phytochemicals in the butanolic extracts of the leaf, stem, and root of *E. variegata,* demonstrating antimicrobial, antibacterial, antifungal, and other therapeutic attributes.

Keywords: bioactive compound, butanolic extract., *Erythrina variegata,* GC-MS analysis, phytochemicals.

Introduction

Since the dawn of human history, plants have been used for a variety of purposes. The application of medicinal plants has included the extraction of active ingredients [SÜNTAR, 2020]. Investigating new drugs from medicinal plants includes evaluating plant extracts for novel compounds and subsequently performing biological activity tests [ATANASOV & al. 2015]. Suspected novel molecules or bioactive compounds are subsequently isolated and purified for the determination of molecular structure and additional pharmacological or toxicological investigations [SHASTHREE & al. 2009]. Parts of plants of interest, including roots, stems, bark, leaves, or fruits, undergo treatment with a suitable solvent to isolate the phytochemicals [BITWELL & al. 2023].

Gas chromatography-Mass spectrometry is a combined analytical technique utilized for examining the bioactive substances in plants employed in cosmetics, pharmaceuticals, drugs, the food sector, and applications in environmental and forensic fields [GOMATHI & al. 2015]. It is a method of separation where a mobile phase containing a mixture is compelled to move while in contact with a selectively adsorptive stationary phase, known as chromatography [HANSEN, 2015]. It holds a crucial position in the phytochemical examination of medicinal plants that have biologically active substances [SAXENA & al. 2013].

The species *Erythrina variegata* (Fabaceae) has shown that alkaloids and flavonoids are key components. Various sections of *Erythrina variegata* have been utilized in traditional

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medicine for their effects as a nervine sedative, febrifuge, anti-asthmatic, and antiepileptic [KUMAR & al. 2010]. The application of GC-MS in studying *Erythrina variegata* provides numerous benefits, including excellent sensitivity, specificity, and the ability to detect minute quantities of substances in intricate mixtures [MUTHUKRISHNAN & al. 2016].

In this research, GC-MS screening was conducted to identify bioactive molecules found in different plant parts, emphasizing their potential bioactivity.

Material and methods

Fresh leaves, stems, and roots of *Erythrina variegata* were collected and carefully rinsed under running tap water to eliminate any soil residue. They can be dried in the shade prior to being ground into a powder at ambient temperature. A total of 10 grams of powdered leaf, stem, and root were obtained, and the mixture was placed in 50 mL of butanol, maintaining a speed of 130 revolutions per minute in an orbital shaker for 48 hours. The GC-MS analysis was conducted using crude extracts from the leaf, stem, and root with butanol.

Method of analysis

The GC-MS examination of a butanolic extract from *Erythrina variegata* was performed utilizing an Agilent 8890 GC device. Using an injector with a capacity of 1 liter and a milligram per minute ratio of 1:1, helium gas was utilized as a gaseous carrier at a temperature of 75 °C. The oven temperature was adjusted to 350 °C. Typically, the GC-MS analysis duration for butanolic extracts from the leaf, stem, and root of *Erythrina variegata* is 41.5 minutes. The bioactive compounds were recognized by their retention time, the MS fragment ions produced, and the proportion of these bioactive compounds was assessed from the overall peak area. The phytochemicals were identified by matching their MS spectrum patterns with the standard mass spectra found in the National Institute of Standards and Technology (NIST) Mass Spectra Database.

Results and discussions

The GC-MS analysis of three different explants (leaf, stem and root) of *Erythrina variegata* confirmed the presence of bioactive compounds.

GC-MS analysis of leaf butanoic extract

A total of approximately six bioactive compounds were discovered in the butanolic extract of *Erythrina variegata* leaves (Table 1). They are Octadecane, 6-methyl-, Oxirane, octyl, Butane, 1,1- dibutoxy-, Neophytadiene, 3-Methylene- 7,11-dimethyl- 1-dodecene and Phytol. Of the seven compounds (Figure 1), the one with the shortest retention time of 6.130 min was Octadecane 6-methyl-($C_{19}H_{40}$) [DAS & al. 2024], which had a peak area of 10.33%, whereas the compound with the longest retention time of 30.354 min and a peak area of 12.44% was phytol ($C_{20}H_{40}$) [SUVARCHALA & al. 2022]. Phytol, a type of diterpene, is commonly utilized for its antimicrobial, antioxidant, antitumor, anticancer, antiarthritic, immunostimulatory, antidiabetic, chemo preventive, pesticidal, and diuretic effects and possesses sunscreen attributes [WILLIE & al. 2021].

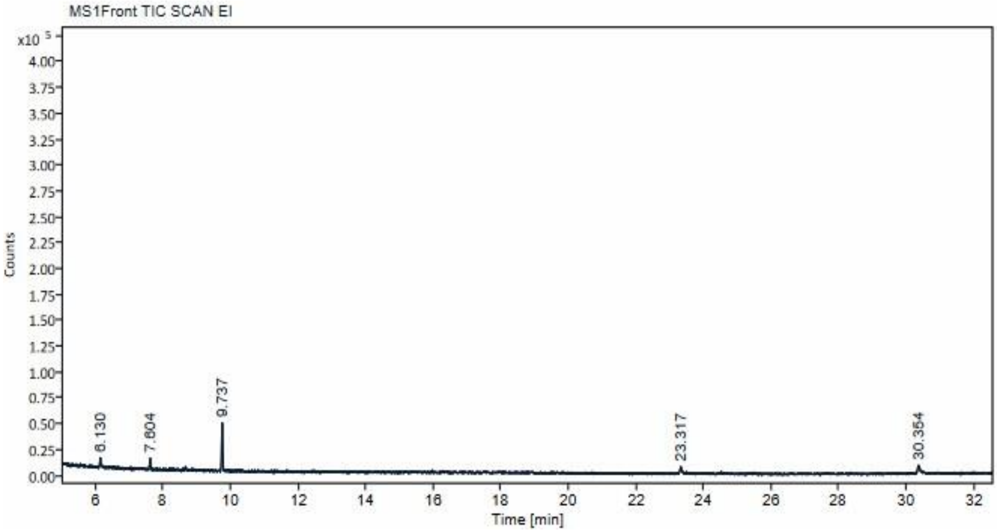

Figure 1. GC-MS Chromatogram of leaf butanolic extract of *Erythrina variegata*

Table 1. Bioactive compounds of *Erythrina variegata* leaf butanolic extract using GC-MS analysis

S. No.	Compound name	Chemical formula	Mwt (g/mol)	RT (min)	Area (%)
1.	Octadecane, 6- methyl-	$C_{19}H_{40}$	268.52	6.130	10.33
2.	Oxirane, octyl	$C_{10}H_{20}O$	156.26	6.131	10.33
3.	Butane, 1,1- dibutoxy-	$C_{12}H_{26}O_2$	202.33	9.737	54.89
4.	Neophytadiene	$C_{20}H_{38}$	278.5	23.317	8.56
5.	3-Methylene- 7,11- dimethyl- 1-dodecene	$C_{15}H_{28}$	208.3	23.316	8.44
6.	Phytol	$C_{20}H_{40}O$	296.5	30.354	12.44

GC-MS analysis of stem butanolic extract

The butanolic stem extracts of *E. variegata* disclosed a total of four phytochemical compounds determined by their molecular weight, rentention time, and peak area percentage. They are Hexanal, 2-ethyl, Butanoic acid, butyl ester, 1,1-Diisobutoxy- isobutene and Butane, 1,1- dibutoxy (Figure 2). Among them, Butane, 1,1- dibutoxy [$C_{12}H_{26}O_2$] [KHAN & JAVAID, 2020] exhibited the longest retention time at 9.738 minutes with a peak area of 66.62%, whereas Hexanal,2-ethyl [$C_8H_{18}O$] [HUANG & al. 2024] displayed the shortest retention time of 3.632 and a peak area of 10.35% (Table 2).

