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IMPORTANT ARTHROPOD PESTS ON ORNAMENTAL MARIGOLD (*TAGETES PATULA NANA*) IN SOUTHERN ROMANIAN GREENHOUSES

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Abstract: Marigolds (*Tagetes* spp.) are among the most popular annual plants, decorative by flowers, belonging to the *Asteraceae* family, native to North and South America. It is cultivated mainly for ornamental and medicinal purposes, since in the last period of time it has been cultivated on increasingly large areas as a trap plant in organic farming and in the polyculture system. This paper aims to identify the major arthropod fauna present on this ornamental plant, in a greenhouse from Mehedinți County, in the year 2022, the period before commercialization. The experimental field was located at a greenhouse in the Pristol area, Mehedinți County. Three varieties were used in the experiment: Delia, Flacăra and Bonita mix. The biological material was collected with the help of direct observation method and shaking method. In the year 2022, a total number of 271 specimens of insects and mites were collected from the marigold crop located in the Pristol greenhouse, of which the highest numbers of specimens, 104, were being registered with the Bonita Mix variety. Analyzing the evolution of arthropod pest species, it can be highlighted that the largest number of specimens belonged to the *Tetranychus urticae* species - 100 specimens, of which the most (41) were collected from the Bonita Mix variety and the fewest (25) from Delia.

Keywords: *arthropod, pests, Tagetes patula var. nana, greenhouse*

INTRODUCTION

Tagetes, also called "marigold", is a member of the *Asteraceae* family with over 50 cultivated and wild species (Cicevan et al., 2022), of which *Tagetes patula* (the French marigold) and *Tagetes erecta* (the African marigold) are the most cultivated garden flowers in the world (Yasheshwar et al., 2017). As one of the most important ornamental flowers, marigolds have won over other ornamental plants because they are easy to grow, adapt to different soil types and climatic conditions, and flower abundantly throughout the growing season, making these flowers a favorite with landscapers (Vasudevan et al., 1997). Due to its importance in agricultural practice for its allelopathic, antibacterial (Faizi et al., 2008) antifungal (De-Rodriguez et al., 2006), nematocidal (Franzener et al., 2007) and insecticidal (Dharmagadda et al., 2005; Mir et al., 2019) effects, marigold is used in the pharmaceutical and dermato-cosmetic industries and has recently been increasingly cultivated as a trap crop in organic farming and polyculture (Karakas & Bolukbasi, 2019).

Intensive, large-scale cultivation has caused a number of problems, leading to an increase in the numerical density of some pests, among which we mention: thrips, aphids, true bugs, leaf-mining moths or lepidopteran larvae (Anonymus, 2014). In addition, new species of insects appear to be invading marigold crops, mainly as a result of climate change combined with changes in cultivation techniques. Particularly in the context of an ever-changing pest scenario, information on the pests affecting the crop is a prerequisite for developing an appropriate pest management strategy.

Since very little information is available on the pest species associated with *Tagetes* spp., this paper aims to identify the main arthropod fauna present on this ornamental plant, in a greenhouse from Mehedinți County, in the year 2022, the period before commercialization. In order to achieve this goal, the following objectives have been set: - to describe the composition of the arthropod fauna and to analyze the distribution of this fauna during the phonological development.

MATERIALS AND METHODS

In order to establish the arthropod fauna on *Tagetes patula nana* plants, the experimental field was located in 2022 in a greenhouse in Pristol area (44°13′33″ N latitude and 22°42′33″ E longitude), Mehedinți County. The total area of the greenhouse cultivated with ornamental plants was 100 m^2, and among the flowers selected for the research was the marigold (*Tagetes patula var. nana*) - the occupied area was 20 m^2 (figure 1). The research lasted for one year, starting on 16 February 2022 and continuing until 26 April 2022 (the period of development of the plants under study, prior to their commercialisation).

