UDC 632:615:89

eISSN 2406-1026

Pesticides & Phytomedicine

Pesticidi i fitomedicina

Scientific Journal of the Serbian Plant Protection Society

Vol. 38 * No. 2 * 2023

eISSN 2406-1026

Pesticides & Phytomedicine

Pesticidi i fitomedicina

Scientific Journal of the Serbian Plant Protection Society

Vol. 38 * No. 2 * 2023

Pesticides & Phytomedicine

eISSN 2406-1026

Published triannually

PUBLISHER

Institute of Pesticides and Environmental Protection, Belgrade, Serbia

Phone: (011) 3076-133, 3076-136

Fax: (011) 3076-136

CO-PUBLISHER

Serbian Plant Protection Society, Belgrade, Serbia

PUBLISHER DIRECTOR

Emil Rekanović

LAYOUT

Miodrag Panić

COPYEDITOR

Dušica Marinkov-Jovanović

Cited in: ***Chemical Abstracts; CAB International; DOAJ; EBSCO; AGRIS; Scindeks***
Full text articles available at: www.pesting.org.rs; www.doaj.org;
http://scindeks.ceon.rs/journaldetails.aspx?issn=1820-3949

CONTENTS

Pestic. Phytomed. (Belgrade), 38(2), 2023, 43–53
DOI: https://doi.org/10.2298/PIF2302043T

UDC 632.4:632.952:635.25
Review paper

Stemphylium vesicarium (wallr.) E.G. Simmons: an onion plant pathogen and options for suppression

Ana Takač [1]* and Slavica Vuković[2]

[1]Kite DOO Novi Sad, Međunarodni put 162A, 21233 Čenej, Serbia
[2]University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia
**Corresponding author: ana.takac@kitedoo.rs*

Received: 5 March 2023
Accepted: 3 August 2023

SUMMARY

Onion (*Allium cepa* L.) is one of the most important vegetable species grown worldwide, including the Republic of Serbia. Leaf blight, caused by the fungus *Stemphylium vesicarium*, is a serious and destructive disease of onion leaves around the world, which limits the quality and quantity of bulbs and seeds. Yield decrease occurs due to a reduced photosynthetic area, which leads to the formation of smaller bulbs of poorer quality. The recommended strategy for control and reduction of SLB inoculum includes crop rotation with other vegetable species or cereals that are not hosts of these fungi, the use of resistant onion genotypes, weed removal, adequate use of nitrogen fertilizers, control of thrips (*Thrips* spp.), as well as seed treatment, considering that seeds play a significant role in the spread of pathogens. Timely and correct application of foliar fungicides is certainly the key strategy. The timing of application of fungicides with different modes of action is crucial for controlling *Stemphylium vesicarium* in onion.

Keywords: plant pathogenic fungi, plant disease suppression, onion, leaf blight, fungicides

INTRODUCTION

Onion belongs to the monocotyledon family Amaryllidaceae, genus *Allium* (Yusupov et al., 2021). This genus includes about 1250 perennial bulbous plant species that are used as food and spice, but also as honey-bearing, ornamental, medicinal and industrial plants. It is characterized by a distinct nutritional value and is present in our diet throughout the year (Gvozdanović-Varga, 2011). Cultivation and use of onion has been known for the past 4000 years (Kazanova, 1978; Brewster & Rabinowitch, 1990).

Onion production in the Republic of Serbia is widespread and the main production regions are Northern Banat and Bačka, Podrinje, the environs of Prizren, Pirot and Belgrade (Miladinović et al., 1997). In the Republic of Serbia in 2022, areas under onion crops amounted to 4114 hectares with an average yield of 8.5 t/ha, while 1364 hectares were sown/planted with onion in the Serbian Province of Vojvodina, with an average yield of 12.2 t/ha (Statistical Office of the Republic of Serbia, 2022).

Causal agents of onion disease

Diseases are one of the most important limiting factors in onion production. Onion diseases are caused by dozens of pseudofungi, fungi, bacteria and viruses (Koike et al., 2007). Literature shows that onions are susceptible to at least 66 diseases caused by different pathogens: 36 types of fungi, about 10 different types of bacteria, a large number of viruses and one phytoplasma (Bulajić, 2015). Among them, pseudomycosis and mycosis, which occur during the growing season, but also during storage, stand out for their importance. The presence of pathogens depends on climatic conditions during the growing season, genotype and region of cultivation. In the Republic of Serbia, the most important causal agents of onion diseases are phytopathogenic fungi listed in Table 1, and the drying of onion leaves caused by a fungal pathogen in the genus *Stemphyllium* has become frequent in recent years.

STEMPHYLIUM VESICARIUM (WALLR.) E.G. SIMMONS – THE CAUSAL AGENT OF LEAF BLIGHT

Importance

Stemphylium leaf blight and stalk rot of onion are caused by the hemibiotrophic fungal pathogen *Stemphylium vesicarium* (Wallr.) E.G. Simmons (teleomorf: *Pleospora herbarum* [Pers.] Rabenh., sin *P. allii*) of the genus *Stemphylium*. Depending on agro-ecological conditions and geographic growing region, it is described as a very significant disease of onion (*Allium cepa* L.) and garlic (*A. sativum* L.) (Gupta et al., 1994; Miller & Schwartz, 2008; Mishra & Singh, 2017).

S. vesicarium (SLB) leads to premature leaf blight (leaves droop after necrosis), thus making it more susceptible to post-harvest diseases (Paibomesai et al., 2012). In onion production, SLB can be easily confused with purple blotch symptoms, caused by *Alternaria porri* (Suheri & Price, 2001). Uddin et al. (2006) reported that *S. vesicarium* is first to initiate infection, which is then followed by infection with *A. porri,* and hence the disease is designated as the purple blotch complex (PBC). The mentioned fungi are very well described in international literature as significant pathogens of onion (Mathur & Sharma, 2006) having economic impact on onion production worldwide (Gupta et al. 1994).

The presence of this pathogen under Serbian agro-ecological conditions has been recorded on annual basis in recent years, and it causes significant economic damage in some onion producing regions of Vojvodina Province. So far, there has not been enough recorded data about the ecology and distribution of SLB in the territory of the Republic of Serbia.

The presence of *S. vesicarium* in onion was first described in the United States (Miller et al., 1978), then

Table 1. Onion diseases caused by phytopathogenic fungi (Mijatović et al., 2007)

Disease name	Pathogen or causal agent
ROOT NECROSIS	
Root rot	*Pythium* spp.
Onion Stunting	*Rhizoctonia solani* (Kühn)
White rot	*Sclerotium cepivorum* (Berk)
Botrytis leaf fleck	*Botrytis cinerea* (Pers. ex Fr)
Smut	*Urocystis cepulae* (Rabenh. ex Fuckel)
Onion smudge	*Colletotrichum circinans* (Berk.) Voglino
Pink root of onion	*Pyrenochaeta terrestris* (J.C. Walker & Larson)
BASAL ROT	
Fusarium basal rot	*Fusarium oxysporum* f. sp. *cepae* (Snyder & Hansen)
LOCAL NECROSIS OF ABOVE-GROUND PARTS OF PLANTS	
Downy mildew	*Peronospora destructor* (Berk). Caspary
Purple blotch	*Alternaria porri* (Ellis) Cifferi
Rust	*Puccinia porri, P. allii* (DC) Rudolph
Onion smut	*Urocystis cepulae* (Hansen)
Botrytis leaf blight	*Botrytis squamosa* (Walker), *B. alli* (Munn)

Portugal (Tomaz & Lima, 1988), India (Gupta et al., 1994), Korea (Cho & Yu, 1998), Venezuela (Cedeño et al., 2003), Egypt (Hassan et al., 2007), Canada (Paibomesai et al., 2012), Japan (Misawa & Yasuoka, 2012) and New Zealand (Wright et al., 2018).

Yield and quality losses of up to 90% have been reported in Texas and New York State (Miller et al., 1978; Lorbeer, 1993), while losses of 80-85% have been reported in seed crops in Portugal (Tomaz & Lima, 1988). Extensive damage has also been reported in Egypt (Hassan et al., 2007), India (Rao & Pavgi, 1975), Japan (Misawa & Yasuoka, 2012), New Zealand (Suheri & Price, 2001), South Africa (Aveling et al, 1993) and Spain (Basallote-Ureba et al, 1999). Premature plant mortality under conditions of high disease pressure was correlated to yield losses of 28-38% and up to 74% (Hoepting, 2018a; 2018b).

Host range

Stemphylium vesicarium can infect a range of plant species from many families (Table 2). Its host plants include several crop species of the *Allium* genus, fruit trees, legumes, and ornamentals (Stricker, 2021). *S. vesicarium* has been detected in a wide range of crops as both a pathogen and saprophyte (Hassan et al, 2020). Additionally, the pathogen can cause asymptomatic infections and develop as an endophyte in living tissues of various other plants (Köhl et al., 2009; Misawa &Yasuoka 2012).

Symptoms

The pathogen is common in seed and commercial crop production. Initial symptoms on leaves include the appearance of small yellow to orange spots, 2-3 mm

Table 2. Host plants of *Stemphylium vesicarium,* Stricker (2021)

Common name	Latin name	Source
Leek	*Allium ampeloprasum* L.	(Suheri & Price, 2001)
Common onion	*Allium cepa* L.	(Raghavendra Rao & Pavgi, 1975)
Welsh onion	*Allium fistulosum* L.	(Misawa &Yasuoka, 2012)
Garlic	*Allium sativum* L.	(Aveling &Naude, 1992)
Oats	*Avena* sp. L.	(Brahmanage et al., 2019)
Asparagus	*Asparagus officinalis* L.	(Falloon et al., 1987)
Beet	*Beta vulgaris* L.	(Hanse et al., 2015)
Carrot	*Daucus carota* L.	(Mulenko et al., 2008)
Canola	*Brassica napus* L.	(Mulenko et al., 2008)
Chinese cabbage	*Brassica rapa* L. ssp. *pekinensis*	(Woudenberg et al., 2017)
Chili pepper	*Capsicum chinense* Jacq.	(Vitale et al., 2017)
Citrus	*Citrus* sp. L.	(Woudenberg et al., 2017)
Soybean.	*Glycine max* L	(Pande & Rao, 1998)
Sunflower	*Helianthus annuus* L.	(Arzanlou et al., 2012)
Lettuce	*Lactuca sativa* L.	(Liu et al., 2019)
Sweet pea	*Lathyrus odoratus* L.	(Köhl et al., 2009)
Lentil	*Lens culinaris* Medikus	(Sinha & Singh, 1993)
Lupin	*Lupine* sp. L.	(Ahmad, 2014)
Apple	*Malus* sp. Mill	(Woudenberg et al., 2017)
Mango	*Mangifera indica* L.	(Ahmad, 2014)
Alfalfa	*Medicago sativa* L.	(Díaz-Valderrama et al., 2021)
Parsley	*Petroselinum crispum* [Mill.] Fuss	(Koike et al., 2013)
Green bean	*Phaseolus vulgaris* L.	(Câmara et al., 2002)
Pea	*Pisum sativum* L.	(Woudenberg et al., 2017
Pear	*Pyrus* sp. L.	(Rossi et al., 2008)
Radish	*Raphanus raphanistrum* subsp. *sativus* (L.)	(Belisario et al., 2008)
Tomato	*Solanum lycopersicum* L.	(Woudenberg et al., 2017)
Spinach	*Spinacia oleracea* L.	(Misawa et al., 2017)
Grape	*Vitis vinifera* L.	(Kranz, 1965)
Corn	*Zea mays* L.	(Unamuno, 1941)

in diameter, which become elongated, oval and sunken over time, developing a dirty white to gray color, and reaching a size of over 4-5 cm in length and 1-1.5 cm in width, with profuse sporulation at the centre of lesion (Rao & Pavgi, 1975; Sharma & Sharma, 1999). Another characteristic symptom includes brown, oval lesions up to 7 cm in diameter towards the tip and center of outer leaves, and yellow lesions of 0.5-4 cm in diameter on inner leaves (Figure 1).

Figure 1. *Stemphylium vesicarium*: symptoms on onion leaves (photo A. Takač)

Stemphylium blight is restricted to onion leaves and inflorescence stalks (Rao & Pavgi, 1975; Aveling et al, 1993), and the most prominent symptoms appear on older leaves (Shishkoff & Lorbeer, 1989). Althought SLB becomes visible when an onion crop is at the 3- to 4-leaf stage, the disease most commonly occurs at plant maturity or at the beginning of leaf senescence (Tayviah, 2017).

A characteristic symptom is the progressive leaf necrosis, which starts from the tip (often termed as „tip burn" in international literature) (Figure 2), associated with host-specific toxins (SV-toxin I and SV-toxin II) produced after infection (Singh et al., 2000; Wolpert et al., 2002).

Toxins play a very important role in the pathogenicity and aggressiveness of isolates. Disease progress and premature aging of leaves lead to reduced growth and size of bulbs, which significantly affects the yield (Figure 3).

Figure 2. *Stemphylium vesicarium*: blight of onion leaf tips (photo A. Takač)

Figure 3. *Stemphylium vesicarium*: merging of spots and formation of large necrotic surfaces (photo A. Takač)

Epidemiology

S. vesicarium belongs to the genus *Ascomycota*, family *Pleosporaceae* and order *Moniliales*. The life cycle is characterized by the succession of sexual and asexual stages. In the sexual stage, it forms ascospores that are located in pseudothecia, while the asexual stage includes the formation of conidia on conidiophores (Prados-Ligero et al., 1998; Basallote-Ureba et al., 1999).