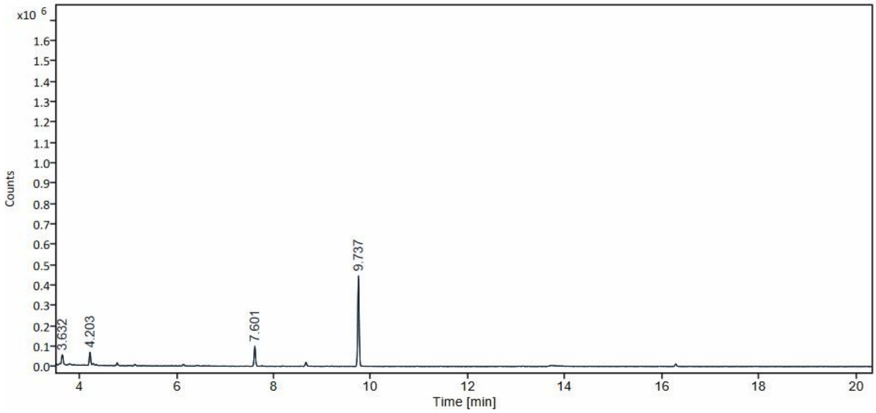

Figure 2. GC-MS Chromatogram of stem butanolic extract of *Erythrina variegata*

Table 2. Bioactive compounds *of Erythrina variegata* stem butanolic extract using GC-MS analysis

S. No.	Compound name	Chemical formula	Mwt (g/mol)	RT (min)	Area (%)
1.	Hexanal, 2- ethyl	$C_8H_{16}O$	130.23	3.632	10.35
2.	Butanoic acid, butyl ester	$C_8H_{16}O_2$	144.21	4.203	9.10
3.	1,1-Diisobutoxy- isobutene	$C_{12}H_{26}O_2$	202.38	7.601	13.93
4.	Butane, 1,1- dibutoxy	$C_{12}H_{26}O_2$	202.33	9.737	66.62

GC-MS analysis of root butanolic extract

Seven bioactive components were identified in the butanolic extracts derived from the root of the *Erythrina variegata* plant (Table 3). The initial compound, hexane, 2-ethyl ($C_8H_{18}O$), exhibited the shortest retention time of 3.632 and a peak area of 10.35%. The last compound recorded was butane, 1,1-dibutoxy ($C_{12}H_{26}O_2$), showing the longest retention time of 9.747 and a peak area of 64.59% (Figure 3).

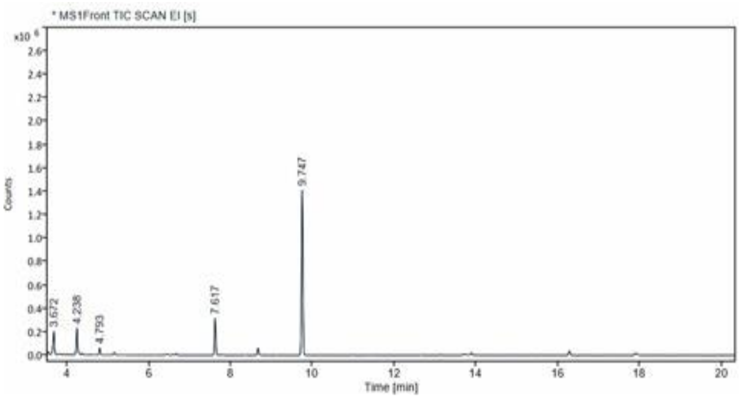

Figure 3. GC-MS Chromatogram of root butanolic extract of *Erythrina variegata*

Table 3. Bioactive compound of *Erythrina variegata* root butanolic extract using GC-MS analysis

S. No.	Compound name	Chemical formula	Mwt (g/mol)	RT (min)	Area (%)
1.	Hexanal, 2-ethyl	$C_8H_{16}O$	130.23	3.672	9.16
2.	Butanoic acid, butyl ester	$C_8H_{16}O_2$	144.21	4.203	10.14
3.	1- Hexanol, 2-ethyl	$C_{12}H_{18}O_2$	130.227	4.793	2.35
4.	2,2- Dimethylpropionic acid, decyl ester	$C_{15}H_{30}O_2$	242.22	4.794	2.35
5.	1- Butoxy-1-isobutoxy-butane	$C_{12}H_{26}O_2$	202.33	7.617	13.76
6.	1-1 Dilscobutoxy- isobutene 1-1	$C_{12}H_{26}O_2$	202.33	7.619	13.76
7.	Butane, 1-1- dibutoxy	$C_{12}H_{26}O$	202.33	9.747	64.59

Conclusions

The present study contributed to the discovery of seventeen bioactive compounds from the butanolic extracts of various plant parts of *E. variegata*. GC-MS analysis of butanolic extracts from the leaf, stem, and root was conducted to examine phytochemical compounds in *E. variegata*. Many substances demonstrate therapeutic benefits. Among the six compounds identified in leaf butanolic extract, the one that exhibited the shortest retention time was Octadecane 6-methyl-($C_{19}H_{40}$) at 6.130 min, showing a peak area of 10.33%. On the other hand, the compound exhibiting the longest retention time was phytol ($C_{20}H_{40}O$), noted at 30.354 min, showing a peak area of 12.44%, recognized for its antinociceptive, antioxidant, and anti-inflammatory effects. All these chemical substances offer potential advantages for various ailments and are valuable in the search for new drugs.

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Research Article

EXPLORING ANTIHYPERTENSIVE DRUG LEADS FROM *BLIGHIA SAPIDA* K. D. KOENIG VIA GC-MS AND IN SILICO APPROACHES

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Abstract: Globally, hypertension is a leading cause of cardiovascular diseases that account for around 17 million deaths. Despite more studies and management measures, the cause of hypertension is barely unknown, auxiliary antihypertensive medications have some drawbacks which include high prices, adverse effects, and resistivity. The little or no side effects posed by alternative medicines and patient compliance to medicinal plants raised interest in investigating *Blighia sapida* K. D. Koenig (Ackee) for its bioactive agents including proteins that could be responsible for its antihypertensive properties. Ethanol leave extract was analyzed using gas chromatography-mass spectrometry (GC-MS) analysis to detect the various bioactive compounds, two proteins that play prominent roles in hypertension were studied and retrieved for Molecular Docking using 3D crystal structures, wizard module of Schrödinger Maestro 12.8 employed to prepare the protein. The results of the docking computations were cleaned and analyzed using Excel spreadsheet software. Following receptor and ligand preparation, molecular docking computations were conducted using Glide's ligand docking plugin with extra precision docking to rigorously score ligand-protein interactions. Further graphical representations of the docking results were created using the R Studio package and GraphPad Prism V8.0. Visualization of the molecular interactions of the ligand-protein complexes was conducted. The GC-MS identified a total of 33 compounds in the ethanol extract: Benzenecarboximidothioic acid, N-phenyl-, 4-nitrophenyl ester, N-Serylserine and Palmitic Acid among others. During molecular docking, in-silico pharmacokinetics, and toxicological profiling, serylserine and pirenzepine were identified for their potential interactions with other important proteins related to hypertension including the calcium ion channel and the angiotensin II receptor (ARB). Serylserine and pirenzepine showed potential binding energy against the targeted proteins. This study could produce new antihypertensive medications that are less expensive, more widely available, and less likely to cause adverse effects, thereby meeting public health requirements, particularly in poor nations.

Keywords: *Blighia sapida*, hypertension, in-silico, molecular docking, pharmacokinetics, proteins, toxicology profiling.

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Introduction

Hypertension is a disease affecting individuals across all age groups; it is the most common cause of cardiovascular diseases all over the world and has been widely reported in Africa as a major cause of morbidity and mortality [ADELOYE & al. 2021]. According to the World Health Organization (WHO) 2016, about 17 million deaths have been recorded due to cardiovascular disease, of which 9.4 million of these deaths were caused by high blood pressure (HBP). In young adults, hypertension is mostly secondary but it is primary in adolescents and is therefore due to obesity [HASELER & SINHA, 2022]. Over the past few decades, the occurrence of hypertension has greatly increased in sub-Saharan Africa and there are expectations that the absolute value might be doubled in the year 2030 [ODILI & al. 2020].

In a recent cross-sectional survey conducted in Nigeria to assess the prevalence of hypertension among youth aged 18-35 years, studies revealed that the overall prevalence of hypertension in this age group was 15.2%, with higher rates observed among males compared to females [ABIODUN & al. 2021]. A wider coverage review ranging from the year 1968 to 2015 shows that the overall prevalence of hypertension ranges from 2.1% to 47.2% in adults and from 0.1 to 17.5% in children [SANI & al. 2024]. Hypertension and its complications constitute approximately 25% of emergency medical admissions in urban hospitals in Nigeria [ADELOYE & al. 2021].

Auxiliary drugs are extensively used in treating hypertension nationwide. Drugs such as amlodipine (Norvasc), felodipine (plendil), nifedipine (Procardia), nicardipine (Cardene), and more help to suppress the effect of high blood pressure. They control blood pressure by acting as angiotensin-converting enzyme (ACE) inhibitors, beta-blockers, and calcium channel blockers [NIAZI & al. 2023]. The adverse effects of these antihypertensive drugs are of great concern [KHAN & al. 2019] and the need therefore arises to consider herbal medicines in the management of high blood pressure due to their safety and fewer side effects.