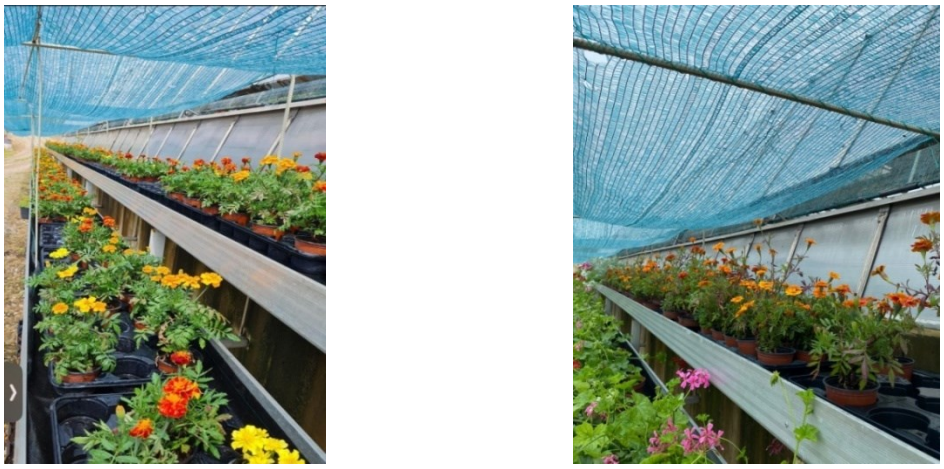

Figura 1. Aspects from the experimental greenhouse, Pristol (Mehedinți County)

To achieve the objectives of this paper, leaves and inflorescences of three varieties of *Tagetes patula nana* were sampled:

- Delia - an annual plant with a dwarf growth habit, a compact shrub, 25-35 cm in height. The flowers are dark orange, arranged in spiral inflorescences, with many flowers open at the same time on the plant;
- Flacăra - an annual, dwarfing, compact shrub, 20-30 cm in height. The flowers are yellow, under the bright orange leaves, grouped in inflorescences wrapped in a pom-pom, with a large number of flowers opening at the same time on the plant;
- Bonita mix - annual, dwarf, compact shrub, 30 cm high, one of the earliest varieties with large, round, 4-5 cm flowers throughout spring and summer.

Biological material was collected using direct observation and shaking methods. The plants were carefully selected to include all 3 varieties under study. Plants showing signs of attack by different pests were selected and examined, and information on plant parts attacked, degree of attack and type of damage, stage of development of pest causing damage, date and variety were noted.

Using the shaking method, samples were collected at intervals of ~7 from the moment of plant emergence until flowering/marketing. Samples were taken by shaking the terminal parts of the plant considered to be affected - the terminal part of the plant on a 10 cm section (leaves together with flowers) on white shaking trays of 25/35 cm size, using 3 flowers/variety.

In addition, surveys were carried out to identify the useful fauna during the entire period under study, using the entomological umbrella. Collected species were transferred to a 5 ml vial containing 70% alcohol using a finely pointed brush.

All pest species were taken to the Phytosanitary Diagnostic and Expertise Laboratory at the Faculty of Agriculture, where specimens were taxonomically identified and mounted using specific methods for specimen preservation.

Data analysis: Statistical calculations were performed with IBM-SPSS-Statistics. Descriptive statistical elements calculated were: means, standard deviations, minimum and maximum values for the studied variables.

RESULTS AND DISCUSSIONS

In Romania, there are protected areas in most of the regions where marigolds are cultivated, and their marketing takes place from April onwards. As with any crop, intensive cultivation has created certain imbalances.

Table 1. Arthropod species and number of specimens collected from Tagetes plants in Pristol, Mehedinți County

Order	Family	Species	No. of specimens / variety		
			De.	Fl.	Bo.
Trombidiformes	*Tetranychidae*	*Tetranychus urticae*	+++	+++	+++
Thysanoptera	*Thripidae*	*Thrips tabaci*	+	+	+
		Frankliniella occidentalis	++	++	+
		Frankliniella schultzei	+	-	+
		Thrips palmi	-	-	+
Hemiptera	*Aphididae*	*Myzodes persicae*	+	+	++
		Aphis gossypii	-	+	-
	Aleyrodidae	*Trialeurodes vaporariorum*	++	+	++
	Pentatomidae	*Nezara viridula*	+	+	-
		Halyomorpha halys	+	+	-
Hymenoptera	*Apidae*	*Apis mellifera*	+	+	+
Coleoptera	*Coccinellidae*	*Coccinella 7-punctata*	+	+	+
		Harmonia axyridis	+	-	-
		Hippodamia variegata	+	+	-
		Propylea quatuordecimpunctata	-	-	+
Lepidoptera	*Noctuidae*	*Spodoptera exigua* - l	+	-	+
		Helicoverpa armigera - l	+	+	+
Diptera	*Agromyzidae*	*Liriomyza trifolii* - l	+	-	+