S. vesicarium is dormant during wintertime, surviving as pseudothecia or mycelia in diseased or asymptomatic leaves (Simmons, 1969). Mycelia also overwinter on infected asymptomatic leaves of winter onion and other host plants (Rossi et al., 2008; Misawa & Yasuoka, 2012; Llorente et al, 2012). Optimal temperature for pseudothecia maturation during the winter period in moderate climates ranges from 5-15 °C, and high relative humidity follows as a factor (Prados-Ligero et al., 2003; Llorente & Montesinos, 2004). Under the same conditions, ascospore maturation lasts from 1-6 months (Simmons, 1969; Prados-Ligero et al., 1998). Ascospores can infect onion plants under laboratory conditions (Prados-Ligero et al., 1998) but their role in field epidemiology is fully unknown.

In *Allium* species, conidia carry primary infection in the field (Prados-Ligero et al., 2003; Misawa & Yasuoka, 2012). Conidia appear throughout the production year from May to September, and the maximum air concentration of conidia is recorded between mid-June and mid-August (Gossen et al., 2021). Conidia of *S. vesicarium* germinate at temperatures as low as 4 °C and infect leaf tissue at 10 °C (Prados-Ligero et al., 2003).

The release of air-borne ascospores and conidia shows a diurnal pattern in response to temperature, leaf wetness duration, and relative humidity (Suheri & Price, 2000). When the weather is warm (18-25 °C) and humid, initial symptoms are first observed on weakened plants (Johnson & Lunden, 1986). Symptoms may occur during periods of leaf wetness >16 h (Suheri & Price, 2000; Prados-Ligero et al., 2003).

Onion seed, transplants and volunteer onion play significant roles in disease epidemiology. *S. vesicarium* can be carried on or in onion seeds, and the pathogen is easily transmitted from infected seeds to seedlings (Aveling et al., 1993; Stricker, 2021). Lorbeer (1993) noted a distinct influence and importance of seeds in its epidemiology in New York State. According to Hay et al. (2021), the presence of the pathogen was not confirmed in a healthy seed collection. Considering that seeds are treated (coated) with fungicides in conventional production, the risk of seed-to-seedling transmission of pathogens is reduced.

According to Hay et al (2021), volunteer onions in fields or in cull-piles may also represent an important source of inoculum, especially in the absence of onion crop rotation. During monitoring activities in two onion crops in New York in late May 2020, *S. vesicarium* was recovered from 80 to 88% of volunteer onion plants, even though the incidence in surrounding crops was only 2-8%. It indicates that *S. vesicarium* had overwintered in volunteers or that the more rapidly developing volunteer plants acted as a green bridge for pathogen establishment in the crop.

S. vesicarium can be maintained in plant debris and in infected perennial weed species. Such common weeds include: redroot pigweed (*Amaranthus retroflexus)*, marsh yellow cress (*Rorippa palustris)*, yellow nutsedge (*Cyperus esculentus)*, perennial sowthistle (*Sonchus arvensis)*, bull thistle (*Cirsium vulgare)*, purslane (*Portulaca oleracea)*, as well as plants in the families: *Amaranthaceae*, *Brassicaceae*, *Cyperaceae*, *Asteraceae* and *Portulacaceaea* (Rao & Pavgi, 1975), where it releases ascospores in the spring. Stricker (2021) lists common sowthistle (*Sonchus oleraceus)*, field horsetail (*Equisetum arvense)*, field pennycress (*Thlapsi arvense)*, and jimsonweed (*Datura stramonium*) as hosts.

The relative importance of weeds as a source of inoculum is not yet known, and the removal of such inoculum reservoirs may not be effective for controlling SLB in onion crops because airborne spores have the potential to travel great distance (Hay et al, 2021).

Suppression of *Stemphylium vesicarium*

Prerequisites for effective protection include the reduction of inoculum level and prevention of disease development (Wright et al., 2018). Taking into account that the pathogen can persist in soil or plant material for several years, crop rotation with other vegetable or plant species belonging to the group of small grains that are not hosts of this fungus leads to decreasing occurrence of this pathogen, and consequently reduced disease incidence (Chand & Kumar, 2016). Crop rotation lasting 3-4 years is recommended.

Another very important measure is keeping the plants in good condition, and protecting crops from other leaf pathogens and thrips (Leach et al., 2020), considering that the pathogen penetrates the plant at the site of damaged leaves.

Seed treatment with hot water at 50 °C for 20 min reduces the inoculum level but also reduces germination. An excessive use of nitrogen and unbalanced nutrition increase the sensitivity of plants (Acharya & Shrestha, 2018).

Irrigation at 7 days interval and the recommended dose of nitrogen of 235 kg/ha have been found to give significantly better results in reducing the intensity of *S. vesicarium* and *Peronospora destructor* infections, and at the same time they lead to an increase in yield (Acharya & Shresthra, 2018). It is recommended to avoid overhead irrigation during the day because it prolongs the wetting of leaves and creates conditions for severe infection and dieback of leaves. Irrigation should either take place at night or a drip irrigation system should be installed as an even better solution.

Under the growing conditions in the Province of Vojvodina, Serbia, a significant difference in the intensity of infection has been observed in onions grown in a drip system and overhead irrigation system in which leaves become moist or wet (unpublished data).

Production should be based on good structural soils, and spatial isolation from winter onion is recommended as preferable.

Regular application of preventive (contact) and curative (systemic) fungicides is an important tool for managing diseases caused by fungal pathogens, especially where genetic resistance is not available (Llorente et al., 2012).

In order to reduce the risk of developing resistance, the application of fungicides with different mechanisms of action is recommended (Jasnić, 2006; Brent & Hollomon, 2007). Forecast models used worldwide to predict the occurrence of disease-causing agents provide very important information for proper timing of fungicide applications. Several forecasting models have been used for the management of *S. vesicarium* in a range of crops: FAST, TOMcast, BOTcast, BSPcast and STREP, all based on the temperature, leaf wet period and relative humidity (Stricker et al., 2020).

One of the most effective measures in controlling this disease complex is the use of tolerant genotypes, as well as good agricultural practices, but in case of their unavailability, chemical control measures are the only solution.

Application of fungicides

Fungicides that are currently registered worldwide to control *S. vesicarium* belong to the following FRAC groups: phthalimides (M4), chloronitriles (M5), demethylation inhibiting fungicides (DMI; FRAC group 3), succinate dehydrogenase inhibitors (SDHI; FRAC group 7), anilino pyrimidine fungicides (AP; FRAC group 9), quinone outside inhibitors, (QoI; FRAC group 11), but only a few have been registered to control *S. vesicarium* (Stricker, 2021).

Fungicides belonging to the triazole group: tebuconazole, difenoconazole and hexaconazole, and the contact fungicides propineb, mancozeb and chlorothalonil, have shown satisfactory effectiveness in controlling *S. botryosum* under *in vitro* and *in vivo* conditions (Mohan et al. 2003).

Mishra and Gupta (2012) studied the effects of eight different fungicides on fungal mycelial growth. Significant inhibition was recorded with contact fungicides: mancozeb, propineb, and the systemic fungicides azoxystrobin and propiconazole.

The 2022 List of Approved Substances in the Republic of Serbia did not include the active substance mancozeb, while propineb had been previously withdrawn from use (Ministry of Agriculture, Forestry and Water Management, 2022).

The results of an experiment carried out by Tesfaendrias et al. (2012) indicate a very high effectiveness of the fungicides azoxystrobin + difenoconazole, fluopyram + pyrimethanil, and difenoconazole in controlling this pathogen, which confirms the results of other authors that the combination of strobilurin and triazole (azoxystrobin + flutriafol) enables better control of *S. vesicarium*. Fungicides in the strobilurin group (Quinone outside Inhibitors, QoI fungicides) are very effective in inhibiting the germination of fungal spores. Their effect on the mycelial growth was also recorded, but their ability to inhibit spore germination is more significant.

On the other hand, fungicides of the triazole group (DeMethylation Inhibitors, DMI fungicides) inhibit ergosterol biosynthesis. Considering that fungal spores contain ergosterol, those fungicides have not shown high efficiency in preventing spore germination. Their effectiveness is based on the inhibition of mycelial growth (Bradley, 2011, loc.cit. Mishra & Singh, 2017), which could be the reason for different *in vitro* and *in vivo* efficacy results.

Laboratory analyses conducted by Mishra & Singh (2017) proved that fluopyram + tebuconazole, applied at a concentration of 50 ppm, completely inhibited the growth of *S. vesicarium* mycelium. Under *in vivo* conditions, azoxystrobin + flutriafol showed the highest efficacy, compared to the control. Based on all tested fungicides, the combinations of azoxystrobin 25% + flutriafol 25% and fluopyram 20% + tebuconazole 20% were recommended for controlling *S. vesicarium*

in vivo. The lowest infection intensity was recorded when four foliar treatments were applied using the fungicides: propiconazole 0.1%, mancozeb 0.25% and copper oxychloride 0.3%, (Gupta & Gupta, 2013).

Hoepting (2016) highlighted the effectiveness of fungicides in the FRAC 3 group (difenoconazole) and FRAC 7 (fluopyram, fluxapiroxad). Hoepting (2020) noted that fungicides in the FRAC 3 group (difenoconazole, propiconazole, tebuconazole), FRAC 7 (fluopyram), FRAC 9 (cyprodinil, pyrimethanil) and FRAC 2 (iprodione) w the most effective in controlling *S. vesicarium*. Treatment with fluopyram 200 g/l + tebuconazole 200 g/l showed an efficiency of 52.5%, while fluopyram 250 g/l + trifloxystrobin 250 g/l and fluazinam showed an efficiency of 80-88.8 % in trials (Hausbeck et al, 2018).

Seed treatment with the a.i. penflufen (Succinate dehydrogenase inhibitor, SDHI) (FRAC 7), in combination with regular foliar fungicide application, gave satisfactory results in controlling *S. vesicarium* (Stricker et al., 2020).

Paneru et al. (2020) recommend the use of hexaconazole at a concentration of 0.1% and a combination of mancozeb + cymoxanil as effective fungicides for controlling the disease complex of onion.

Various bioagents such as *Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma harzianum*, *Gliocladium* spp. and *Saccharomyces cerevisiae* significantly inhibit mycelial growth under *in vitro* conditions (Hussein et al., 2007). Plant extracts of *Azadirachta indica* and *Datura stramonium* have shown effectiveness in reducing the growth of the fungi *A. porri* and *S. vesicarium* in a protected area (Abdel-Hafez et al., 2014, loc. cit Meena & Verma, 2017).

Leaf defoliation caused by *S. vesicarium* does not always have a direct effect on the yield, especially if the pathogen appears at the end of the growing season, but on the other hand, it leads to decrease in the effectiveness of maleic hydrazide, which is used in production as a sprout inhibitor (Isenberg et al., 1974). It is recommended to apply sprout inhibitor to green leaves immediately before laying of onions (Ilić et al., 2011). In this way, both the yield and quality of onions are preserved. If maleic hydrazide is not applied, onions will have a shorter shelf life. In Serbia, products based on a.i. maleic hydrazide potassium 245 g/l and maleic hydrazide potassium 270 g/l have been registered for this purpose (Aleksić et al., 2022).

Table 3: Active substances registered worldwide and in the Republic of Serbia for the control of *S. vesicarium* on onion (Anonymous 2021; Aleksić et al., 2022)

MOA	Target site and code	Group name	Chemical Group	Common name	FRAC code
C	C2	SDHI	pyrazole-4-carboxamides	flyxapyroxad	7
C	C2	SDHI	pyrazole-4-carboxamides	benzovindiflupyr	7
C	C2	SDHI	N-methoxy-(phenyl-ethyl) -pyrazole-carboxamides	pydiflumetofen	7
G	G1	DMI	triazoles	difenoconazole*	3
C	C2	SDHI	pyridinyl-ethyl-benzamides	fluopyram*	7
D	D1	AP	aniilino-pyrimidines	pyrimethanil	9
C	C3	QoI	methoxy-acrylates	azoxystrobin*	11
M	multi -site contact activity	Inorganic (elektrophiles)	inorganic	copper oxychloride*	M 01
G	G1	DMI	triazolinthiones	prothioconazole*	3

AP – fungicides (Anilino-Pyrimidines)
C2 – complex II: succinate-dehydrogenase
C3 – complex III: cytochrome bc1 (ubiquinol oxidase) at Qo site (*cyt b gene*)
C – respiration
D – amino acids and protein synthesis
D1 – methionine biosynthesis
DMI – fungicides (DeMethylation Inhibitors)
FRAC Code- fungal control agents sorted by cross-resistance pattern and mode of action
G1 – C14- demethylase in sterol biosynthesis(*erg11/cyp51*)
G – sterol byosyntesis in membranes
M – Chemicals with multi-site activity
MOA – mode of action
QoI – fungicides (Quinone outside Inhibitors)
SDHI – Succinate-dehydrogenase inhibitors
* registered in the Republic of Serbia for suppression of *S. vesicarium*

REFERENCES

Acharya B., & Shrestha R.K. (2018). Nitrogen level and irrigation interval on mitigating Stemphylium blight and downy mildew in onion. *International Journal of Applied Sciences and Biotechnology, 6*(1), 17-22. doi: 10.3126/ijasbt.v6i1.18795

Aleksić, G., Brkić, D., Gašić, S., Kljajić, P., Marčić, D., Pavlović, D., & Radivojević, I. (Eds.) (2022). *Pesticidi u poljoprivredi i šumarstvu u Srbiji* (Pesticides in agriculture and forestry in Serbia) (21st amended edition). Belgrade, Serbia: Plant Protection Society of Serbia.

Anonymous (2021). *Vegetable crop protection guide*. Publication 838, 161-166. Retrieved from Ontario Crop Protection Hub http://www.omafra.gov.on.ca/english/crops/pub838/p838order.htm

Aveling, T.A.S., Snyman, H.G., & Naude, S.P. (1993). Evaluation of seed treatments for reducing *Alternaria porri* and *Stemphylium vesicarium* on onion seed. *Plant Disease*, *77*(10), 1009-1011. doi: 10.1094/PD-77-1009

Basallote-Ureba, M.J., Prados-Ligero A.M., & Melero-Vara J.M. (1999). Aetiology of leaf spot of garlic and onion caused by *Stemphylium vesicarium* in Spain. *Plant Pathology, 48*(1), 139-145. doi: 10.1046/j.1365-3059.1999.00313.x

Brent, K.J., & Hollomon, D.W. (2007). *Fungicide resistance in crop pathogens: How can it be managed?* (2nd ed.). Brussels, Belgium: Fungicide Resistance Action Committee.