Among the list of plants with medicinal potentials used in the management of hypertension in Nigeria is *Blighia sapida* K. D. Koenig (Ackee). Ackee is a native fruit of tropical West Africa that belongs to the family of Sapindaceae. This plant is known for its medicinal abilities in treating a wide range of ailments. All parts of this plant (bark, seed, root, fruit, capsule, leaves) can be used in treating: fever, malaria, internal haemorrhage, dysentery, burns, eyes inflammation, yellow fever, constipation, cutaneous infections, diarrhea, ulcers, yaws, intercostal pain, epilepsy and so on [RAMIREZ-SANTOS & al. 2023]. This study therefore focuses on the anti-hypertensive potential of *Blighia sapida* of leaf extract using gas chromatography-mass spectrometry and in-silico studies.

Material and methods

Plant collection and preparation

Leaves of *Blighia sapida* (Ackee) were collected from Olabisi Onabanjo University, Ago Iwoye, Ogun State and authenticated with herbarium deposit made at Forest Herbarium Institute of Nigeria with the voucher number 114066. Leaves were dried for three weeks in a cool, dry place with good ventilation. The plant material (200 g) was subjected to Soxhlet extraction using ethanol. The concentrated extracts were obtained by evaporating the solvent using a rotary evaporator.

The percentage yield of *Blighia sapida* was calculated as:

% Yield = weight of dried extract/weight of dried plant sample * 100 *where*: Weight of dried plant = 200 g, Weight of dried extract = 38.9 g, % Yield = 15.9/200 * 100, % Yield = 7.95%.

Gas Chromatography - Mass Spectrometry Analysis

The extracts were analyzed using an Agilent 8860 gas chromatograph equipped with a 5977B mass spectrometry detector (MSD) system, fitted with an Elite 5MS (5% diphenyl/95% dimethyl polysiloxane) fused capillary column (30 × 0.25μm ID × 0.25 μm df). For GCMS detection, an electron ionization system operated in electron impact mode with an ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, with an injection volume of 1μl (split ratio of 10:1). The injector temperature was maintained at 300 °C. The ion source temperature was 250 °C. The GC oven temperature was programmed from 110 °C (1 min), then ramped at 15 °C/min to 310 °C (2 min). Mass spectra were taken at 70 eV with a scanning interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 3 min.

Identification and retrieval of proteins

Two proteins that play prominent roles in hypertension were studied and retrieved for Molecular Docking. These biomolecules were retrieved in their 3D crystal structures from PDB in PDB file format. The proteins are Angiotensin II Receptor with PDB ID 4YAY and L-type Calcium Ion Channel with PDB ID 8E59.

Preparation of proteins

The biomolecules underwent preparation using the protein preparation wizard module of Schrödinger Maestro 12.8. This process included preprocessing to assign bond orders and hydrogen bonds, reconstruct missing side chains and loops, and delete water molecules beyond 4Å from the het group [OLANREWAJU & al. 2024; MADHAVI SASTRY & al. 2013]. Subsequently, the hydrogen bond network was optimized, and heavy atoms were minimized. Binding sites were mapped, and grids were generated using the Glide plugin grid generation module in Maestro Schrödinger [OLANREWAJU & al. 2024; OLUGBOGI & al. 2023]. For proteins with unknown binding sites, computational mapping was performed using the Sitemap tool before grid generation [OLANREWAJU & al. 2024; HALGREN, 2009].

Retrieval and preparation of ligand

Thirty-three phytocompounds from Ackee were retrieved from the PubChem database in SDF format after being identified from the GCSM result. These compounds were prepared using LigPrep in the Schrödinger suite, utilizing the OPLS4 forcefield module for minimization.

Molecular docking

Following receptor and ligand preparation, molecular docking computations were conducted using Glide's ligand docking plugin with extra precision docking to rigorously score ligand-protein interactions.

Binding free energy calculations

The relative binding free energy (ΔG bind) of the ligand-protein complex was calculated using the Prime MMGBSA method (OLANREWAJU & al. 2024; BORKOTOKY & al. 2016). The formula used was: $\Delta G(bind) = \Delta G\ (solv) + \Delta E\ (MM) + \Delta G\ (SA)$

Where:

- ΔG solv. Represents the difference in GBSA solvation energy of the protein-ligand complex and the sum of the solvation energies for the free proteins and ligands.
- ΔE MM is the difference in the minimized energies between the protein-ligand complex and the sum of the energies for free proteins and ligands.
- ΔG SA is the difference in surface area energies of the complex and the sum of the surface area energies for the free proteins and ligands.

Prime MMGBSA computes the energy of optimized free receptors, free ligands, and ligand-protein complexes. Additionally, the ligand strain energy was calculated by immersing the ligands in a solution generated by the VSGB suite. This comprehensive approach allows for a detailed assessment of the energetics and stability of ligand-protein interactions.

Pharmacokinetic screening

The hit ligands underwent a virtual screening procedure based on Lipinski's "rule of five," Veber's rule, Ghose's rule filters, and toxicity parameters. Physicochemical properties, including drug-likeness and toxicity, were calculated using the Protox II web server, DataWarrior program version 4.6.1 [OLANREWAJU & al. 2024; SANDER & al. 2015], and AdmetSar web server. These calculations were performed by importing the ligands' mol.sdf and canonical smile format, allowing for a comprehensive assessment of their pharmacological and safety profiles.

Data snalyses and visualization

The results of the docking computations were cleaned and analyzed using Excel spreadsheet software. Further graphical representations of the docking results were created using the R Studio package and GraphPad Prism V8.0. Visualization of the molecular interactions of the ligand-protein complexes was conducted using Discovery Studio version 2021 and PyMOL visualizer.

Results and discussion

Computer-aided drug design (CADD) employs variety of computational techniques and algorithms to streamline drug discovery and development [NIAZI & al. 2023]. A crucial aspect of CADD is molecular docking, which forecasts the binding affinity and interactions between small molecule ligands and target proteins, offering valuable insights into their therapeutic potential [BAIG & al. 2018]. Through systematic computational analyses, lead compounds from *Blighia sapida* that could serve as promising candidates for further preclinical and clinical evaluation were identified, ultimately contributing to the advancement of personalized medicine strategies for hypertensive patients. For example, used CADD methods to assess the antihypertensive properties of peptides derived from *Acheta domesticus*. Similarly, CADD was employed as a supplemental technique by AKINTUNDE & al. (2022) to validate in vitro experiments that targeted proteins associated with hypertension.

The present study endeavors to harness computational methodologies, particularly molecular docking and in silico ADMET screening, to explore the therapeutic promise of phytochemicals derived from *Blighia sapida*. An essential part of the renin-angiotensin system (RAS), which controls blood pressure by fluid balance and vasoconstriction, is the Angiotensin II receptor, mainly type 1 (AT1). Blood pressure rises when angiotensin II activates this receptor. Conventional medications that target this receptor, such as losartan and other angiotensin receptor blockers (ARBs), function by opposing AT1, which reduces blood pressure and vasoconstriction [DINGEO & al. 2023].

The activity of calcium ion channels affects cardiac output and vascular resistance, which are crucial for controlling heart rate and vascular smooth muscle tone. Calcium channel blockers (CCBs), which include verapamil and nifedipine, work by blocking these channels. This results in vasodilation and a slowed heart rate which lowers blood pressure. These medications help treat hypertension because they directly alter the vascular smooth muscle's contractility, which affects systemic vascular resistance [HARRAZ & JENSEN, 2021]. By targeting these proteins, *Blighia sapida* compounds not only counteract the immediate processes that contribute to high blood pressure but also provide insights into the larger regulatory networks that may be used to build more refined therapeutic approaches.

The docking computation results demonstrated promising therapeutic capabilities of the phytocompounds from *Blighia sapida*. Table 2 and table 3 present the docking scores and the binding free energy calculations, measured in kcal/mol. *Blighia sapida* phytocompounds demonstrated substantial inhibitory effects against the two key protein targets in hypertension.

The phytocompounds contained in *Blighia sapida* also showed minimal potential in reducing blood pressure and preventing vasoconstriction via inhibiting angiotensin II receptor. Among the phytocompounds, serylserine has the highest docking score (-8.932 kcal/mol) and the lowest binding free energy (-22.72 kcal/mol). This suggests it might have the strongest potential for binding to the AT1R. The remaining phytocompounds (palmitic acid, benzenecarboximidothioic acid, and D-allose) have lower docking scores and higher binding free energies than serylserine, suggesting weaker interactions with the AT1R.

The standard drug, losartan, has a docking score (-9.133 kcal/mol) that is slightly lower than serylserine, but its binding free energy (-66.53 kcal/mol) is significantly lower. This indicates that losartan has a much stronger overall binding affinity.