*De. – Delia; Fl. – Flacăra; Bo. – Bonita Mix; l – larvae; + - 0- 10 specimens/ variety; ++ - 11 – 20 specimens/ variety; +++ - ≥21 specimens/ variety

Thus, in the greenhouse located in Pristol, Mehedinți County, the attack of insects and mites represented one of the most important obstacles in producing these flowers. Since the aim of this work was to identify the most important arthropods collected from the plants of *Tagetes patula nana*, the harmful and beneficial fauna collected in 2022 for the three varieties studied are presented in Table 1.

A total of 271 individuals representing 18 species from 9 families and 7 orders, namely *Trombidiformes* (1 species), *Thysanoptera* (3 species), *Hemiptera* (5), *Hymenoptera* (1 species), *Coleoptera* (4 species), *Lepidoptera* (2 species) and *Diptera* (1), were collected during the 3-month survey.

Table 2. Descriptive characteristics associated with the varieties studied, Pristol (Mehedinți), 2022

Variety	Mean± SD	95% Confidence Interval for Mean		Minimum	Maximum
		Lower Bound	Upper Bound		
Delia	4.28 ± 6.27	1.16	7.39	0.00	25.00
Flacara	5.00 ± 8.31	0.87	9.13	0.00	34.00
Bonita Mix	5.78 ± 10.18	0.72	10.84	0.00	41.00
Total	5.02± 5.02	2.76	7.28	0.00	41.00

Among the three varieties studied, we found consistent patterns in the total number of insects. Overall, the Bonita Mix variety was preferred by a high number of species and individuals, with a maximum mean of 5.78 individuals with a standard deviation of 10.18, and the minimum mean was recorded on the Delia variety, 4.28 individuals/trap with a standard deviation of 6.27 (Table 2 and figure 1).

Table 3. Analysis of the variation of the three varieties under study

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	20.259	2	10.13	0.143	0.867
Within Groups	3602.722	51	70.642		
Total	3622.981	53			

Our results show no significant differences between the three varieties studied (F=0.143, p=0.867>0.05).

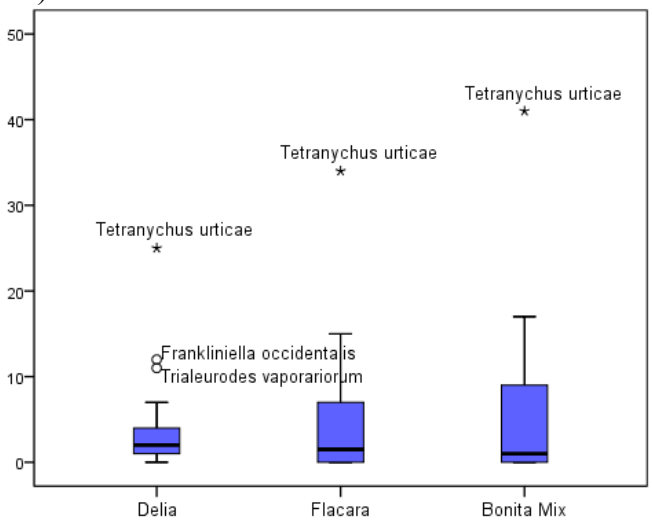

Figure 1. Box plot associated with the *Tagetes patula nana* varieties studied

It is well known that greenhouses provide a microclimate favourable to the mass reproduction of thrips and aphids, which increase in numbers as they find the food and conditions necessary to reproduce. An analysis of the arthropod species evolution (figure 1) shows that the most abundant species is *Tetranychus urticae*, with a mean of 33.33 ± 8.02 individuals, most of them collected from Bonita Mix and fewer from Delia (Table 3).

The works of Taleb & Sardar (2008), Patel & Ghetiya (2016, 2018) also mention the importance of tetracychid mites and the significant damage these species cause to Tagetes sp. They mention the importance of temperature for the development of acarine populations under protected conditions and emphasise the importance of controlling, especially given the polyphagous nature of the species.