Brewster, J.L., & Rabinowitch, H.D. (1990). *Onions and allied crops*, Volume III: Biochemistry, Food Science, and Minor Crops. Boca Raton, FL: CRC Press.

Bulajić, A. (2015). Kontrola biljnih bolesti u organskoj proizvodnji lukova (Control of plant diseases in organic production of onions). *Savremeni povrtar, 54*, 18-31.

Cedeño, L., Carrero, C., Quintero, K., Pino, H., & Espinoza, W. (2003). *Stemphylium vesicarium*, causal agent of severe foliar blight on garlic and onion in Mérida, Venezuela. *Interciencia 28*(3), 174-177.

Chand, G., & Kumar, S. (2016). *Crop diseases and their management: Integrated approaches*. Oakville, ON, Canada: Apple Academic Press.

Cho, H.S., & Yu, S.H. (1998). *Stemphylium vesicarium* on garlic and other *Allium* spp. in Korea. *Korean Journal Plant Pathology 14*(6), 567-570. Available at http://www.koreascience.or.kr/article/JAKO199811920118431.pdf

Gossen, B.D., Tayviah, C.S., & McDonald, M.R. (2021). The role of ascospores and conidia, in relation to weather variables, in the epidemiology of Stemphylium leaf blight of onion. *Plant Disease. 105*(7), 1912-1918.

Gupta, R.C., & Gupta, R.P. (2013). Effect of integrated disease management packages on disease incidence and bulb yield of onion (*Allium cepa* L.). *SAARC Journal of Agriculture. 11*(2), 49-59.

Gupta, R.P., Srivastava, K.J., & Pandey, U.B. (1994). Diseases and insect pests of onion in India. *Acta Horticulturae*, *358,* 265-372. doi: 10.17660/ActaHortic.1994.358.43

Gvozdanović-Varga, J. (2011). Proizvodnja crnog luka (Onion production). In Milošević, M. & Kobiljski, B., *Semenarstvo III* (pp 433-480). Novi Sad: Institute of Field and Vegetable Crops.

Hassan, M.H.A., Allam, A.D.A., Abo-Elyousr, K.A.M., & Hussein, M.A.M. (2007). First report of stemphylium leaf blight of onion caused by *Stemphylium vesicarium* in Egypt. *Plant Pathology 56*(4), 724. doi: 10.1111/j.1365-3059.2007.01581.x

Hassan, M., Yousuf, V., Bhat, Z.A., Bhat, N.A., Shah, T.A., Khan, M.A. ... Shaf, S. (2020). Morpho-cultural and pathogenic variability among isolates of *Stemphylium vesicarium* (Wallr.) E. Simmons causing Stemphylium blight in onion collected from different geographical regions of Kashmir valley. *Indian Phytopathology*. *73*(9), 469-481. doi: 10.1007/s42360-020-00253-8

Hausbeck, M.K., Perla D.E., & Cook A.J. (2018). Evaluation of fungicides for control of Stemphylium leaf blight of onion, 2018. *Plant Disease Management Reports, 13,* V135.

Hay, F., Stricker, S., Gossen, B.D., McDonald, M.R., Heck, D., Hoepting, C., Sharma, S., & Pethybridge, S. (2021). Stemphylium leaf blight: A re-emerging threat to onion production in Eastern North America. *Plant Disease, 105* (12), 3780-3794. https://doi.org/10.1094/PDIS-05-21-0903-FE

Hoepting, C. (2016). Managing Stemphylium leaf blight. Spray by number to prevent fungicide resistance. *Onion World*, 32(6-10). Available at http://c919297.r97.cf2.rackcdn.com/kv3fapalp3iyewmyq2gipq2f1g9czb-optimized-pub.pdf

Hoepting, C. (2018a). Efficacy of fungicide treatments for control of Stemphylium leaf blight on onion, 2017. *Plant Disease Management Reports*, *12,* V144.

Hoepting, C. (2018b). Effect of fungicide programs for control of Stemphylium leaf blight on onion, 2017. *Plant Disease Management Reports, 12,* V146.

Hoepting, C. (2020). *Fungicide status for control of Stemphylium leaf blight of onion*. Retrieved from CCE Cornell Vegetable Program https://rvpadmin.cce.cornell.edu/uploads/doc_888.pdf

Hussein, M.A.M., Hassan, M.H.A., Allam, A.D.A., & Abo-Elyousr, K.A.M. (2007). Management of Stemphylium blight of onion by using biological agents and resistance inducers. *Egyptian Journal of Phytopathology, 35*(1), 49-60.

Ilić, Z., R. Filipović-Trajković, Lazić S., Bursić V., & Šunjka D. (2011). Maleic hydrazide residues in the onion bulbs induce dormancy and hamper sprouting for long periods. *Journal of Food, Agriculture and Environment 9*(1), 113-118. doi: 10.1234/4.2011.1918

Isenberg, F.M.R., Thomas, T. H., Pendergrass M., & Abdel-Rahman, M. (1974). Hormone and histological differences between normal and maleic hydrazide treated onions stored over winter. *Acta Horticulturae 38*, 95–125. doi: 10.17660/ActaHortic.1974.38.11

Jasnić, S. (2006). Pojava rezistentnosti gljiva prema fungicidima i antirezistentna strategija (Occurrence of fungi resistance to fungicides and antiresistance strategies). *Biljni lekar/ Plant Doctor, 34*(6), 458-468.

Johnson, D.A., & Lunden J.D. (1986). Effects of wounding and wetting duration on infection of asparagus by *Stemphylium vesicarium*. *Plant Disease. 70*, 419-420.

Kazakova, A. (1978). *Kulturna flora SSSR: Luk*. Leningrad, USSR: Kolos.

Köhl, J., Groenenboom-de Haas, B.H., Kastelein, P., Rossi, V., & Waalwijk, C., (2009). Quantitative detection of pear-pathogenic *Stemphylium vesicarium* in orchards. *Phytopathology, 99*(12), 1377-1386. doi: 10.1094/PHYTO-99-12-1377

Koike, S. T., Gladders, P., & Paullus, A. O. (2007). *Vegetable disease: A color handbook*. San-Diego, USA: Academic Press.

Leach, A., Hay, F, Harding, R., Damann, K.C., & Nault, B. (2020). Relationship between onion thrips (*Thrips tabaci*) and *Stemphylium vesicarium* in the development of Stemphylium leaf blight in onion. *Annals of Applied Biology, 176*, 55-64. doi: https://doi.org/10.1111/aab.12558

Llorente, I., & Montesinos, E. (2004). Development and field evaluation of a model to estimate the maturity of pseudothecia of *Pleospora allii* on pear. *Plant Disease 88*(2), 215-219. doi:10.1094/PDIS.2004.88.2.215

Llorente I., Moragrega C., Ruz L., & Montesinos E. (2012). An update on control of brown spot of pear. *Trees*, *26,* 239-245. doi:10.1007/s00468-011-0607-1

Lorbeer, J.W. (1993). A serious outbreak of Stemphylium leaf blight of onion in New York. In: *Proceedings of the 1993 National Onion Research Conference* (pp 32-37). Ithaca, NY, USA: Cornell University.

Mathur, K. & Sharma, S. N. (2006). Evaluation of fungicides against *Alternaria porri* and *Stemphylium vesicarium* disease of onion in Rajasthan. *Journal of Mycology and Plant Pathology. 36*(2), 323-324.

Meena, L., & Verma, A.K. (2017). Fungal diseases of onion and their biological management: A review. *International Journal of Recent Scientific Research, 8*(8), 19441–19445. doi: http://dx.doi.org/10.24327/ijrsr.2017.0808.0693

Mijatović, M., Obradović, A., & Ivanović, M. (2007). *Alliaceae* – lukovičasto povrće (crni luk, beli luk, praziluk). In: *Zaštita povrća (Protection of vegetables)* (pp 188-202). Smederevska palanka, Serbia: AgroMivas.

Miladinović, Ž., Damjanović, M., Brkić, S., Marković, Ž., Stevanović, D., Sretenović-Rajičić, T. ... Milić, B. (1997). *Gajenje povrća* (Vegetable growing). Belgrade: Školska knjiga.

Miller M.E., & Schwartz H.F. (2008). *Stemphylium* leaf blight and stalk rot. In: Swartz, H.F., Mohan, S.K. (eds.), *Compendium of onion and garlic diseases and pests*, (2nd edition) (pp 45-47). St Paul, Minnesota, USA: American Phytopathological Society.

Miller, M.E., Taber R.A, & Amador J.M. (1978). Stemphylium blight of onion in South Texas. *Plant Disease Reporter, 62,* 851-853.

Ministry of Agriculture, Forestry and Water Management of Republic of Serbia (2022). *Liste odobrenih supstanci* (Lists of approved substances). Available at http://www.minpolj.gov.rs/download/liste-odobrenih-supstanci/?script=lat

Misawa, T., & Yasuoka, S. (2012). The life cycle of *Stemphylium vesicarium*, the causal agent of Welsh onion leaf blight. *Journal of General Plant Pathology, 78,* 18-29. doi: 10.1007/s10327-011-0352-8

Mishra B., & Singh R.P. (2017). Fungicidal management of Stemphylium blight of onion caused by *Stemphylium vesicarium* (Wallr.) Simmons. *Biosciences Biotechnology Research Asia, 14*(3), 1043-1049. doi:10.13005/bbra/2539

Mishra, R.K., & Gupta R.P. (2012). *In vitro* evaluation of plant extracts, bio-agents and fungicides against Purple blotch and Stemphylium blight of onion. *Journal of Medicinal Plant Research, 6*(45): 5658-5661. doi: 10.5897/JMPR10-246

Mohan, C., Thind, T.S., Prem, R., & Arora, J.K. (2003). Promising activities of triazoles and other fungicides against fruit rot of chilli and stemphylium blight of onion. *Plant Disease Reporter, 19,* 200-203.

Paibomesai, M., Celetti M., & Tesfaendrias M. (2012). Update on stemphylium leaf blight of onions in Ontario. *Hort Matters, 12*(19), 11-12.

Paneru, N., Adhikari, P., & Tandan, P. (2020). Management of purple blotch complex of onion (*Allium Cepa* cv Red Creole) under field condition in Rukum-West, Nepal. *Malaysian Journal of Sustainable Agriculture*, *4*(2), 71-74.

Prados-Ligero, A.M., González-Andújar, J.L., Melero-Vara, J.M., & Basallote-Ureba, M.J., (1998). Development of *Pleospora allii* on garlic debris infected by *Stemphylium vesicarium*. *European Journal of Plant Pathology, 104*(9), 861-870.

Prados-Ligero, A.M., Melero-Vara, J.M., Corpas-Hervías, C., & Basallote-Ureba, M.J. (2003). Relationships between weather variables, airborne spore concentrations and severity of leaf blight of garlic caused by *Stemphylium vesicarium* in Spain. *European Journal of Plant Pathology, 109,* 301-310. doi: 10.1023/A:1023519029605

Rao, N.N.R., & Pavgi, M.S. (1975). Stemphylium leaf blight of onion. *Mycopathologia. 56*(2), 113-118.

Rossi, V., Pattori, E., & Bugiani, R. (2008). Sources and seasonal dynamics of inoculum for brown spot disease of pear. *European Journal of Plant Pathology 121,* 147-159.

Sharma, R.C. & Sharma, S. (1999). Stemphylium leaf blight and stalk rot. In Verma, L.R. & Sharma, R.C. (Eds.), *Diseases of horticultural crops: Vegetables, ornamentals and mushrooms* (pp 356-357), New Delhi, India: Indus Publishing Company.

Shishkoff, N., & Lorbeer, J.W. (1989). Etiology of stemphylium leaf blight of onion. *Phytopathology. 79,* 301-304.

Simmons, E.G., (1969). Perfect states of Stemphylium. *Mycologia 61*(1), 1-26. doi: 10.1080/00275514.1969.12018697

Singh, P., Park, P., Bugiani, R., Cavanni, P., Nakajima, H., Kodama, M., & Kohmoto, K., (2000). Effects of host-selective SV-toxin from *Stemphylium vesicarium*, the cause of brown spot of European pear plants, on ultrastructure of leaf cells. *Journal of Phytopathology 148*(2), 87-93.

Statistical Office of the Republic of Serbia (2022). *Crop production, Onion*. Available at: https://data.stat.gov.rs/Home/Result/130102?languageCode=sr-Cyrl&displayMode=table&guid=f0ee54df-289d-4264-b8d0-1aae69092077

Stricker, S.M. (2021). *Improved integrated pest management of Stemphylium leaf blight of onion*. (Ph.D. thesis). University of Guelph, Ontario, Canada.

Stricker, S.M., Tayviah C.S., Gossen B.D., & Mcdonald M.R. (2020). Fungicide efficacy and timing for the management of *Stemphylium vesicarium* on onion, *Canadian Journal of Plant Pathology*, *43*(2), 275-287. doi: 10.1080/07060661.2020.1804461

Suheri, H., & Price T.V. (2000). Infection of onion leaves by *Alternaria porri* and *Stemphylium vesicarium* and disease development in controlled environments. *Plant Pathology. 49* (3), 375-382. doi: 10.1046/j.1365-3059.2000.00458.x

Suheri, H., & Price T. V. (2001). The epidemiology of purple leaf blotch on leeks in Victoria, Australia. *European Journal of Plant Pathology. 107,* 503-510. doi: 10.1023/A:1011244104920

Tayviah C.S. (2017). *Epidemiology and management of Stemphylium leaf blight on onion (Allium cepa L.) in the Holland Marsh, Ontario*. (M.Sc. thesis). University of Guelph. Ontario, Canada.