Biomedical researchers have shown a strong interest in inhibiting or blocking calcium ion channel subunits in the treatment of high blood pressure. Among the phytocompounds, pirenzepine has the highest docking score (-8.024 kcal/mol) and the lowest binding free energy (-23.38 kcal/mol), suggesting it might have the strongest potential for binding to the L-type calcium ion channel, while the remaining phytocompounds (benzenecarboximidothioic acid, 4-O-methylmannose, and phytol) have lower docking scores and higher binding free energies than pirenzepine, suggesting weaker interactions with the L-type calcium ion channel.

Medication absorption and metabolism are highly influenced by molecular weight (MW), with lower MW frequently resulting in more effective metabolism. This is critical in the treatment of hypertension, because effective medication distribution and avoidance of unnecessary tissue buildup are critical. Table 4 summarizes the key properties of the identified hit compounds from *Blighia sapida* and compares them to standard drugs using the SwissADME server.

Serylserine has a low molecular weight, good hydrogen bond donor/acceptor potential (indicated by Number of H-Bond Acceptors (HA) and Number of H-Bonds (HD)), and low Topological Polar Surface Area (TPSA). This suggests good water solubility and potentially good oral bioavailability also confirmed in Figure 12(a), Serylserine also has a low predicted P-glycoprotein (Pgp) substrate score and low reactive oxygen species (#ROS), suggesting potentially good absorption and lower risk of oxidative stress. However, it is not predicted to be blood–brain barrier (BBB) permeable. Palmitic Acid has a high iLogP value, indicating high-fat solubility and potentially lower oral bioavailability, it is predicted to be a Pgp substrate and may have lower absorption due to P-glycoprotein efflux. It also interacts with multiple CYP enzymes, potentially leading to drug interactions. Benzenecarboximidothioic acid has a high molecular weight, TPSA, and good predicted GI absorption, it has a low predicted Pgp substrate score and #ROS, but may have lower bioavailability due to its inability to cross the BBB. D-Allose similar to serylserine, has good water solubility and potentially good oral bioavailability, it has a low predicted Pgp substrate score and #ROS. The standard drug, amlodipine, has a lower docking score (-7.478 kcal/mol) but a significantly lower binding free energy (-8.932 kcal/mol). This indicates that amlodipine has a much stronger overall binding affinity.

The strategic application of ADMET profiling does not only speed up the drug development process but also increases the possibility of developing effective and safe medicines tailored to manage and reduce high blood pressure, eventually improving patient outcomes in hypertension management.

Table 1. Gas chromatography-mass spectrometry results

S/N	RT (mins)	Proposed compound	Molecular formula	Molecular weight (/mol)	Area %	Quality
1.	3.230	beta-D-Glucopyranose, 1,6-anhydro-	$C_6H_{12}O_6$	180.16	2.22	9
2.	6.824	1,3,5-Triazine-2,4(1H,3H)-dione	$C_3H_3N_3O_2$	113.08	0.71	25
3.	10.469	1-Nonadecene	$C_{19}H_{38}$	266.5	0.58	94
4.	9.919	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	$C_{11}H_{16}O_2$	180.24	0.41	96
5.	7.465	2-Methoxy-4-vinyl phenol	$C_9H_{10}O_{12}$	150.17	0.44	90
6.	12.031	3-Hexanol, 2,4-dimethyl-	$C_8H_{18}O$	130.229	7.61	38
7.	7.465	3-Methoxyacetophenone	$C_9H_{10}O_2$	150.17	0.44	80
8.	6.824	4-Fluoro-6-aminopyrimidine	$C_4H_4FN_3$	113.09	0.71	43
9.	10.154	4-Hydroxy-2-mercapto pteridine	$C_6H_4N_4OS$	180.19	0.64	43
10.	10.154	4-Methyl-2,5-dimethoxybenzaldehyde	$C_{10}H_{12}O_3$	180.2	0.64	47
11.	11.819	4-O-Methylmannose	$C_7H_{14}O_6$	194.18	39.77	53
12.	12.202	5,5,8a-Trimethyldecalin-1-one	$C_{13}H_{22}O$	194.31	1.81	43
13.	6.824	6H-Pyrido[2,3-b][1,4]benzodiazepine	$C_{19}H_{21}N_5O_2$	351.4	0.71	22
14.	10.469	9-Eicosene, (E)-	$C_{20}H_{40}$	280.5	0.58	94
15.	10.154	Benzenamine, N-(phenyl)(4-nitrophenylthio)methylene-	$C_{19}H_{14}N_2O_2S$	334.4	0.64	43
16.	4.747	Benzoic acid, methyl ester	$C_8H_80_2$	136.15	1.80	87
17.	19.555	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.6	0.78	72
18.	9.484	D-Allose	$C_6H_{12}O_6$	180.16	1.34	90
19.	10.469	E-14-Hexadecenal	$C_{16}H_{30}O$	238.41	0.58	91
20.	3.230	Glycerin	$C_3H_8O_3$	92.09	2.22	9
21.	13.484	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5	1.92	91
22.	3.230	Hydroxyethyl butyl sulfide	$C_6H_{14}OS$	134.24	2.22	9
23.	12.946	Neophytadiene	$C_{20}H_{38}$	278.5	0.61	99
24.	17.083	n-Heptyl methylphosphonofluoridate	$C_8H_{18}FO_2P$	196.2	0.43	43
25.	13.805	n-Hexadecanoic acid	$C_6H_{32}O_2$	256.42	6.72	98
26.	11.819	N-Serylserine	$C_6H_{12}N_1O_5$	192.17	39.77	50
27.	15.361	Octadecanoic acid	$C_{18}H_{36}O_{20}$	284.5	1.00	99
28.	15.184	Oleic Acid	$C_{18}H_{34}O_2$	282.5	2.20	98
29.	19.229	Oxirane, [(dodecyl oxy)methyl]-	$C_{15}H_{30}O_2$	242.4	0.55	49
30.	7.465	Phenol, 2,3,5,6-tetramethyl-	$C_{10}H_{14}O$	150.22	0.44	64
31.	14.978	Phytol	$C_{20}H_{40}O$	296.5	3.41	91
32.	11.104	Thiophene, 2-ethyltetrahydro-	$C_6H_{12}S$	116.23	9.55	35
33.	11.104	Trimethylsilyl 14-acetoxy-3,6,9,12	$C_{15}H_{30}O_8Si$	366.48	9.55	43

Table 2. Type-1 angiotensin II receptor (human)

S/N	Plant Phytocompounds	Docking Scores (kcal/mol)	Binding free Energy (kcal/mol)
1.	Serylserine	-8.932	-22.72
2.	Palmitic Acid	-7.379	-46.31
3.	Benzenecarboximidothioic acid, N-phenyl-, 4-nitrophenyl ester	-7.322	-68.99
4.	D-Allose	-6.879	-26.54
5.	Losartan	-9.133	-66.53

Table 3. L-type Calcium ion Channel

S/N	Plant Phytocompounds	Doocking Scores (kcal/mol)	Binding free Energy (kcal/mol)
1.	Pirenzepine	-8.024	-23.38
2.	Benzenecarboximidothioic acid, N-phenyl-, 4-nitrophenyl ester	-6.921	-51.07
3.	4-O-Methylmannose	-6.633	-23.71
4.	Phytol	-6.272	-55.75
5.	Amlodipine	-7.478	-8.932

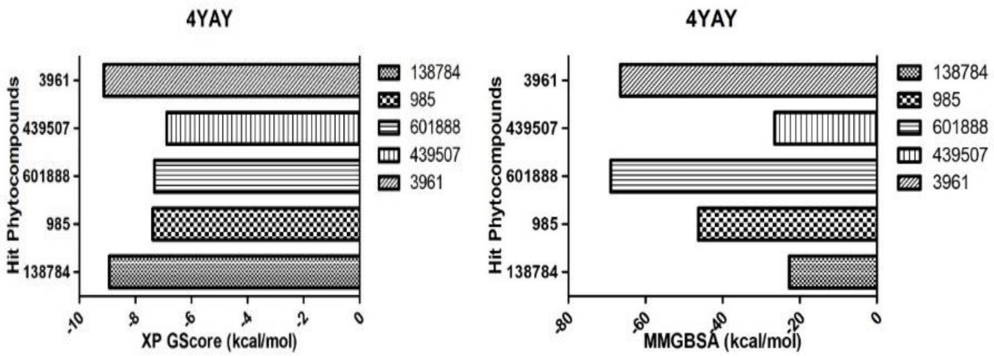

Figure 1. The differences in binding scores (MMGBSA and XP SCORE) of the hit compound from *Blighia sapida* alongside standard drug against Type-1 angiotensin II receptor (human).