In terms of number of species and specimens, mites were followed by thrips, with 4 species - *Thrips tabaci, Frankliniella occidentalis, Frankliniella schultzei* and *Thrips palmi*, respectively, a mean number of individuals comprease between 0.33 and 11.67. In terms of variety, preferences were diverse, with three of the *Thrips* species, *T. tabaci, Frankliniella schultzei* and *T. palmi* preferring the Bonita Mix variety, and the Flacăra variety being preferred by the *Frankliniella occidentalis* species. Early leaf drop was observed, with silvery spots and a general pale brown appearance, indicating thrips attack. *T. tabaci* has been reported to cause significant damage to marigold crops by Coradini et al. (2012).

Table 3. Descriptive characteristics associated with the species studied

	Mean	95% Confidence Interval for Mean		Minimum	Maximum
		Lower Bound	Upper Bound		
Tetranychus urticae	33.33 ± 8.02	13.41	53.26	25	41
Thrips tabaci	7 ± 2	2.03	11.97	5	9
Frankliniella occidentalis	11.67 ± 3.06	4.08	19.26	9	15
Frankliniella schultzei	2 ± 2	-2.97	6.97	0	4
Thrips palmi	0.33 ± 0.58	-1.1	1.77	0	1
Myzodes persicae	8.33 ± 5.13	-4.41	21.08	4	14
Aphis gossypii	1.67 ± 2.89	-5.5	8.84	0	5
Trialeurodes vaporariorum	12.33 ± 4.51	1.13	23.53	8	17
Nezara viridula	1± 1	-1.48	3.48	0	2
Halyomorpha halys	2.33 ± 2.08	-2.84	7.5	0	4
Apis mellifera	1.67 ± 1.15	-1.2	4.54	1	3
Coccinella 7-punctata	3.67 ± 3.79	-5.74	13.07	1	8
Harmonia axyridis	0.33 ± 0.58	-1.1	1.77	0	1
Hippodamia variegata	1 ± 1	-1.48	3.48	0	2
Propylea quatuordecimpunctata	1± 1.73	-3.3	5.3	0	3
Spodoptera exigua - l	1 ± 1	-1.48	3.48	0	2
Helicoverpa armigera - l	1 ± 0	1	1	1	1
Liriomyza trifolii - l	0.67 ± 0.58	-0.77	2.1	0	1
Total	5.02 ± 8.27	2.76	7.28	0	41

Aphids were also abundant with an average of 10 individuals collected. They belonged to two species - *Myzus persicae* and *Aphis gossypii* - with a mean number of 4.33 *M. persicae* individuals from the Bonita Mix variety, 2.33 from the Flacăra variety and 1.33 individuals from the Delia variety. In the case of *A. gossypii*, 1.66 individuals of the Flacăra variety were recorded. Aphid colonies, both adult and larval, were observed feeding on marigold flowers, often causing the flowers to become sooty and wither prematurely. Pal and Sarkar (2009) identified a number of aphid species that attack flowering plants in protected areas. These include *Myzodes persicae, Macrosiphoniella sanborni* and *Aphis gossypii*.

Unlike thrips and aphids, the larvae of the noctuids *Spodoptera exigua* and *Helicoverpa armigera* were also collected from the leaves and inflorescences of *Tagetes patula nana*, but in rather small numbers, being present on all 3 varieties studied. Being polyphagous and polymorphic species that attack plants belonging to more than 47 botanical families, among which we mention: legumes, oilseeds, fruits, vegetables, soybeans, cotton, maize and many other agricultural and horticultural crops (Pălegeșiu et al., 2007; Costea & Grozea, 2021; Vîrteiu et al., 2021), their presence in the solar house is most likely due to the biological reserve already present in the soil inside the solar house and in the surrounding area. The species has been reported on marigolds by Halder et al. (2018), who mention that the female lays her eggs on the flower buds of the plants, and the larvae are later found feeding on these inflorescences. The quantitative and qualitative decline due to the attack of this species, which can reach 20% of the inflorescences, was also reported by Bhagat (2017).