Tesfaendrias, M.T., Paibomesai, M., Celetti, M. & McDonald, M.R. (2012). *The battle against Stemphylium leaf blight of onion in Ontario, Canada*. Available at http://www.hort.cornell.edu/expo/proceedings/2014/Onions/Stemphylium%20Tesfaendrias.pdf

Tomaz, I., & Lima A. (1986). An important disease of onion caused by *Stemphylium vesicarium* (Wallr.) Simmons in Portugal. *Horticultural Abstracts, 68,* 618.

Uddin M.N., Islam M.R., Akhtar N., & Faruq A.N. (2006). Evaluation of fungicides against Purple blotch complex of onion (*Alternaria porri* and *Stemphylium botryosum*) for seed production. *Journal of Agricultural Education and Technology 9*, 83-86.

Wolpert, T.J., Dunkle, L.D., & Ciuffetti, L.M. (2002). Host-selective toxins and avirulence determinants: what's in a name? *Annual Review of Phytopathology, 40,* 251-285. doi: 10.1146/annurev.phyto.40.011402.114210

Wright, P., Tyson, J., & Fullerton, R. (2018). *Stemphylium leaf blight of onion*. A Plant & Food Research report prepared for: Onions NZ. Milestone No. 76204. Contract No. 35374. Job code: P/340501/01. SPTS No. 16075. Available at https://www.vri.org.nz/dmsdocument/10-joy-tyson-stemphylium-leaf-blight-of-onion-infosheet-final-amended

Yusupov, Z., Deng, T., Volis, S., Khassanov, F., Makhmudjanov, D., Tojibaev, K., & Sun, H. (2021). Phylogenomics of *Allium* section *Cepa* (Amaryllidaceae) provides new insights on domestication of onion. *Plant Diversity 43(2)*, 102-110. doi: 10.1016/j.pld.2020.07.008

Stemphylium vesicarium (wallr.) E.G. Simmons: patogen crnog luka i mogućnost suzbijanja

REZIME

Crni luk (*Allium cepa*) je jedna od najznačajnijih povrtarskih vrsta koja se uzgaja u svetu i kod nas. Sušenje lista, uzrokovano gljivom *Stemphylium vesicarium*, je ozbiljna i destruktivna bolest lista crnog luka u svetu, koja ograničava kvalitet i kvantitet lukovice i semena. Smanjenje prinosa nastaje usled smanjene fotosintetske površine, što dovodi do obrazovanja manjih lukovica lošijeg kvaliteta. Preporučena strategija za suzbijanje i smanjenje inokuluma SLB uključuje plodored sa drugim povrtarskim vrstama ili žitaricama koje nisu domaćini ove gljive, korišćenje otpornih genotipova crnog luka, uklanjanje korova, pravilna upotreba azotnih đubriva, suzbijanje tripsa (*Thrips* spp.), kao i tretman semena, obzirom da seme ima značajnu ulogu u širenju patogena. Ključna strategija je svakako pravovremena i pravilna primena folijarnih fungicida. Vreme primene fungicida, različitih mehanizama delovanja je ključno za suzbijanje *Stemphylium vesicarium* na crnom luku.

Ključne reči: patogene gljive, suzbijanje bolesti, luk, sušenje lista, fungicidi

Pestic. Phytomed. (Belgrade), 38(2), 2023, 55–63
DOI: https://doi.org/10.2298/PIF2302055D

UDC 632.7:632.937.1:595.752 (497.11)
Original scientific paper

Bionomy of *Coccus pseudomagnoliarum* (Kuwana) (Hemiptera: Coccidae), a new species in the fauna of Serbia

Marina Dervišević[1]*, Aleksandar Stojanović[2] and Draga Graora[3]

[1]Institute of Pesticides and Environmental Protection, Banatska 31b, 11080 Belgrade, Serbia
[2]Natural History Museum, Njegoševa 51, 11000 Belgrade, Serbia
[3]University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Serbia
**Corresponding author: marina.dervisevic@pesting.org.rs*

Received: 16 May, 2023
Accepted: 15 June, 2023

SUMMARY

Citricola scale, *Coccus pseudomagnoliarum* (Kuwana) (Hemiptera: Coccidae) was for the first time registered in Serbia in 2015 in the area of Belgrade on *Celtis occidentalis* L. *C. pseudomagnoliarum* develops one generation annually and overwinters as the second-instar nymph on host twigs. It forms numerous colonies on infested plants, and symptoms of its feeding appear in the form of drying leaves and twigs. In addition, large amounts of honeydew that this scale secretes reduce photosynthesis and transpiration in plants, which accelerates their decay. Citricola scale attracts many entomophagous insects which are able to reduce pest population. The parasitoid wasps *Coccophagus lycimnia* (Walker), *Coccophagus piceae* Erdos, *Coccophagus scutellaris* (Dalman), *Coccophagus shillongensis* (Hayat and Singh) (Aphelinidae), *Cheiloneurus claviger* Thomson and *Metaphycus stanleyi* Compere (Encyrtidae) were reared. *C. piceae* and *M. stanleyi* are new species in the fauna of Serbia. *C. pseudomagnoliarum* is a new host for the species *M. stanleyi*. The predators *Coccinella septempunctata* L., *Exochomus quadripustulatus* (L.) (Coccinellidae) and *Chrysoperla carnea* (Stephens) (Chrysopidae) were found in scale colonies. The most efficient natural enemy of *C. pseudomagnoliarum* nymphs was *C. lycimnia*, reducing scale populations by 11-26%.

Keywords: citricola scale, Coccidae, natural enemies, Serbia

INTRODUCTION

Citricola scale, *Coccus pseudomagnoliarum* (Kuwana) (Hemiptera: Coccidae), originates from Asia, and is now considered a cosmopolitan species. In Europe, it is most widespread in Mediterranean countries, where it causes significant damage to citrus fruits, especially lemons, oranges, tangerines, and grapefruits (Barbagallo, 1974; Tena & Garcia-Marí, 2008a; Kapranas, 2012; García-Morales et al., 2016). In addition, it was found on pomegranate (*Punica granatum* L.), walnut (*Juglans regia* L.), as well as many ornamental plants in the genera *Berberis*, *Celtis*, *Pinus*, *Tamarix* and *Ulmus* (Japoshvili et al., 2008; Batsankalashvili et al., 2017).

In the former Yugoslavia, *C. pseudomagnoliarum* was first registered in the 1970s on citrus trees in Bar, Tivat and Herceg Novi (Velimirović, 1985).

The pest causes considerable damage to infested plants, especially citrus species, by feeding on plant sap, and thereby causing a decline in growth, and physiological weakening of plants. Indirect damage is caused by honeydew excretion as it covers the aboveground plant organs, which enables sooty mold to settle. All infested plant parts turn black, resulting in reduced photosynthesis and transpiration. Infested fruits are smaller, stunted and unsightly, and their quality and market value are lower (Masten-Milek et al., 2017; Kondo, 2022).

Citricola scale populations are controlled by a number of natural enemies. Fourty-five of such parasitoid species belonging to the families Aphelinidae, Encyrtidae, Eulophidae and Pteromalidae (Hymenoptera: Chalcidoidea) have been registered (Noyes, 2019). Predators from the families Anthocoridae, Geocoridae (Hemiptera), Coccinellidae (Coleoptera), Noctuidae and Pyralidae (Lepidoptera) have also been identified (Öncüer, 1977; Lázaro-Castellanos, 2022; Saleh, 2022).

In 2015, *Coccus pseudomagnoliarum* was detected for the first time in Serbia on *Celtis occidentalis* L. at the Ušće location in Belgrade (Graora et al., 2016), which led to more detailed subsequent studies. This paper provides data on the presence, infestation intensity and harmfulness of citricola scale, as well as information on its morphology, development cycle, and presence of its natural enemies.

MATERIALS AND METHODS

The study of citricola scale bionomy was carried out on *Celtis occidentalis* L. at three locations in Belgrade (Ušće, New Belgrade, Zemun) and in Požarevac over the period 2015-2017.

The presence, distribution, intensity of *C. pseudomagnoliarum* infestation and symptoms of damage were determined by visual examination of plants and sampling of infested plant material. The intensity of infestation was determined using the Borchsenius scale (1963). Plant material was sampled every 7-10 days during the vegetative period, and once a month during plant dormancy. Five one- or two-year-old twigs, 20 cm long were sampled from each infested plant.

In the laboratory, we examined the sampled plant material, reared and made permanent microscopic slides of citricola scale, and identified the scale and its natural enemies.

To analyze the morphological characteristics of the scale, permanent microscopic slides of females were made following a method of Kosztarab & Kozár (1988), and species identification was performed using the identification keys of Gill (1988) and Kosztarab & Kozár (1988).

For rearing purposes, the sampled twigs with scale colonies were placed in glass cylinders covered with dense synthetic meshes. The time of oviposition, number of eggs laid, and duration of embryonic and postembryonic development of scales were monitored by daily examination of the twigs. The average number of eggs laid by females was determined by counting the eggs of 10 females.

In addition, plant material was examined under binoculars to determine scale parasitism. The percentage of parasitism was calculated using the formula $P = B \times 100 / a$, where P – parasitism percentage, B – the number of parasitized scales, and a – the total number of examined scales in all samples (Hadzibeyli, 1983). Plant material with scale colonies was then placed in glass cylinders for rearing parasitoids. Examination was performed daily to determine the time and number of eclosed parasitoid specimens. Wasps were collected using an aspirator, killed with ethyl acetate and stored in gelatin capsules; they were then mounted on cards and identified by the second author. The mounted specimens are preserved in the Laboratory for Entomology and Agricultural Zoology, Faculty of Agriculture, University of Belgrade, Serbia.

Predator larvae collected with scales were reared individually in petri dishes in order to prevent cannibalism. Eclosed adults mounted on cards, and predatory ladybugs were identified using the Bieńkowski (2018) key.

RESULTS

The conducted analysis of morphological characteristics of female specimens led to conclusive identification of the species as *Coccus pseudomagnoliarum* (Kuwana, 1914).

Taxonomic status

Coccus pseudomagnoliarum belongs to the order Hemiptera, family Coccidae, subfamily Coccinae, tribe Coccini, genus Coccus (Choi & Lee, 2020).

Synonyms: *Lecanium (Eulecanium) pseudomagnoliarum*, Kuwana, 1914; *Coccus citricola*, Campbell, 1914; *Coccus aegaeus*, De Lotto, 1973a; *Coccus magnoliarum* (Kuwana, 1914) (García-Morales et al., 2016).

Morphological characteristics of *C. pseudomagnoliarum* female

The female has an elongated oval, convex body shape, 2-7 mm long. The color varies from gray to brown, with a central pale longitudinal ridge and two transverse pale bands extending from the stigmas. There are brown spots on the integument that merge with the edge of the female, so the scale takes on a marble pattern (Figure 1). The female antennae are 8-segmented. There are three setae in each group in the stigmatic depressions, and the central one is much longer than the lateral ones and curved at the top. Discoid pores are scattered throughout the body. Simple discoid pores form a transverse band on the abdomen. Quinquelocular pores are present in the area of stigmas. Multilocular pores are located in the area around anal plates.

C. pseudomagnoliarum life cycle

In the course of research, the species was found to reproduce by parthenogenesis and develop one generation annually. Its second-instar nymphs overwinter on twigs of *C. occidentalis*. In the spring, during March and April, nymphs continue feeding, and after molting they form females (Figure 1). The first appearance of females was recorded at the end of April in 2015 and 2017 and at the beginning of May in 2016 (Table 1). Females feed intensively on plant sap over the following three to four weeks, and then start laying eggs at the end of May. *C. pseudomagnoliarum* females are ovoviviparous. They lay eggs individually under their body. The duration of embryonic development depends primarily on weather conditions. When the weather is warm, nymphs hatch within an hour, while in cold and rainy weather they hatch after a day. The oviposition period is quite long and lasts from the end of May to the end of June. During that period, eggs laid and already hatched nymphs are located under the female scales. The average number of eggs laid per female is 799.6±3.3. The hatched nymphs migrate to leaves to feed there during the summer months, mostly on the reverse side of leaf along its main veins (Figure 2). Due to the extended egg-laying period, first-instar nymphs are present on leaves almost until mid-September, when they molt, forming second-instar nymphs (Figure 3). Second-instar nymphs continue to feed on leaves until October, when they descend to thicker twigs to overwinter (Figure 4).

Figure 1. Female of *C. pseudomagnoliarum* (orig.)

Figure 2. First-instar nymphs of *C. pseudomagnoliarum* (orig.)

Figure 3. Second-instar nymphs of *C. pseudomagnoliarum* (orig.)

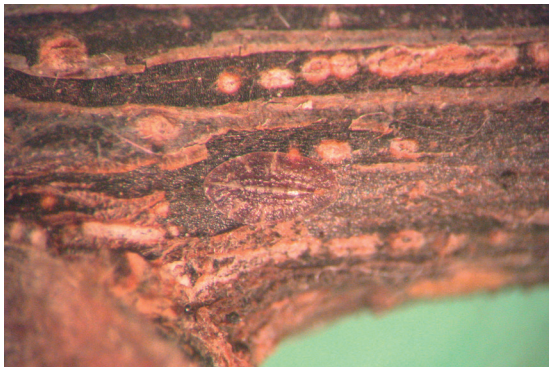


Figure 4. Overwintering second-instar nymphs (orig.)

Table 1. The life cycle of *Coccus pseudomagnoliarum* on *Celtis occidentalis*

Development stage	Year		
	2015	2016	2017
female	27.04.	04.05.	24.04.
egg	28.05.	25.05.	22.05.
N_1	29.05.	26.05.	22.05.
N_2	18.09.	15.09.	10.09.

N_1 – first-instar „crawler"
N_2 – second-instar

Figure 5. Colony of *C. pseudomagnoliarum* on *C. occidentalis* (orig.)

Figure 6. *C. occidentalis* covered with honeydew (orig.)