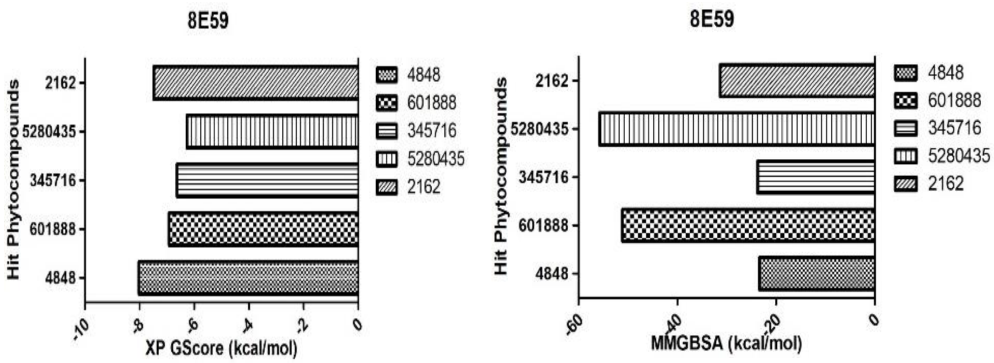

Figure 2. The differences in binding scores (MMGBSA and XP SCORE) of the hit compound from *Blighia sapida* alongside standard drug against L-type Calcium Ion Channel

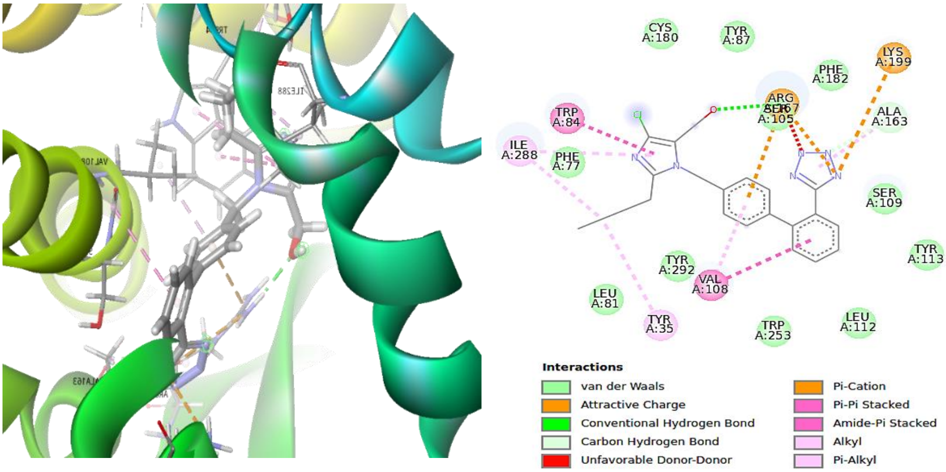

Figure 3. The 3D and 2D depictions illustrate the molecular interaction between the pharmacophoric moieties of Losartan and the key amino acid residues located at the binding site of Type-1 angiotensin II receptor (human)

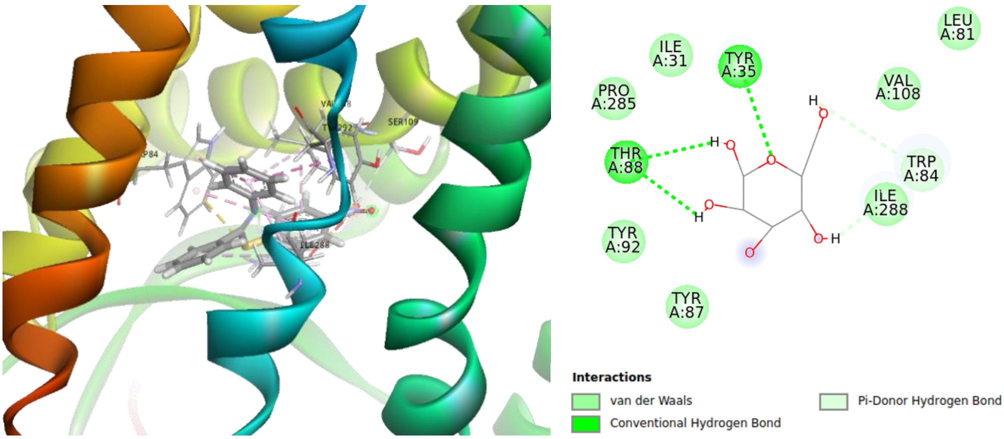

Figure 4. The 3D and 2D depictions illustrate the molecular interaction between the pharmacophoric moieties of D-Allose and the key amino acid residues located at the binding site of Type-1 angiotensin II receptor (human)

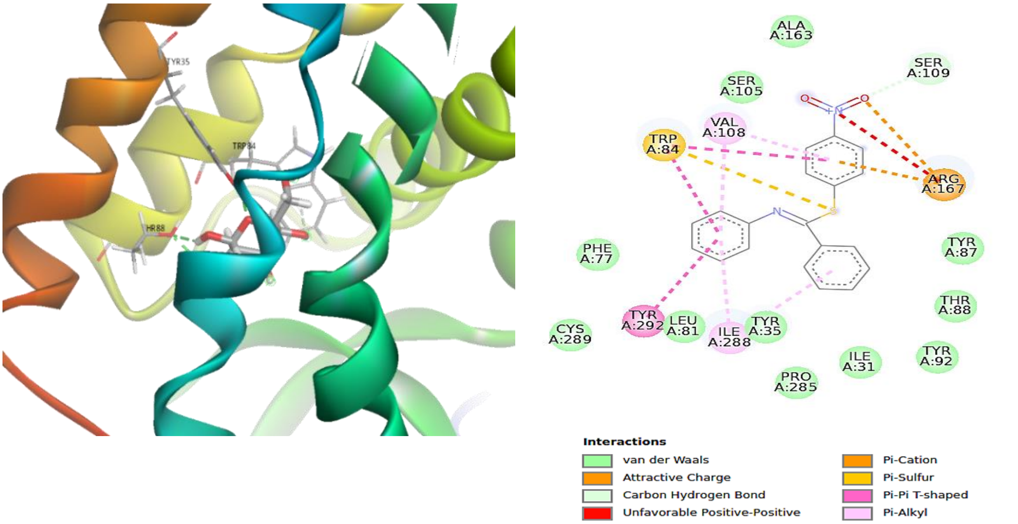

Figure 5. The 3D and 2D depictions illustrate the molecular interaction between the pharmacophoric moieties of Benzenecarboximidothioic acid, N-phenyl-, 4-nitrophenyl ester, and the key amino acid residues located at the binding site of Type-1 angiotensin II receptor (human)

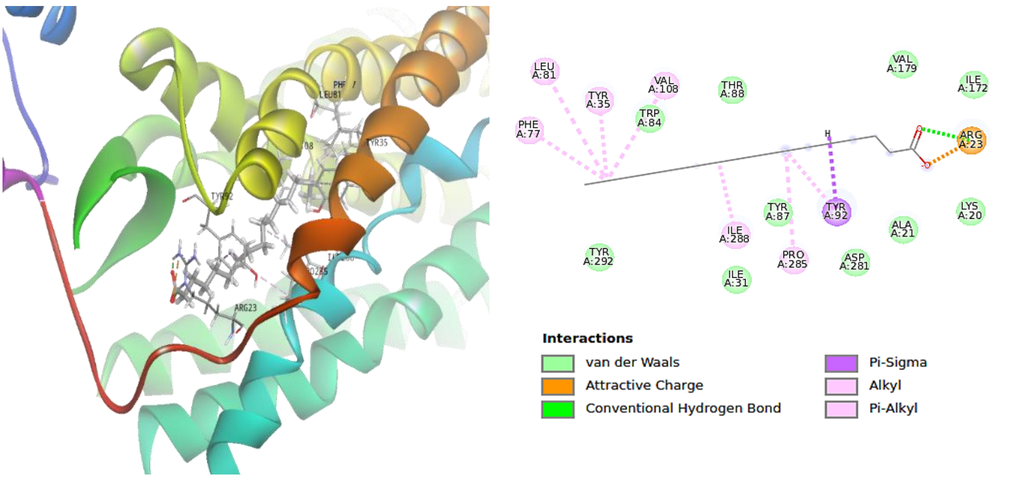

Figure 6. The 3D and 2D depictions illustrate the molecular interaction between the pharmacophoric moieties of palmitic acid and the key amino acid residues located at the binding site of Type-1 angiotensin II receptor (human)