The species *Liriomyza trifolii* had the lowest number of specimens collected in 2022 (0.67). The results are in agreement with the researches of Ganai et al (2017) and Anonymous (2014) who reported the significant infestation of insect pests such as thrips, aphids and mites. The presence of the species *Nezare viridula* on *Tagetes* inflorescences was also mentioned by Ganai et al. (2017).

In 2022, in the solar farm of Pristol, a total of 4 species of predators belonging to the family *Coccinelidae* were reported on *Tagetes* plants. The species of *Coccinela 7 - punctata* was the most abundant (3.67 ± 3.79 adults). The species *Hippodamia variegata* and *Propylea quatuordecimpunctata* presented a mean number of one specimen each. We also highlight that on the 3 months period only one specimen of *Harmonia axyridis* was found. These have been observed feeding on aphids and greenhouse whiteflies.

Of the known pollinating insects, only the species *Apis mellifera* was present in the greenhouse in which the research was carried out. The shape and size of the flower, the colour, the scent, the nectar and the pollen are just some of the factors that influence the interest of the pollinating insects (Yan et al., 2016). In the case of the present study, the low number of pollinators, taking into account the factors mentioned above, can be explained by the fact that the plants were taken out for sale as soon as the first inflorescences formed, in mid-April. Therefore, the observations only refer to the period until flowering, or until about 50% of the plants had flowered. Further research is needed to establish whether the decline in pollinator numbers is not due to other causes. Many researchers (Potts et al., 2010; Vanbergen & Initiative, 2013) point to a decline in bee populations due to ecological factors and anthropogenic pressures such as pesticides, increasingly aggressive pathogens, but also the decline in food resources.

The results highlight the preferences of entomophages and pollinators for the flowers of *Tagetes patula nana*, and these plants can be used to provide a food/habitat source for these beneficial insects. The results highlight the need for the conservation of these insect species and their use in biological control systems, similar to those described by Boisclair et al. (2014).

Table 4. Man-Whitney test

Species	*Thrips palmi*	*Harmonia axyridis*	*Propylea quatuordecimpunctata*
Tetranychus urticae	U=41, p=0.001	U=41, p=0.001	
Thrips tabaci	U=30.167, p=0.017	U=30.167, p=0.017	U=25.833, p=0.041
Frankliniella occidentalis	U=36.5, p=0.005	U=35.5, p=0.005	U=31.167, p=0.014
Myzodes persicae	U=30.5, p=0.005	U=30.5, p=0.016	U=26.167, p=0.038
Trialeurodes vaporariorum	U=-35.5, p=0.005	U-35.5, p=0.005	

* The significance level is 0.05

The results of the Mann-Whitney test for the arthropod fauna collected from the plants of *Tagetes patula nana* show a significant difference between *Thrips palmi* and the other pest species (where $p < 0.05$). Similar results were found for *Harmonia axyridis and Propylea quatuordecimpunctata* (Table 4). However, there were significant differences between *Harmonia axyridis* and arthropod pests: *Tetranychus urticae* (p= 0.001); *Thrips tabaci* (p= 0.017); *Frankliniella occidentalis* and *Trialeurodes vaporariorum* (p= 0.005); *Myzodes persicae* (p=0.016). In the case of *Propylea quatuordecimpunctata*, significant differences were registered only in relation to the pests *Thrips tabaci* (p = 0.041), *Frankliniella occidentalis* (p = 0.014) and *Myzodes persicae* (p = 0.038).

Lundgren (2009) emphasises the importance of understanding the resources that these plants provide to beneficial insects in the form of non-prey food (nectar, pollen, honey, etc.) as being essential for the success of biological control programmes.

As habitat manipulation is an essential component of pest management, Winkler (2005) recommends rigorous selection of plant species in a microhabitat, considering not only the attractiveness of a flowering species to beneficials, but also its potential to act as a pest trap, thereby reducing pest numbers.

CONCLUSIONS

In this study, significant differences in arthropod fauna species were recorded for *Tagetes patula nana* plants. The most abundant predator species was *Coccinela 7 - punctata* and the most abundant arthropod pest species was *Tetranychus urticae*. It is also highlighted that marigold can be used as a trap plant in organic farming. The potential use of plant diversity around crops is needed to achieve effective conservation biological control in organic farming systems.

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