C. pseudomagnoliarum distribution, infestation intensity and damage symptoms

C. pseudomagnoliarum was found on *C. occidentalis* at three locations in Belgrade (Ušće, New Belgrade, Zemun) and a location in Požarevac. The highest intensities of infestation at all locations in Belgrade and Požarevac were level 3 and 4, so that the aboveground plant parts were covered with dense scale colonies (Figure 5). *C. pseudomagnoliarum* nymphs generally cluster on leaves along their main nerves, while females inhabit the twigs of host plants. As a result of feeding, the observed symptoms include chlorotic spots and lesions, discoloration and premature leaf fall, and drying of individual twigs. Apart from direct damage caused by feeding, *C. pseudomagnoliarum* also produces large amounts of honeydew (Figure 6), which is a suitable substrate for development of sooty mold that reduces photosynthesis and transpiration, devestating plant aesthetics.

Natural enemies of *C. pseudomagnoliarum*

During the research, 6 species of parasitoid wasps (Hymenoptera: Chalcidoidea) were found and reared from *C. pseudomagnoliarum* colonies. Four species were identified as belonging to the genus *Coccophagus* (family Aphelinidae): *C. lycimnia* (Walker), *C. piceae* Erdos, *C. scutellaris* (Dalman), and *C. shillongensis* (Hayat and Singh). Another two species, *Cheiloneurus claviger* Thomson and *Metaphycus stanleyi* Compere of the family Encyrtidae, were also observed (Table 2). In this study, two parasitoid wasps, *C. piceae* and *M. stanleyi*, were detected for the first time in the fauna of Serbia. For the species *C. piceae*, citrus scale is a new host. In the family Aphelinidae, the most numerous and efficient natural enemy was *C. lycimnia*, whose parasitism in second-instar nymphs of citricola scale was 11-26%. In the family Encyrtidae, the newly-identified species *M. stanleyi* was the most numerous as a gregarious parasitoid.

Three distinct predator species were successfully reared and identified during the study: *Coccinella septempunctata* L., *Exochomus quadripustulatus* (L.) (Coleoptera, Coccinellidae) and *Chrysoperla carnea* (Neuroptera, Chrysopidae). These species were individually present in *C. pseudomagnoliarum* colonies (Table 2). Larvae and adults of these predators fed on all developmental stages of citricola scale.

Table 2. Natural enemies of *C. pseudomagnoliarum*

Order	Family	Species	Location	Total eclosed individuals
Hymenoptera	Aphelinidae	*Coccophagus lycimnia*	New Belgrade	37
			Ušće	37
			Zemun	1
		*Coccophagus piceae**	Ušće	5
		Coccophagus scutellaris	New Belgrade	1
		Coccophagus schillongensis	New Belgrade	2
			Ušće	5
	Encyrtidae	*Cheiloneurus claviger*	Ušće	3
		*Metaphycus stanleyi**	New Belgrade	20
			Ušće	383
Coleoptera	Coccinelidae	*Coccinella septempunctata*	Ušće	2
		Exochomus quadripustulatus	Ušće	1
			New Belgrade	2
Neuroptera	Chrysopidae	*Chrysoperla carnea*	Ušće	2

*new species in Serbia

DISCUSSION

After its first detection in Serbia in 2015 on *C. occidentalis* at the location Ušće (Belgrade), *C. pseudomagnoliarum* was found at two additional locations in the territory of Belgrade (New Belgrade and Zemun), and also in Požarevac. Significant citricola scale infestations were observed at all of these locations, forming numerous colonies on infested plants. Extensive feeding of numerous nymphs and females caused leaves to dry out and fall off, and individual twigs to dry out.

C. pseudomagnoliarum has been detected on over 30 host plants from 16 families worldwide (García-Morales et al., 2016) but it most commonly causes high damage with economic impact in citrus-growing areas (Velimirović, 1985; Bernal et al., 2001; Mohamed et al., 2012). In Croatia, it is a widespread pest that inflicts significant damage on citrus fruits. Through its sap-sucking activities, the pest induces substantial weakening of host plants, leading to their decay (Masten-Milek, 2007; Masten-Milek et al., 2017). In California, it has been found to cause a 43% decrease in orange yields (Grafton-Cardwell et al., 2022). In addition, an intense infestation by this pest was detected in a commercial citrus orchard in Croatia, where neither agrotechnical nor chemical control measures had been applied. It is hypothesized that the lush canopy of fruit trees along with high humidity in the canopy, create favourable conditions for intensive reproduction of this species (Markotić, 2023).

In Serbia, *C. pseudomagnoliarum* develops one generation per annum and reproduces by parthenogenesis, which is consistent with data from Israel (Ben-Dov, 1980), Montenegro (Velimirović, 1985), Hungary (Fetykó et al., 2013), Croatia (Masten-Milek et al., 2017), Greece (Stathas & Karipidis, 2020) and California (Gill, 1988). Its females are ovoviviparous. Oviposition was observed from the end of May to the end of June, and females were found to lay an average of about 800 eggs each. In Hungary, a female lays between 250 and 280 eggs from mid-May to mid-July (Fetykó et al., 2013). Its nymphs hatch within a few hours and promptly migrate to plant leaves, where they feed until the end of summer. After this period they molt and form second-instar nymphs, which descend onto twigs to prepare for overwintering there. Typically, the species overwinters as the second-instar nymph, while young overwintering females were reported in Italy by Barbagallo (1974).

The abundance of *C. pseudomagnoliarum* is regulated by numerous natural enemies. In this study, 6 species of parasitoid wasps and 3 species of predators were reared from colonies of this scale. Among the identified species of parasitoids are four species from the genus *Coccophagus* (family Aphelinidae). This genus includes about 200 described species that are parasitoids of scale insects, most commonly from the family Coccidae, but they have also been found to be parasitoids of Diaspididae, Pseudococcidae, Eriococcidae, and Kermesidae (Hayat, 1997; Noyes, 2019). Females of the genus *Coccophagus* are endoparasitoids of scales, while males can also be

secondary hyperparasitoids (Kapranas et al., 2007). Most species are polyphagous and parasitize different hosts, and some species are described as potential biological agents for scale insect control (Schweizer et al., 2003). During our survey, *C. lycimnia* demonstrated the highest efficiency as a parasitoid targeting second-instar nymphs, with parasitation levels reaching 11-26%.

Among the established species of this genus, *C. piceae* is new to the fauna of Serbia. According to literature data, *C. piceae* has been identified on *Physokermes piceae* (Schrank), *Pulvinaria vitis* (L.), *Pulvinaria* sp. and *Didesmococcus unifasciatus* (Archangelskaya) (Ülgentürk, 2001; Bolu, 2012; Noyes, 2019). Notably, during this study, *C. pseudomagnoliarum* was determined as a new host for this parasitoid. Furthermore, the discovery of the species *C. shillongensis*, which was registered for the first time in Serbia in 2016, is also of significant importance. At the time, it was found on five other new hosts besides *C. pseudomagnoliarum* (Dervišević et al., 2021). The first finding of the species *M. stanleyi* in the genus *Metaphycus* was also reported. Up to 20 specimens of this gregarious parasitoid wasp were observed to hatch from a single *C. pseudomagnoliarum* female. Generally, one or more larvae of this parasitoid may develop in a female scale, depending largely on body size of the host (Bernal et al., 1999). *M. stanleyi* is the primary endoparasitoid for over 30 species of the Coccidae family (Noyes, 2019). As a biological control agent, it was introduced from South Africa to California in 1937 to control *Saissetia oleae* (Olivier) (Bartlett, 1978).

Along the Montenegrin coast, the species *C. lycimnia* and *Metaphycus flavus* (Howard) have significantly reduced the number of citricola scale populations (Velimirović, 1994). In Greece, two species of parasitoids, *Coccophagus shillongensis* and *Metaphycus dispar* (Mercet), reached a parasitism rate of 35% (Stathas & Karipidis, 2020), while in Spain the species *Metaphycus helvolus* (Compere) exhibited a noteworthy parasitism rate of 50% (Tena & García-Marí, 2008b).

Besides, the predators *Coccinella septempunctata*, *Exochomus quadripustulatus* (Coleoptera, Coccinellidae) and *Chrysoperla carnea* (Neuroptera, Chrysopidae) were also reared. However, all predatory species were present in relatively low numbers in scale insect colonies. Numerous predators of *C. pseudomagnoliarum* have been recorded worldwide, including the ladybugs *Chilocorus bipustulatus* (L.), *C. renipustulatus* (Scriba), *Cryptolaemus montrouzieri* (Mulsant), *Exochomus quadripustulatus*, *Serangium parcesetosum* (Sicard), and *Rhyzobius lophanthae* (Blaisdell) (Deeb et al., 2017; Basheer et al., 2022; Saleh, 2022). *C. bipustulatus* and *S. parcesetosum* were found to reduce the number of scale nymphs under controlled conditions by 97.8 and 99.2%, respectively (Deeb et al., 2017).

CONCLUSION

The data in the present study provide information concerning the biology of and damage caused by *C. pseudomagnoliarum,* which is considered a new pest in Serbia. Although the scale is currently found only on *C. occidentalis*, it may be considered as a potentially serious threat after being reported as an important pest of citrus and ornamental plants in many parts of the world. Also, data on the diversity and number of natural enemies are significant for the control of *C. pseudomagnoliarum,* specifically in urban areas, where chemical pest control measures are rarely applied.

ACKNOWLEDGMENT

This research was funded by the Ministry of Science, Technological Development and Innovations of the Republic of Serbia, Grant No. 451-03-47/2023-01/ 200214.

REFERENCES

Barbagallo, S. (1974). Notizie sulla presenza in Sicilia di una nuova cocciniglia degli agrumi. *Coccus pseudomagnoliarum* (Kuwana) (Homoptera, Coccidae). Osservazioni biologiche preliminari. *Entomologica – Annali di Entomologia Generale ed Applicata, 10,* 121-139.

Bartlett, B. R. (1978). Coccidae. In C.P. Clausen (Ed.*), Introduced parasites and predators of arthropod pests and weeds: A world review. Agriculture Handbook* (pp 57-74). Washington, D.C.: U.S. Department of Agriculture, U.S. Government Printing Office.

Basheer, A., Saleh, A., & Fandi, J. (2022). A study of some ecological indices of numerical diversity of predators associated with *Coccus pseudomagnoliarum* (Kuwana) (Hemiptera: Coccomorpha: Coccidae) in Latakia Governorate. *Damascus University Journal of Agricultural Sciences, 38*(4), 214-238.

Batsankalashvili, M., Kaydan, M.B., Kirkitadze, G., & Japoshvili, G. (2017). Updated checklist of scale insects (Hemiptera: Coccomorpha) in Sakartvelo (Georgia). *Annals of Agrarian Science, 15*(2), 252-268. doi: 10.1016/j.aasci.2017.05.002

Ben-Dov, Y. (1980). Observations on scale insects (Homoptera: Coccoidea) of the Middle East. *Bulletin of Entomological Research, 70,* 261-271.

Bernal, J., Luck., R.F., & Morse, J.G. (1999). Host influences on sex ratio, longevity, and egg load of two *Metaphycus* species parasitic on soft scales: implications for insectary rearing. *Entomologia Experimentalis et Applicata, 92,* 191-204.

Bernal, J.S., Luck., R.F., Morse, J.G., & Drury, M.S. (2001). Seasonal and scale size relationships between citricola scale (Homoptera: Coccidae) and its parasitoid complex (Hymenoptera: Chalcidoidea) on San Joaquin Valley citrus. *Biological Control, 20,* 210-221. doi: 10.1006/bcon.2000.0905

Bieńkowski, A.O. (2018). Key for identification of the ladybirds (Coleoptera: Coccinellidae) of European Russia and the Russian Caucasus (native and alien species). *Zootaxa, 4472*(2), 233-260. doi: 10.11646/zootaxa.4472.2.2

Bolu, H. (2012). A new pest on almond tree, the soft scale *Didesmococcus unifasciatus* (Archangelskaya) (Hemiptera: Coccidae) and its new records parasitoids, Turkey. *Journal of the Entomological Research Society, 14*(1), *107-114.*

Borchsenius, N.S. (1963). *Praktičeskij opredelitely kokcid (Coccoidea) kulturnyh rastenij i lesnyh porod SSSR. (Practical determination of coccids (Coccoidea) on cultivated plants and forest trees in SSSR)*. Moscow - Leningrad, SSSR: Akademij Nauk.

Choi, J., & Lee, S. (2020). Molecular phylogeny of the family Coccidae (Hemiptera, Coccomorpha), with a discussion of their waxy ovisacs. *Systematic Entomology, 45*(2), 396-414. doi: 10.1111/syen.12404

Deeb, S., Abboud, R., Mofleh, M., & Ahmad, M. (2017). Evaluation the impact of both predators *Serangium parcesetosum* Sicard and *Chilocorus bipustulatus* L. (Coleoptera: Coccinellidae) in controlling citricola scale insect *Coccus pseudomagnoliarum* (Kuwana) and olive scale *Saissetia oleae* (Olivier) (Hemiptera: Coccidae) on citrus, Syria. *Syrian Journal of Agricultural Research, 4*(4), 133-139.

Dervišević, M., Stojanović, A., & Graora, D. (2021). First record and new hosts of *Coccophagus shillongensis* Hayat and Singh, 1989 (Hymenoptera: Aphelinidae) in Serbia. *Phytoparasitica, 49,* 547-551. doi: 10.1007/s12600-021-00886-z

Fetykó, K., Szita, É, & Konczné-Benedicty, Z. (2013). New species of Coccidae, *Coccus pseudomagnoliarum* (Kuwana) recorded on common hackberry (*Celtis occidentalis* L.) in urban environment. *Növényvédelem, 49*(12), 565-569. (In Hungarian)

García-Morales, M., Denno, B.D., Miller, D.R., Miller, G.L., Ben-Dov, Y., & Hardy, N.B. (2016). *ScaleNet: A literature-based model of scale insect biology and systematics*. Retrieved from: http://scalenet.info/

Gill, R.J. (1988). *The scale insects of California, Part I: The soft scales (Homoptera: Coccoidea: Coccidae)*. Technical Series in Agricultural Biosystematics and Plant Pathology. Sacramento, California, USA: California Department of Food and Agriculture.