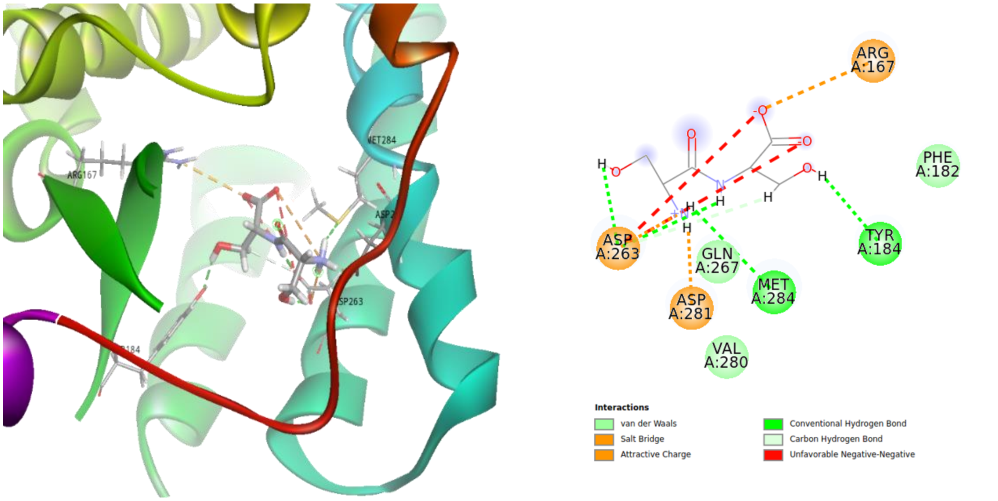

Figure 7. The 3D and 2D depictions illustrate the molecular interaction between the pharmacophoric moieties of Serylserine and the key amino acid residues located at the binding site of Type-1 angiotensin II receptor (human)

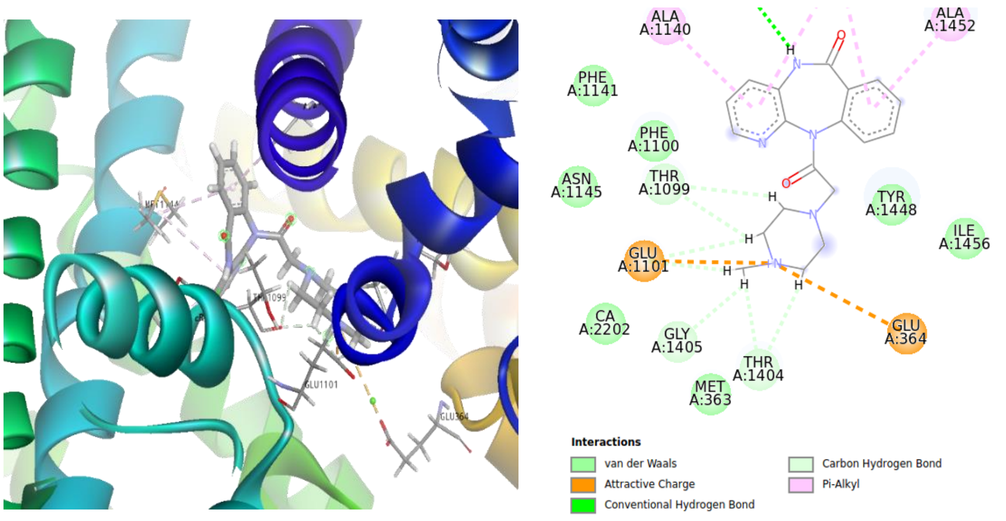

Figure 8. The 3D and 2D depictions illustrate the molecular interaction between the pharmacophoric moieties of Pirenzepine and the key amino acid residues located at the binding site of L-type Calcium Ion Channel

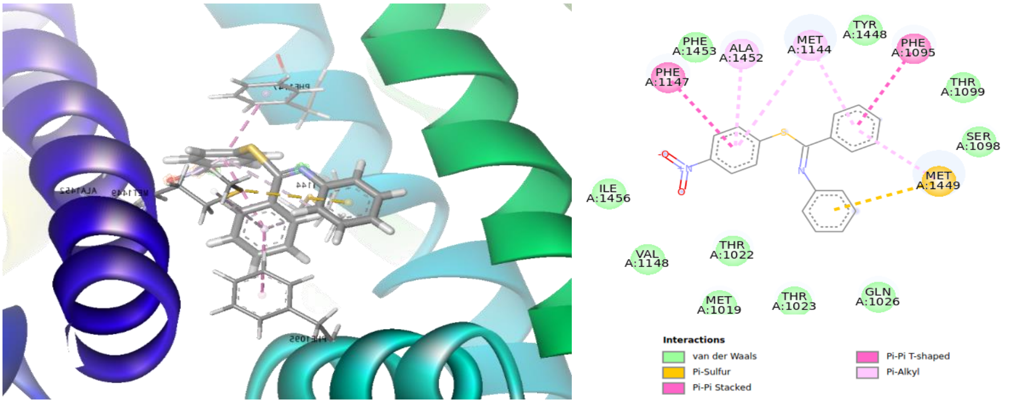

Figure 9. The 2D and 3D depictions illustrate the molecular interaction between the pharmacophoric moieties of Benzenecarboximidothioic acid, N-phenyl-, 4-nitrophenyl ester, and the key amino acid residues located at the binding site of L-type Calcium Ion Channel

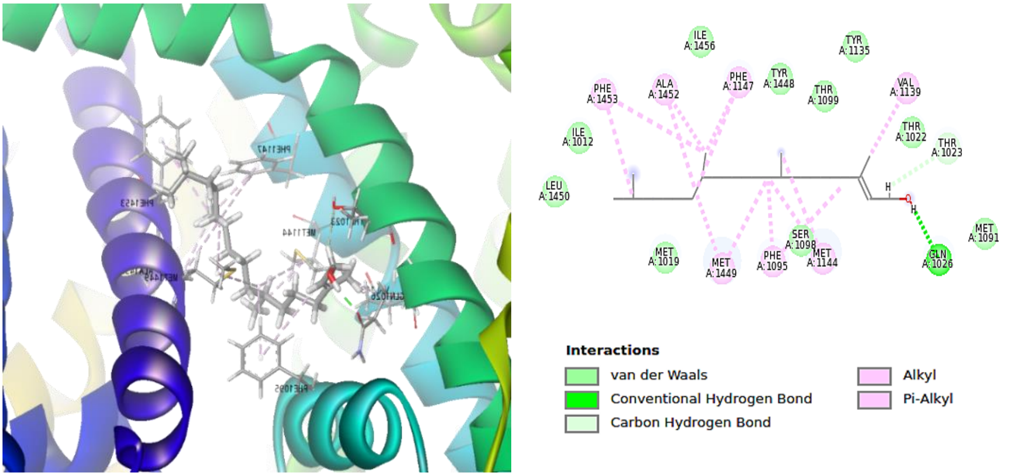

Figure 10. The 2D and 3D depictions illustrate the molecular interaction between the pharmacophoric moieties of Phytol and the key amino acid residues located at the binding site of L-type calcium ion channel

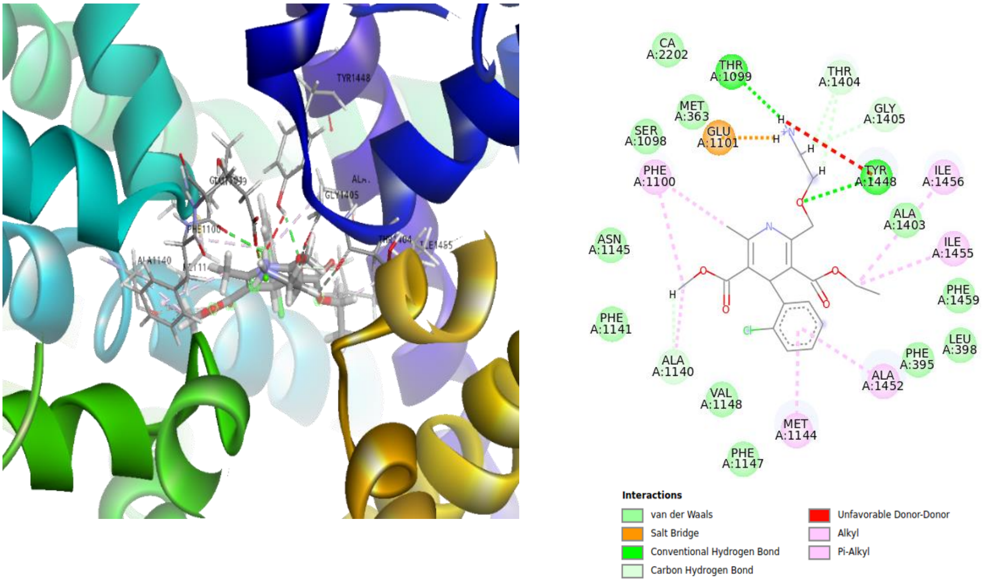

Figure 11. The 2D and 3D depictions illustrate the molecular interaction between the pharmacophoric moieties of Amlodipine and the key amino acid residues located at the binding site of L-type calcium ion channel