Grafton-Cardwell, E.E., Gu, P., & Daugherty, M.P. (2022). Impact of citricola scale *Coccus pseudomagnoliarum* (Hemiptera: Coccidae) on Valencia orange, *Citrus sinensis* production and fruit quality. *Journal of Economic Entomology*, *115*(6), 2121–2124. doi: 10.1093/jee/toac169

Graora, D., Dervišević, M., Gajić, M., & Spasić, R. (2016). *Coccus pseudomagnoliarum* Kuwana (Hemiptera: Coccidae), a new soft scale species in Serbia. In: *Book of Abstracts of the XV Symposium of Plant Protection* (pp 33), Zlatibor. Belgrade, Serbia: Plant Protection Society of Serbia. (In Serbian)

Hadzibeyli, Z. (1983). *Koktsidi subtropicheskoi zoni Gruzii (Coccids of the subtropical zone of Georgia).* Tbilisi, Georgia: Akademia Nauk Gruzii, Metsniereba (Georgian Academy of Sciences).

Hayat, M. (1997). Aphelinidae. In Y. Ben-Dov & C.J. Hodgson (Eds.), *Soft scale insects: Their biology, natural enemies and control, Vol. 7B* (pp 111–145). Amsterdam, Netherlands: Elsevier.

Japoshvili, G., Gabroshvili, N., & Japoshvili, B. (2008). Scale insect pests on ornamental plants in city Tbilisi. *Proceedings of the Institute of Zoology, 23,* 147-152.

Kapranas, A. (2012). Coccidae. In V. Vacante & U. Gerson (Eds.), *Integrated control of citrus pests in the Mediterranean region* (pp 183-191). Bentham Science Publishers. doi: 10.2174/97816080529431120101

Kapranas, A., Morse, J.G., Pacheco, P., Forster, L.D., & Luck, R.F. (2007). Survey of brown soft scale *Coccus hesperidum* L. parasitoids in southern California citrus. *Biological Control 42*, 288-299. doi: doi.org/10.1016/j.biocontrol.2007.05.012

Kondo, T. (2022). Family Coccidae. In T. Kondo & G. Watson (Eds.), *Encyclopedia of scale insect pests* (pp 225-349). Wallingford, UK: CABI.

Kosztarab, M., & Kozár, F. (1988). Systematic part. In: *Scale insects of Central Europe*, Series Entomologica, Vol. 40 (32-387). Dordrecht. Netherlands: Springer. doi: https://doi.org/10.1007/978-94-009-4045-1_2

Lázaro-Castellanos, C., Gómez-Domínguez, N.S., & Vanegas Rico, J.M. (2022). *Laetilia Coccidivora* (Comstock,

1879) preying on *Coccus pseudomagnoliarum (*Kuwana, 1914) in Mexico, first record of association prey-predator host plant (Lepidoptera: Pyraloidea). *SHILAP Revista de Lepidopterología*, *50*(197), 167-170. doi: 10.57065/shilap.205

Markotić, V. (2023). *Fauna of insects from the suborder Sternorrhyncha in citrus orchards in the Republic of Croatia*. (Doctoral thesis). University of Zagreb, Faculty of Agriculture.

Masten-Milek, T. (2007). *Fauna of scale insects (Insecta: Coccoidea) in the Republic of Croatia*. (Doctoral thesis). Josip Juraj Strossmayer University in Osijek, Faculty of Agriculture.

Masten-Milek, T., Markotić, V., Šimala, M., & Pintar, M. (2017). Grey citrus scale (*Coccus pseudomagnoliarum* (Kuwana, 1914)) – economicaly important pest of citrus. *Glasilo biljne zaštite 17*(6), 548-556.

Mohamed, E.M., Basheer, A.M., & Abukaf, N. (2012). Survey of parasitoid species of citricola scale insect, *Coccus pseudomagnoliarum* (Kuwana) (Homoptera: Coccidae) and their effect in citrus orchards at Lattakia, Syria. *Egyptian Journal of Biological Pest Control*, *22,* 61-65.

Noyes, J.S. (2019). *Universal Chalcidoidea Database*. World Wide Web electronic publication http://www.nhm.ac.uk/our-science/data/chalcidoids/

Öncüer, C. (1977). Investigation on the identification, distribution, and efficiency of the natural enemies of some Coccoidae (Homoptera) which infect the fruit trees in Izmir Province. *Journal of Agriculture Faculty of Ege University, 336,* 122-129.

Saleh, A. (2022). Relative abundance of insect predators associated with citricola scale *Coccus pseudomagnoliarum* (Kuwana) (Hemiptera: Coccidae) within citrus orchards in Lattakia Governorate. *Damascus University Journal of Agricultural Sciences, 38*(3), 69-96. (In Arabic)

Schweizer, H., Luck, R.F., & Morse, J.G. (2003). Augmentative releases of *Metaphycus* sp. nr. *flavus* against citricola scale on oranges in the San Joaquin Valley of California: are early releases better than late ones? *Journal of Economic Entomology, 96,* 1375-1387. doi: 10.1093/jee/96.5.1375

Stathas, G.J., & Karipidis, C.F. (2020). The scale insect *Coccus pseudomagnoliarum* (Kuwana) (Hemiptera: Coccomorpha: Coccidae) on citrus in Greece. *Hellenic Plant Protection Journal*, *13*(2), 93-97. doi: 10.2478/hppj-2020-0010

Tena, A., & García-Marí, F. (2008a). Suitability of citricola scale *Coccus pseudomagnoliarum* (Hemiptera: Coccidae) as host of *Metaphycus helvolus* (Hymenoptera: Encyrtidae): Influence of host size and encapsulation. *Biological Control 46*(3), 341-347.

Tena, A., & García Marí, F. (2008b). Parasitoides de *Coccus pseudomagnoliarum* (Hemiptera: Coccidae), una nueva plaga en los cítricos valencianos. *Levante Agrícola, 400,* 47-54.

Ülgentürk, S. (2001). Parasitoids and predators of Coccidae (Homoptera: Coccoidea) species on ornamental plants in Ankara, Turkey. *Acta Phytopathologica et Entomologica Hungarica, 36*(3-4), 369-375. doi: 10.1556/APhyt.36.2001.3-4.18

Velimirović, V. (1985). *Štitaste uši (Coccoidea) na agrumima u južnom dijelu Crne Gore s posebnim osvrtom na Coccus pseudomagnoliarum Kuwana, 1914*. (Doktorska disertacija). Fakultet poljoprivrednih znanosti, Zagreb.

Velimirović, V. (1994). Natural enemies on *Coccus pseudomagnoliarum* Kuwana in costal part of Montenegro. *Plant Protection, 45*(2), 139-150.

Bionomija *Coccus pseudomagnoliarum* (Kuwana) (Hemiptera: Coccidae), nove vrste u fauni Srbije

REZIME

Tokom 2015. godine, u Srbiji je prvi put registrovana štitasta vaš citrusa, *Coccus pseudomagnoliarum* (Kuwana) (Hemiptera: Coccidae), na *Celtis occidentalis* L. u Beogradu. *C. pseudomagnoliarum* tokom godine razvija jednu generaciju i prezimljava u stadijumu larve drugog stupnja na grančicama domaćina. Na infestiranim biljkama obrazuje brojne kolonije, usled čije ishrane se javljaju simptomi u vidu sušenja listova i grančica. Osim toga, velika količina medene rose koju ova vaš luči, smanjuje fotosintezu i transpiraciju biljaka što ubrzava njihovo propadanje. Štitasta vaš citrusa privlači brojne entomofagne insekte koji mogu redukovati brojnost njenih populacija. Odgajene su parazitoidne osice *Coccophagus lycimnia* (Walker), *Coccophagus piceae* Erdos, *Coccophagus scutellaris* (Dalman), *Coccophagus shillongensis* (Hayat and Singh) (Aphelinidae), *Cheiloneurus claviger* Thomson i *Metaphycus stanleyi* Compere (Encyrtidae). *C. piceae* i *M. stanleyi* su nove vrste u fauni Srbije. *C. pseudomagnoliarum* je novi domaćin za vrstu *M. stanleyi*. Od predatora, utvrđene su vrste *Coccinella septempunctata* L., *Exochomus quadripustulatus* (L.) (Coccinellidae) i *Chrysoperla carnea* (Stephens) (Chrysopidae). Najefikasniji prirodni neprijatelj larvi *C. pseudomagnoliarum*, bila je *C. lycimnia*, redukujući brojnost populacija za 11-26%.

Ključne reči: štitasta vaš citrusa, Coccidae, prirodni neprijatelji, Srbija

Pestic. Phytomed. (Belgrade), 38(2), 2023, 65–73
DOI: https://doi.org/10.2298/PIF2302065D

UDC 632.951:632.95.024:632.7+632.937.1
Original scientific paper

Comparative toxicity of spinetoram to *Trialeurodes vaporariorum* Westwood and its parasitoid *Encarsia formosa* Gahan

Tanja Drobnjaković[1]*, Mirjana Prijović[1], Emanuele Porcu[2], Michele Ricupero[2], Gaetano Siscaro[2], Lucia Zappalà[2] and Antonio Biondi[2]

[1]Institute of Pesticides and Environmental Protection, Banatska 31b, 11000 Belgrade, Serbia
[2]Department of Agriculture Food and Environment, University of Catania, via S. Sofia 100, 95123 Catania, Italy
**Corresponding author: tanjadrobnjakovic@gmail.com*

Received: 4 May 2023
Accepted: 10 July, 2023

SUMMARY

The role of selective new generation bioisecticides, beside their effectiveness against key pests, relies on their safety to beneficial arthropods. Spinetoram, a semi-synthetic analogue of the microbial-derived bioinsecticide spinosad is registered worldwide for application in numerous crops, but assessment of its ecotoxicological risk to beneficial arthropods has scarcely been documented. Moreover, this is the first report on toxic effects of spinetoram on a pest, the greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae), and/or its successful biocontrol agent, the parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae). Under laboratory conditions, we assessed the acute toxicity of spinetoram insecticide (25% a.i.) to adults, nymphs and eggs of the greenhouse whitefly, as well as to parasitoid adults and pupae. In all concentration-response bioassays, the spinetoram insecticide was applied to tobacco leaves settled onto 1% agar layer in ventilated Petri dishes using a Potter spray tower. The parameters of spinetoram acute toxicity to adults of both the pest and the parasitoid were evaluated in residual contact bioassays, while whitefly eggs and nymphs, and parasitoid pupae were topically treated with a series of spinetoram concentrations, covering a range of 10-90% mortality. Lethal spinetoram effects on the parasitoid *E. formosa* were assessed through selectivity ratio (SR) estimations, showing the ratios beetween median lethal concentrations (LC_{50s}) estimated for the parasitoid, and LC_{50s} estimated for the pest. The following LC_{50} values were obtained: 4.593, 15.027 and 11.73 mg a.i./l for whitefly adults, nymphs and eggs, respectively, and 0.686 and 1.715 mg a.i./l for parasitoid adults and pupae, respectively. The calculated SR estimations were below 1, indicating that spinetoram insecticide is non-selective to both tested stages of the parasitoid *E. formosa.* A more detailed understanding of spinetoram impact on *E. formosa* in whitefly integrated management requires further evaluation of sublethal effects and greenhouse trials, with an emphasis on population-level responses.

Keywords: bioinsecticide, *Encarsia formosa*, IPM, selectivity ratio, whitefly

INTRODUCTION

Insecticide resistance in whiteflies, caused by overused synthetic chemicals and the associated side effects on the environment and non-target organisms, urgently promotes the use of alternative and environment-friendly control strategies (Kapantaidaki et al., 2018; Patra & Kumar Hath, 2022; Mota-Sanchez & Wise, 2023). In the concept of Integrated Pest Management (IPM), which is considered a more ecologically sound and sustainable strategy for whitefly control, biological control with parasitoids plays an essential role (van Lenteren & Martin, 1999; Gerling et al., 2001). The dominant parasitoid species *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) has been utilized for biological control of two most harmful whitefly species, the greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) and the tobacco whitefly *Bemisia tabaci* (Gennadius). For many decades, it has been considered one of the most successful biological control agents in greenhouse crops in many world regions (Hoddle et al., 2001; Bacci et al., 2007; Li et al., 2011; Sugiyama et al., 2011). Due to unfavorable ecological conditions for the parasitoid and/or high pest population density, insecticide treatments are necessary when *E. formosa* fails to keep a whitefly population below the economic threshold (Hoddle et al., 1998; Albajes et al., 1999). Biopesticides, i.e. commercial pest control agents manufactured from living organisms and/or their products (Chandler et al., 2011; Kumar et al., 2021), have been marked as potentially compatible for use with biocontrol agents in IPM or organic production. Low mammalian toxicity, looser harvest and re-entry restrictions and higher safety towards non-target organisms and the environment are some of the most frequently mentioned advantages of biopesticides over synthetic chemical compounds (Copping & Menn, 2000; Villaverde et al., 2014; Kumar et al., 2021).