Table 4. Physiochemical, pharmacokinetics, and medicinal chemistry properties of the hit compounds and the standards (in bold letters) using the SwissADME server

PubChem CID	Compound Name	Mol/W	MR	iLogP	HA	HD	TPSA	GI absorption
138784	Serylserine	192.17	40.76	0.04	6	6	132.88	Low
985	Palmitic Acid	256.42	80.8	3.85	2	1	37.3	High
601888	Benzenecarboximidothioic acid, N-phenyl-, 4-nitrophenyl ester	334.39	100.2	2.84	3	0	83.48	High
439507	D-Allose	180.6	35.74	-0.7	6	5	110.38	Low
4848	Pirenzepine	351.4	108.71	2.37	5	1	74.23	High
345716	4-O-Methylmannose	194.18	40.47	0.52	6	4	99.38	Low
5280435	Phytol	296.53	98.94	4.85	1	1	20.23	Low

Table 4. (Cont'd)

PubChem CID	BBB Permeant	Pgp substrate	#ROS	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 Inhibitor	Bioavailability Score
138784	No	No	06	No	No	No	No	No	0.55
985	Yes	No	14	Yes	No	Yes	No	No	0.85
601888	No	No	05	Yes	Yes	Yes	No	No	0.55
439507	No	Yes	01	No	No	No	No	No	0.55
4848	No	Yes	03	No	No	No	No	No	0.55
345716	No	Yes	02	No	No	No	No	No	0.55
5280435	No	Yes	13	No	No	Yes	No	No	0.55

Keys: MW: Molecular weight; HA; Number of H-Bond Acceptors, HBD: Number of H-Bonds; TPSA: Topological Polar Surface Area; GI: Gastrointestinal, BBB: Blood–Brain Barrier; P-gp: P Glycoprotein; #RO5: Violations of Lipinski rule of five, MR: modified release, CYP1A2 inhibitors: a selective serotonin reuptake inhibitor used to treat obsessive-compulsive disorder, CYP3A4: is the primary cyp expressed postnatally, CYP2D6: mediates the metabolism of substrate psychotropic drugs, CYP2C9: the enzyme primarily responsible for the metabolism of warfarin's active S-enantiomer, CTP2C19: the principal enzyme involved in the hepatic metabolism of drugs.

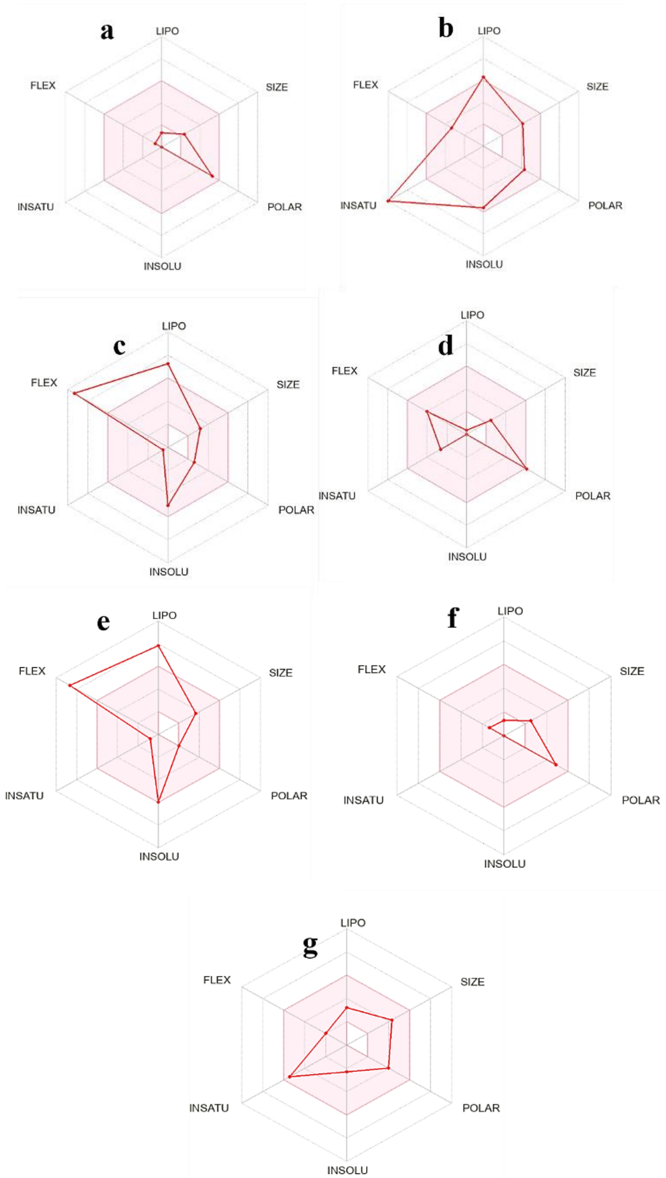

Figure 12. The bioavailability radar of the hit phytocompounds and standard drugs describing the physicochemical properties and drug-likeness of these compounds. The pink lines border within the acceptable range for each of the parameters measured by radar numbers and their corresponding compounds, where (a) represents the bioavailability radar for Serylserine (b) represents the bioavailability radar for Palmitic Acid (c) represents the bioavailability radar for Benzenecarboximidothioic acid, N-phenyl-, 4-nitrophenyl ester (d) represents the bioavailability radar for D-Allose (e) represents the bioavailability radar for Pirenzepine (f) represents the bioavailability radar for 4-O-Methylmannose (g) represents the bioavailability radar for Phytol

Table 5. The toxicity result of hit phytocompounds using Protox II server

Compounds	Hepatotoxicity	Neurotoxicity	Nephrotoxicity	Respiratory toxicity	Cardiotoxicity	Carcinogenicity
Serylserine	Inactive	Inactive	Active	Inactive	Active	Inactive
Palmitic Acid	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Benzenecarboximidothioic acid, N-phenyl-, 4-nitrophenyl ester	Active	Inactive	Inactive	Inactive	Active	Inactive
D-Allose	Inactive	Inactive	Inactive	Active	Active	Inactive
Pirenzepine	Active	Inactive	Inactive	Inactive	Active	Inactive
4-O-Methylmannose	Active	Inactive	Inactive	Inactive	Active	Inactive
Phytol	Active	Active	Inactive	Active	Inactive	Inactive

Table 6. The toxicity result of hit phytocompounds using Protox II server

Compounds	Immuno-toxicity	Mutageni-city	Cyto-toxicity	Eco-toxicity	Clinical toxicity	Nutritional toxicity
Serylserine	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Palmitic Acid	Inactive	Inactive	Inactive	Active	Inactive	Inactive
Benzenecarboximidothioic acid, N-phenyl-, 4-nitrophenyl ester	Inactive	Active	Inactive	Active	Inactive	Inactive
D-Allose	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Pirenzepine	Inactive	Active	Inactive	Active	Inactive	Inactive
4-O-Methylmannose	Inactive	Active	Inactive	Active	Inactive	Inactive
Phytol	Active	Inactive	Inactive	Inactive	Inactive	Inactive

Conclusion

Through the application of Computer-Aided Drug Design (CADD), this extensive study has shed light on the potential medicinal uses of *Blighia sapida* (Ackee) phytochemicals for the treatment of hypertension, by identifying important *Blighia sapida* phytocompounds that exhibit minimal potential against critical protein targets involved in hypertension through docking computations and pharmacokinetic screening. Among the compounds present in *Blighia sapida*, Serylserine and Pirenzepine have the strongest potential in binding with protein target involved in hypertension. The pharmacokinetic assessments further reinforced the potential of these compounds, both Serylserine and Pirenzepine exhibited excellent profiles in terms of absorption, distribution, metabolism, and excretion (ADME). Notably, D-Allose also showed exceptional qualities, especially with regard to renal clearance and bioavailability, which makes it a viable option for more research and development.

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IN MEMORIAM

Academician Constantin Toma – a life dedicated to plant knowledge

This year, on November 19, have passed ninety years since the birth of Academician Constantin Toma – the person who, for more than six decades, contributed essentially to the knowledge of the plant world and to the training of generations of researchers or teachers, who appreciated him for the unique way in which he was always ready to offer new perspectives in discovering the secrets of the natural sciences.