Spinetoram, a semi-synthetic analogue of the microbial-derived bioinsecticide spinosad, has been classified by the US Environmental Protection Agency (EPA) as a "reduced risk pesticide" (Chloridis et al., 2007), which could be used as an alternative choice for controlling homopteran insect pests, such as whiteflies. Currently, spinetoram-based biopesticides are registered worldwide for application in many crops. It has been shown that they are highly effective against thrips, leafminer flies, some lepidopteran pest and whiteflies, among others (Dripps et al., 2011; Shimokawatoko et al., 2012; Abd-Ella, 2015). Although spinetoram has shown a reduced-risk profile in preliminary risk assessments, having toxicological properties similar to spinosad (Chloridis et al., 2007), the role of this potentially selective new generation bioinsecticide, besides its effectiveness against key pests, depends on its safety to non-target organisms. An increasing number of studies assessing the risk of spinosad to beneficial arthropods have been published in recent decades, suggesting that spinosad is non-selective to natural enemies of insects, especially parasitoids (Williams et al., 2003, Biondi et al., 2012). On the other hand, ecotoxicological assessment of spinetoram risk to beneficials has hardly been documented. There have been only a few reports from laboratory or field studies dealing with lethal and/or sublethal effects of spinetoram on predatory insects (Srivastava et al., 2008; Lefebvre et al., 2011; Ricupero et al., 2020) and parasitoids (Hernández et al., 2011; Abbes et al., 2015; Abd-Ella, 2015). Furthermore, to the best of our knowledge, no previous studies have quantified the effects of spinetoram on the greenhouse whitefly or its parasitoid *E. formosa*. Since many bioinsecticides that are effective against target pests are also harmful to parasitoids, causing lethal and/or sublethal effects and reducing the effectiveness of biological control agents (Desneux et al., 2007; Biondi et al., 2013; Drobnjaković et al., 2018, 2019; Giunti et al., 2022; Shankarganesh et al., 2022), integration of spinetoram and *E. formosa* in IPM programs requires to understand the toxicology of spinetoram regarding both the pest and its parasitoid.

Within that framework, this study aimed to evaluate the acute toxicity of spinetoram to the greenhouse whitefly and its parasitoid under laboratory conditions. The results of this study should serve as a starting point for further research of its effects, contributing to the optimization of integrated whitefly control strategies.

MATERIALS AND METHODS

Biological materials

A laboratory colony of *T. vaporariorum* was initiated from infested tomato plants collected from a commercial greenhouse in Padinska Skela, Serbia (N44°56'56.35"; E020°25'41.41") in the autumn of 2018. After the laboratory colony was established, the whitefly population in the greenhouse had no further contact with insecticides and did not experience any further selection pressure. A commercial strain of *E. formosa* with normal sensitivity to pesticides was successfully bred, starting from the pupal stage of the parasitoid obtained from Koppert Biological Systems Inc (The Netherlands). The parasitoid wasp population was grown together with its host pest at 27±1°C and 60±10% R.H. under a 16L:8D photoperiod.

Both the pest and the parasitoid were maintaned on four weeks old *Nicotiana tabacum* L. plants (with 6-8 fully developed leaves) of "Samsun" variety in ventilated muslin cages (Bug Dorm, Megaview Science Co., Ltd., Taiwan) in accordance with the recommended methodology of the European Plant Protection Organisation (EPPO, 2004).

Insecticide

The commercial insecticide Delegate™ 250 (manufactured by Corteva Agriscience) is formulated as water dispersible granules (WG). Its content of spinetoram as the active ingredient was 250 g/l.

Acute toxicity bioassays

All concentration-response bioassays for both the pest and its parasitoid were performed in five replicates in a climate chamber under the same controlled laboratory conditions as described for insect rearing. Bioassays were conducted in Petri dishes (14 cm diameter), each with a lid opening (10 cm diameter) and covered with muslin on top to ensure ventilation and prevent condensation inside. Whiteflies at different stages of development (adult, nymph and egg), and adults and pupae of the parasitoid wasp were added to Petri dishes as needed. Dilutions of the insecticide spinetoram were prepared with distilled water to obtain final concentrations (at least 6) of the active ingredient (a.i.). Since products based on spinetoram are not yet authorized for whitefly control in protected crops, preliminary trials were conducted to determine the range of insecticide concentrations by exposing each test stage to a concentration equivalent to a higher application rate recommended for the control of some other pests of greenhouse solanaceous vegetables (tomato, eggplant, cucumber, pepper and lettuce - 132 g/ha; 33 mg a.i/l)), until the observed mortality was < 100 %. A Potter Precision Spray Tower (Burkard Scientific, UK) was used to spray 2 ml of liquid under 100 kPa air pressure to create a water deposit of 2.6±0.2 mg/cm^2 in each dish. Control treatments for each acute toxicity bioassay were sprayed with distilled water only.

A) ***Acute toxicity bioassays with T. vaporariorum.*** Ten couples of two-day-old adult whiteflies (per replicate) were anaesthetised with CO_2 for 2 seconds and then carefully transferred into each muslin bag placed over fully developed tobacco leaves (4-week-old plants) to lay eggs for 24 hours. Adult whiteflies were then removed from each bag, while tobacco plants remained in the cages for additional 48 hours. After that period, the tobacco leaves were cut from the plants and observed under a stereomicroscope to record the number of eggs laid. Each leaf containing 40 eggs was considered an experimental unit. Tobacco leaves were then treated with a range of spinetoram concentrations (37.5, 18.75, 9.37, 4.69, 2.34, and 1.17 mg a.i./l) and, after complete air drying, transferred individually to clean Petri dishes containing agar layers. Tobacco leaves remained in Petri dishes until the L_1 crawling stage (crawler) hatched from the treated eggs; hatching was observed and recorded under a stereomicroscope. Mortality was calculated using the number of crawlers emerged in relation to the number of treated eggs, 7 days after treatment.

Similar to the egg bioassay, adult pests were anaesthetised in a concentration-response bioassay with whitefly nymphs and transferred to muslin bags for a period of 24 hours to lay eggs. After that period, adult whiteflies were removed from the bags, while tobacco plants remained in the cages for a period of about 18 days until the laid eggs have developed into fourth-instar nymphs. Tobacco leaves were then cut from the plants to count the number of fourth-instar whitefly nymphs under a stereomicroscope. Tobacco leaves with nymphs were then treated with a range of spinetoram concentrations (37.5, 18.75, 9.37, 4.69, 2.34, and 1.17 mg a.i./l) and, after air drying, transferred individually to clean Petri dishes on agar layers where they remained until adults emergence. Mortality was calculated from the number of emerged adults against the number of treated whitefly nymphs, 7 days after treatment.

Acute toxicity of the insecticide spinetoram to the adult stage of the whitefly was assessed in a residual contact test in which a range of spinetoram concentrations was sprayed: 37.5, 18.75, 9.37, 4.69, 2.34 and 1.17 mg a.i./l. The insecticide (or pure water in control dishes) was applied to the entire surface of each Petri dish (i.e. the lid and the lower dish with a tobacco leaf on agar medium). After the treated surfaces dried for 2 hours at room temperature, 40 (0-2 day old) adult whiteflies were anaesthetised with CO_2 for two seconds and then carefully transferred to each Petri dish. Mortality was recorded 48 hours after exposure, and adults were considered dead if they remained immobile after a light touch with a fine brush.

B) Acute toxicity bioassays with E. formosa. The baseline toxicity of the insecticide spinetoram to parasitoid adults was assessed by a residual contact test in the same way as described for the adult stage of the pest. Briefly, forty newly hatched (12-24 h old) adult wasps were exposed to dry spinetoram residues (3.75, 1.87, 0.94, 0.47, 0.23 and 1.15 mg a.i./l) in each Petri dish with a few drops of honey on a piece of aluminium foil (0.5 × 0.5 mm) attached to the lid of each dish.

Honey was applied after the insecticide has dried to avoid possible contamination of honey drops and ingestion of insecticide residues by parasitic wasps. As with the adult stage of the pest, mortality was calculated based on the number of live wasps relative to the number of wasps exposed for 48 hours (EPPO, 2004; Drobnjaković et al., 2018, 2019, 2021).

For the concentration-mortality response of the parasitoid pupal stage, tobacco leaves with parasitised whitefly nymphs (parasitoid pupae, 3 days old, i.e. 11 days after parasitoid oviposition in host nymphs) were fixed on aluminium foil with the natural, non-toxic adhesive Traganth-kit. After drying, the leaves were cut into pieces to adjust the number of pupae to about 40 per piece and placed on clean filter papers in Petri dishes. Petri dishes containing these tobacco leaves with pupae were treated with serially diluted solutions (7.5, 3.75, 1.87, 0.94, 0.47 and 0.23 mg a.i./l). After air drying for 2 hours, the treated leaves with pupae were transferred to clean Petri dishes to observe the emergence of adult parasitoids. Mortality was calculated using the ratio of emerged adults to treated pupae 9 days after treatment (EPPO, 2004; Drobnjaković et al., 2018, 2019, 2021).

Selectivity ratio. Direct lethal effects of the insecticide spinetoram on the tested life stages of *E. formosa* were assessed through the selectivity ratio (SR) as an Environmental Risk Assessment (ERA) parameter. In our study, selectivity ratios showed the relation beetween LC_{50} values estimated for the adult and pupal stages of the parasitoid, compared to LC_{50} values estimated for all tested stages of the pest (adult, nymph and egg). The selectivity ratio (SR) was calculated using the formula of Şengonca & Liu (2001):

$$SR = \frac{LC_{50} \text{ of the parasitoid}}{LC_{50} \text{ of the pest}}$$

$SR < 1$ indicates that the chemical is more toxic to the parasitoid than to the greenhouse whitefly (non-selective); $SR > 1$ indicates that the chemical is less toxic to the parasitoid.

Statistical analysis

The baseline toxicity of spinetoram to all life stages of the pest and parasitoid tested was estimated using a log-probit regression model (Finney, 1971) in Polo Plus software (LeOra Software, Berkeley, USA), estimating lethal concentrations (LC_{10}, LC_{50}, LC_{90}) and slopes of the regression lines. The concentration-mortality relationships were considered valid (i.e. they fitted the observed data) if there was no significant difference between the observed and expected data at $p < 0.05$ level. A pairwise comparison of the estimated LC_{50} values was performed using the lethal concentration ratio test: when 95% confidence interval between two LC values included 1, these values were considered nonsignificantly different (Robertson et al., 2007).

RESULTS

Acute toxicity bioassays

Log-probit regression analyses of concentration-mortality data showed that the spinetoram insecticide demonstrated significiant acute toxicity both to *T. vaporariorum* and *E. formosa*, but toxicity to the tested development stages of the parasitoid was significantly higher, compared to the pest.

Table 1. Baseline toxicity of spinetoram to life stages of the greenhouse whitefly and parasitoid *Encarsia formosa*, after topical or residual exposure

Insects	Life stages	*n*	LC_{50} (mg/l) (95% CLs)	LC_{90} (mg/l) (95% CLs)	LC_{10} (mg/l) (95% CLs)	Slope (± SE)	χ^2	*df*
Trialeurodes vaporariorum	Adult	1248	4.59 (2.76 - 6.84)	51.92 (27.91 - 164.95)	0.41 (0.09 - 0.91)	1.22 (± 0.13)	5.33	4
	Nymph	1156	15.28 (9.39 - 22.20)	179.89 (105.93 - 428.15)	1.30 (0.34 - 2.79)	1.20 (± 0.10)	6.04	4
	Egg	1402	11.73 (8.73 -1 5.35)	123.99 (81.80 - 221.10)	1.11 (0.54 - 1.84)	1.25 (± 0.06)	5.60	4
Encarsia formosa	Adult	1215	0.69 (0.57 - 0.81)	5.10 (3.91 - 7.22)	0.09 (0.06 - 0.13)	1.47 (± 0.12)	1.78	4
	Pupa	1400	1.72 (1.42 - 2.05)	15.60 (11.47 - 23.24)	0.19 (0.12 - 0.27)	1.34 (± 0.10)	3.79	4

n = number of individuals ; CLs = confidence limits; SE = standard error, $\chi 2$ = chi-square testing goodness of fit of concentration-mortality response; *df* = degrees of freedom

In the case of *T. vaporariorum*, significantly different median lethal concentrations of spinetoram were determined for the tested developmental stages. A LC_{50} ratio test showed that the adult stage of the pest was the most sensitive to the toxic effect of spinetoram, while the response of the nymphal stage to spinetoram showed the lowest lethal effect in acute toxicity bioassays. The LC_{50} values determined for the tested whitefly development stages were significantly lower (7.2, 2.2 and 2.8 times lower for adults, nymphs and eggs, respectively) (Table 1), compared to the application rate of the spinetoram insecticide recommended for use in protected solanaceus vegetable crops (33 mg a.i./l).

In the case of *E. formosa*, the parasitoid pupae showed 2.5-fold higher LC_{50}s than adults, but the ratio test did not indicate a significantly higher toxicity of spinetoram to the parasitoid adult stage. The label concentration of spinetoram insecticide was 47.8- and 19.2-fold (for adults and pupae, respectively) the estimated LC_{50} values for the parasitoid (Table 1).

Selectivity ratio estimations, showing ratios between median lethal concentrations estimated for the parasitoid, compared to LC_{50}s estimated for the greenhouse whitefly, are summarized in Figure 1. The calculated selectivity ratio values in all comparisons were less than 1, showing that the spinetoram insecticide is much more toxic to the parasitoid than to the pest.

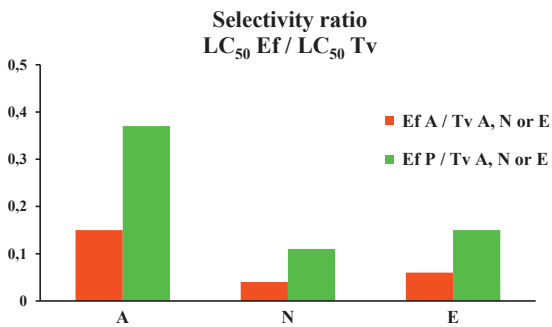


Figure 1. Selectivity ratios of spinetoram for the adult and pupal stages of *Encarsia formosa*

DISCUSSION

The current study showed that the insecticide spinetoram has high acute toxicity both to *T. vaporariorum* and *E. formosa* under the conditions described. However, the toxicity found for the tested parasitoid stages was significantly higher than for the pest. Acute toxicity parameters showed that spinetoram strongly affected the viability of eggs, nymphs and adults of the greenhouse whitefly pest, and the adult stage was the most sensitive to its toxic effect, while the nymphal stage was the least sensitive. In the case of *E. formosa*, adults and pupae were equally sensitive to spinetoram. Analysis of the obtained results based on the selectivity ratio, i.e. values below 1, indicated a non-selective nature of the spinetoram insecticide to both tested stages of the parasitoid.