Academician Constantin Toma dedicated his entire life to the science of plants, and is considered the most brilliant plant morphologist and anatomist, who founded in Iași, the most famous *School of Plant Anatomy and Morphology* in Romania, where he developed, together with his disciples, relevant research in the field of plant biology and electron microscopy, with results recognized by specialists from the country and abroad.

These researches are notable through the diversity of the addressed topics, which targeted original studies on 800 species of native or cultivated plants and 50 varieties, grouped into 90 botanic families of pteridophytes, gymnosperms and angiosperms. Particular attention was paid to certain taxa presenting food, medicinal, melliferous or fodder importance.

The microscopic and ultramicroscopic researches were primarily focused on aspects of plant cytology, embryology, histochemistry, cytogenetics, plant cell and tissue cultures, taxonomical and ecological anatomy, carpology and plant sexuality biology, xylotomy and phytochemistry.

The group of researchers formed by Academician Constantin Toma unites specialists from the Faculty of Biology and the "Anastasie Fătu" Botanical Garden within "Alexandru Ioan Cuza" University of Iași, from the Institute of Biological Research in Iași, from "Stejarul" Biological Research Center in Piatra-Neamț, and from "Ion Borcea" Natural Sciences Museum Complex in Bacău.

The scientific opera of Academician Constantin Toma includes 21 scientific treatises, monographs or atlases published by prestigious publishing houses, 14 courses and manuals of practical works for students, 12 studies in volumes with scientific reviewers, and 480 scientific articles published in various journals in the country or abroad; most of those articles were published with his doctoral students and collaborators.

Professor Constantin Toma paid special attention to aspects related to the history of biology and to the evocation of the scientific contributions of his teachers and of many national and international personalities in the field of biology. In this respect, he was a model in terms of the way he honored the memory, or expressed gratitude to all those who contributed essentially to his development. He also published, as unique author or in collaboration, 120 articles presenting aspects from the history of biology, and 100 articles popularizing the natural sciences.

Academician Constantin Toma express a lot of emotion in the articles evoking his native village Gugești in Vaslui County, also in the memories from his years of study about the remarkable teachers from the "Cuza Vodă" High School in Huși, or in the memories about the conferences organized with former colleagues and students at the "Mihai Ralea" Municipal Library in the same locality.

I often had the opportunity to find out, in direct discussions, how he managed difficult events in the history of the University, of the Faculty of Biology and of the Botanical Garden.

Our *Professor* contributed to the development of the Botanical Garden on the site situated in Copou Hill. As director, between 1970 and 1973, he initiated new approaches in botanical research, while from an administrative perspective he contributed to the completion of the scientific sections and planted the horse chestnut trees at the main entrance. He was very attached by those threes, and he repeatedly told me that they should never be removed, even though in recent decades, almost all the specimens have been affected by the mining moth (*Cameraria ohridella*). He also said that those specimens remind him of the most beautiful years of his life, and the pest control should be applied to the entire city, not just on that alley in the Botanical Garden.

Academician Constantin Toma passed away on September 8, 2020, and is buried in the cemetery of Podgoria Copou Monastery located near the Botanical Garden, a historical monument founded by Prince Vasile Lupu in 1638.

His disciples remember him as the founder of a renowned School, for the clarity of his academic courses, for his model as a dedicated researcher, and especially for the nobility of his soul !

Cătălin TĂNASE
"Alexandru Ioan Cuza" University of Iaşi, Faculty of Biology
Corresponding member of the Romanian Academy

JOURNAL OF PLANT DEVELOPMENT
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	☐	**Round numbers** that require one word (e.g. up to sixteen, twenty, thirty, etc.) are written out unless they are measurements. All other numbers are in figures except at the beginning of a sentence. **Dates** are in the form of 10 Jan. 1999.
	☐	In the text, references are made using the author(s) name of a certain paper (e.g.: other authors as GÉHU (2006) mentioned that…). Footnotes should be avoided.
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	☐	References appear as follows in the text: [GÉHU, 2006] or [BOX & MANTHEY, 2006], or when there are more than two authors [AMORFINI & al. 2006]. In cases where more than one reference is cited, references are listed in chronological order and separated by semicolons e.g. [TACKHOLM, 1920; WISSEMANN, 2000; RITZ & WISSEMANN, 2003; CAISSARD & al. 2006]. Reference to work in press is only used when the paper has been accepted for publication in a named journal, otherwise the terms ‘unpubl. res.’, giving the initials and location of the person concerned is given (e.g. M. PETRESCU, University of Iasi, Romania, unpubl. res.) or ‘pers. comm.’ (e.g. M. PETRESCU, University of Iasi, Romania, pers. comm.).
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	☐	References conform to one of the following styles according to the type of publication:
	☐	**Papers in periodicals**: the name of the author(s) would be given in capital letters. The Christian name(s) would be abbreviated. Before the last but one and the last author you must insert the sign “&”. In the reference list you must mention all the authors of a certain paper. The year of a paper publication is put after the author (s). Title should be fully written. Between the year and the title we recommend to be inserted a point sign. Each periodical name is to be written in italics. A certain volume must be given in bolds. After it is placed the number of the periodical, inserted between the round brackets; next to it would be inserted the page numbers of the paper. BOSCAIU N. 2001. Ecotonul si importanta functiilor sale ecoprotective. *Ocrotirea Naturii.* **42**: 13-21. MEHREGAN I. & KADEREIT J. W. 2008. Taxonomic revision of *Cousinia* sect. *Cynaroideae* (*Asteraceae*, *Cardueae*). *Willdenowia.* **38**(2): 293-362.

	□	***Books*****:** title should be fully written. The title of a book is written in italics. After the title, is placed the name of the town, the publishing house and the page numbers. BOSCAIU N. 1971. *Flora si vegetatia Muntilor Tarcu*, *Godeanu si Cernei*. Bucuresti: Edit. Acad. Române, 494 pp. HILLIER J. & COOMBES A. 2004. *The Hillier Manual of Trees & Shrubs*. Newton Abbot, Devon, England: David & Charles, 512 pp.
	□	**Chapters in books:** author(s), year, title, pages, a point sign, followed by "In": author (s) of the book, city, publishing house, number of pages. TUTIN T. G. 1996. *Helleborus* L. pp. 249-251. In: TUTIN T. G. & al. (eds). *Flora Europaea*. 2nd ed., 1993, reprinted 1996. Vol. **1**. *Psilotaceae to Platanaceae*. Cambridge: Cambridge University Press, XLVI, 581 pp.
	□	Article only available on-line with digital objet identifier (DOI): SONKÉ B., DJUIKOUO K. M. N. & ROBBRECHT E. 2008. *Calycosiphonia pentamera* sp. nov. (afrotropical Rubiaceae) from the 'Lower Guinea' area. *Nordic Journal of Botany*. http://doi.org/10.1111/j.0107-055X.2007.00141.x
	□	Thesis: NTORE S. 2004. Contribution à la connaissance systématique du genre afrotropical *Pauridiantha* (Rubiaceae). PhD Thesis, K. U. Leuven, Leuven, Belgium.

CHECKLIST FOR *ILLUSTRATIONS*

□	Photographs, drawings, maps, graphs, charts, plates and diagrams are all treated as 'Figures' and should be numbered consecutively in accordance with their appearance in the text.
□	The mentions at the drawings, figures, pictures and tables will be placed inside the round brackets – for instance (Figure 2), (Table 2).
□	All illustrations should be clearly marked with the figure number and the author's name.
□	Figures composed of several smaller figures are treated as one figure and the subfigures are labeled with capital letters (A, B, C, D, etc.). Scientific names in captions are not followed by authors.
□	Each table has a complete caption at the top and is Table 1, Table 2, etc. according to the order in which they are first mentioned in the text.
□	All the schemes, drawings, electron micrographs etc. would be accompanied by a scale bar.
□	Line diagrams are black and white. Use of colour in line diagrams is acceptable where this enhances clarity significantly.
□	Related graphics are grouped into a single figure whenever possible. Each part of a grouped figure is labelled A, B, C, etc. and not treated as separate figures. Labels are preferably placed in the upper left or lower left corner of the picture using.
□	Colour images are used where they enhance significantly the clarity of the scientific information and must be very clear, being accompanied by the explanations.
□	The diagrams should be made in Excel; pictures, ink drawings must be saved in JPG, JPEG, or BMP format, having a good resolution.
□	If figures or tables are not imported into your text, these would be placed on separate sheets, with indications of their inserting place in the text.
□	Table text is typed using Times New Roman. Capitals are avoided within table cells (exceptions: first letter of the heading of row or column, names of taxa requiring a capital, and abbreviations e.g. pH).
□	Tables must be cell-based. Do not provide tables as graphic objects.
□	Be sparing in the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article.

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