Side effects of spinetoram on agricultural arthropod pests, as well as their arthropod natural enemies, have been scarcely documented (Srivastava et al., 2008; Hernández et al., 2011; Lefebvre et al., 2011; Abbes et al., 2015; Abd-Ella, 2015; Ricupero et al., 2020). To the best of our knowledge, this is the first report on toxic effects of spinetoram on the greenhouse whitefly, and its important biocontrol agent, the parasitoid *E. formosa*. Only one other study quantified the toxicity of spinetoram to whitefly and/or to *Encarsia* spp. species. Abd-Ella (2015) assessed the susceptibility of the pomegranate whitefly, *Siphoninus phillyreae* (Haliday) (Hemiptera: Aleyrodidae), a pest of numerous ornamental and fruit crops, and its major parasitoid species *Encarsia inaron* (Walker) (Hymenoptera: Aphelinidae) to spinetoram insecticide (Radiant SC; 0.021 ml a.i./l), using the leaf-dipping technique. Of five tested neurotoxic insecticides (imidacloprid, spinetoram, emamectin benzoate, abamectin and teflubenzuron), representing five insecticide chemical classes, spinetoram had the highest acute toxicity to pomegranate whitefly nymphs and adults. Although selectivity ratios between the LC_{50}s of the parasitoid and the pest were > 1, indicating a potentially selective nature of spinetoram, a risk quotient analysis, used to assess the risk to non-target arthropods (Hassan et al., 1998; Peterson, 2006) showed that spinetoram was slightly - moderately toxic (Preetha et al., 2010) to the *E. inaron* adult stage, 24 and 48 h post-treatment.

Similar to our findings, other studies quantifying toxic effects of spinetoram to parasitoids also suggested that spinetoram's toxicological profile regarding parasitoids is not promising. In a study that evaluated the lethal effects of insecticides on adults of *Ganaspidium nigrimanus* (Kieffer) (Hymenoptera: Figitidae) and *Neochrysocharis formosa* (Westwood) (Hymenoptera: Eulophidae), two important parasitoid species of the dipteran pest *Liriomyza trifolii* (Burgess), spinetoram proved to be the most harmful insecticide tested, suggesting

that it should be cautiously used in pest management systems. The field recommended rate of spinetoram (Radiant SC; 164 mg a.i./l) significantly decreased the cumulative survival of adult parasitoids when they were exposed to spinetoram either topically in leaf residue bioassays, or through feeding on a spinoteram contaminated food source (Hernández et al., 2011). Adverse effects of spinetoram have also been recorded in a study with another hymenopteran parasitoid wasp - *Bracon nigricans* (Braconidae), a natural enemy of the invasive tomato pest *Tuta absoluta* (Lepidoptera: Gelechiidae). Three days after residual contact with dry spinetoram insecticide residue (insecticide solutions were applied at maximum label rate, Radiant SC; 0.09 ml a.i./l), very high mortality of newly emerged parasitoid adults was recorded at three tested constant temperatures (77% mortality at 25°C, and total mortality at higher temperatures, 30 and 40°C) (Abbes et al., 2015).

The 'reduced risk' alternatives are not always of lower risk to arthropods, particularly natural enemies, and may have variable effects (Lefebvre et al., 2011; Biondi et al., 2012; Parsaeyan et al., 2020). The results of our study indicate that spinetoram may be a good choice in chemical management of greenhouse whitefly, while greenhouse populations of *E. formosa* parasitoid may be eliminated in the IPM system if sprayed with the recommended field rate of the spinetoram insecticide. Although toxic effects of an insecticide on an arthropod may be estimated by the mortality of adult females at the median lethal concentration (Robertson & Worner, 1990; Stark et al., 1997), such assessment provides only a partial measure of toxic effects (Stark & Banken, 1999; Desneux et al., 2007). A more precise assessment of risks for *E. formosa* involved in integrated use of spinetoram in whitefly management requires a further assessment of sublethal effects and greenhouse trials, highlighting the population-level response. For the most realistic scenario, spinetoram toxicity should be validated under greenhouse conditions, taking into account different application rates, routes of exposure, along with the ecology of the parasitoid during the growing season.

ACKNOWLEDGEMENT

This study was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, Grant No. 451-03-47/2023-01/200214.

REFERENCES

Abbes, K., Biondi, A., Kurtulus, A., Ricupero, M., Russo, A., Siscaro, G. ... Zappalà, L. (2015). Combined non-target effects of insecticide and high temperature on the parasitoid *Bracon nigricans*. *PloS ONE*, *10*(9), e0138411. doi:10.1371/journal.pone.0138411

Abd-Ella, A.A. (2015). Susceptibility of the pomegranate whitefly, *Siphoninus phillyreae* (Haliday)(Homoptera: Aleyrodidae) and its parasitoid, *Encarsia inaron* (Walker) (Hymenoptera: Aphelinidae) to certain insecticides under laboratory conditions. *Egyptian Journal of Biological Pest Control*, *25*(3), 689-695.

Albajes, R., Lodovica Gullino, M., van Lenteren, J. C., & Elad, Y. (Eds.). (1999*). Integrated pest and disease management in greenhouse crops*. Dordrecht, Netherlands: Kluwer Academic Publisher.

Bacci, L., Crespo, A.L., Galvan, T.L., Pereira, E.J., Picanço, M.C., Silva, G.A., & Chediak, M. (2007). Toxicity of insecticides to the sweetpotato whitefly (Hemiptera: Aleyrodidae) and its natural enemies. *Pest Management Science, 63*(7), 699-706. doi.org/10.1002/ps.1393

Biondi, A., Mommaerts, V., Smagghe, G., Viñuela, E., Zappalà, L., & Desneux, N. (2012). The non-target impact of spinosyns on beneficial arthropods. *Pest Management Science*, *68*(12), 1523-1536. doi: 10.1002/ps.3396. doi: 10.1002/ps.3396

Biondi, A., Zappalà, L., Stark, J.D., & Desneux, N. (2013). Do biopesticides affect the demographic traits of a parasitoid wasp and its biocontrol services through sublethal effects? *PLoS One*, *8*(9), e76548. doi: 10.1371/journal.pone.0076548

Chandler, D., Bailey, A. S., Tatchell, G. M., Davidson, G., Greaves, J., & Grant, W. P. (2011). The development, regulation and use of biopesticides for integrated pest management. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *366*, 1987–1998. doi:10.1098/rstb.2010.0390

Chloridis, A., Downard, P., Dripps, J.E., Kaneshi, K., Lee, L.C., Min, Y.K., & Pavan, L.A. (2007). Spinetoram (XDE-175): a new spinosyn. In The BCPC congress, *Proceedings of the XVI International Plant Protection Congress* (pp 44-49). Glasgow, UK: British Crop Production Council.

Copping, L.G., & Menn, J.J. (2000). Biopesticides: a review of their action, applications and efficacy. *Pest Management Science*, *56*(8), 651-676. doi:10.1002/1526-4998(200008)56:8<651::AID-PS201>3.0.CO;2-U

Desneux, N., Decourtye, A., & Delpuech, J. M. (2007). The sublethal effects of pesticides on beneficial arthropods. *Annual Review of Entomology*, *52*, 81-106. doi. org/10.1146/annurev.ento.52.110405.091440

Dripps, J.E., Boucher R.E., Chloridis A., Cleveland C.B., DeAmicis C.V., Gomez L.E. ... Watson, G.B. (2011). The spinosyn insecticides. In O. L'opez & J. G. Fernández-Bolanos (Eds), *Green trends in insect control, Green chemistry series, Vol. 11* (pp 163–212). London, UK: Royal Society of Chemistry.

Drobnjaković, T., & Marčić, D. (2021). Effects of spirotetramat insecticide on life history traits and population growth of *Encarsia formosa* (Hymenoptera: Aphelinidae). *Biocontrol Science and Technology, 31*(6), 604-618. doi: 10.1080/09583157.2021.1873248

Drobnjaković, T., Marčić, D., Prijović, M., Perić, P., Milenković, S., & Bošković, J. (2018). Sublethal effects of NeemAzal-T/S botanical insecticide on Dutch and Serbian populations of *Encarsia formosa* (Hymenoptera: Aphelinidae). *Biocontrol Science and Technology*, *28*(1), 1-19. doi:10.1080/09583157.2017.1409336

Drobnjaković, T., Prijović, M., Milenković, S. & Marčić, D. (2019). Sublethal effects of *Beauveria bassiana*-based mycopesticide on Dutch and Serbian populations of *Encarsia formosa* (Hymenoptera: Aphelinidae). *Biocontrol Science and Technology*, *29*(10), 991-1008. doi:10.1080/09583157.2019.16352

EPPO (2004). *Side-effects on Encarsia formosa, PP1/142(2). Efficacy evaluation of plant protection products*. Paris, France: Europian and Mediterranean Plant Protection Organisation.

Finney, D. J. (1971). *Probit analysis*. Cambridge, UK: University Press.

Gerling, D., Alomar, Ò., & Arnò, J. (2001). Biological control of *Bemisia tabaci* using predators and parasitoids. *Crop Protection*, *20*(9), 779–799. doi:10.1016/S0261-2194(01)00111-9

Giunti, G., Benelli, G., Palmeri, V., Laudani, F., Ricupero, M., Ricciardi, R., ... Campolo, O. (2022). Non-target effects of essential oil-based biopesticides for crop protection: impact on natural enemies, pollinators, and soil invertebrates. *Biological Control*, *176*, 105071. doi:10.1016/j.biocontrol.2022.105071

Hassan, S.A., Hafes, B., Degrande, P.E., & Herai, K. (1998). The side-effects of pesticides on the egg parasitoid *Trichogramma cacoeciae* Marchal (Hym., Trichogrammatidae), acute dose-response and persistence tests. *Journal of Applied Entomology*, *122*(1-5), 569-573. doi.org/10.1111/j.1439-0418.1998.tb01547.x

Hernández, R, Guo, K., Harris, M., & Liu, T.X. (2011). Effects of selected insecticides on adults of two parasitoid species of *Liriomyza trifolii: Ganaspidium nigrimanus* (Figitidae) and *Neochrysocharis formosa* (Eulophidae). *Insect Science, 18*(5), 512–520. doi.org/10.1111/j.1744-7917.2010.01391.x

Hoddle, M.S., van Driesche, R.G., Lyon, S.M., & Sanderson, J.P. (2001). Compatibility of insect growth regulators with *Eretmocerus eremicus* for whitefly control on poinsettias: I. Laboratory assays. *Biological Control*, *20*(2), 122-131. doi:10.1006/bcon.2000.0885

Hoddle, M.S., van Driesche, R.G., & Sanderson, J.P. (1998). Biology and use of the whitefly parasitoid *Encarsia formosa*. *Annual Review of Entomology*, *43*, 645-649. doi:10.1146/annurev.ento.43.1.645

IRAC (2023). *The IRAC mode of action classification online*. Retrieved from: https://irac-online.org/modes-of-action/

Kapantaidaki, D. E., Sadikoglou, E., Tsakireli, D., Kampanis, V., Stavrakaki, M., Schorn, C. ... Tsagkarakou, A. (2018). Insecticide resistance in *Trialeurodes vaporariorum* populations and novel diagnostics for *kdr* mutations. *Pest Management Science*, *74*(1), 59-69. doi: 10.1002/ps.4674

Kumar, J., Ramlal, A., Mallick, D., & Mishra V. (2021). An overview of some biopesticides and their importance in plant protection for commercial acceptance. *Plants*, *10*(6), 1185. doi: 10.3390/plants10061185

Lefebvre, M., Bostanian, N.J., Thistlewood, H.M., Mauffette, Y., & Racette, G. (2011). A laboratory assessment of the toxic attributes of six 'reduced risk insecticides' on *Galendromus occidentalis* (Acari: Phytoseiidae). *Chemosphere, 84*(1), 25-30. doi.org/10.1016/j.chemosphere.2011.02.090

Li, S.J., Xue, X., Ahmed, M.Z., Ren, S.X., Du, Y.Z., Wu, J.H. ... Qiu, B. L. (2011). Host plants and natural enemies of *Bemisia tabaci* (Homoptera: Aleyrodidae) in China. *Insect Science*, *18*(1), 101-120. doi.org/10.1111/j.1744-7917.2010.01395.x

Mota-Sanchez, D. & Wise, J.C. (2023). *Arthropod pesticide resistance database*. Retrieved from https://www.pesticideresistance.org/

Parsaeyan, E., Saber, M., Safavi, S.A., Poorjavad, N., & Biondi, A. (2020). Side effects of chlorantraniliprole, phosalone and spinosad on the egg parasitoid, *Trichogramma brassicae*. *Ecotoxicology, 29*, 1052-1061. doi: 10.1007/s10646-020-02235-y

Patra, B., & Kumar Hath, T. (2022). Insecticide resistance in whiteflies *Bemisia tabaci* (Gennadius): Current global status. Insecticides. In Ramón Eduardo Rebolledo Ranz (Ed), *Insecticides – Impact and benefits of its use for humanity* (chapter 19). Temuco, Chile: Universidad de La Frontera. doi: 10.5772/intechopen.101954

Peterson, R.K.D. (2006). Comparing ecological risks of pesticides: the utility of a risk quotient ranking approach across refinements of exposure. *Pest Management Science*, *62*, 46-56. doi: 10.1002/ps.1126

Preetha, G., Stanley, J., Suresh, S., & Samiyappan, R. (2010). Risk assessment of insecticides used in rice on mired bug, *Cyrtorhinus lividipennis* Reuter, the important predator of brown planthopper, *Nilaparvata lugens* (Stal). *Chemosphere*, *80*(5), 498-503. doi:10.1016/j.chemosphere.2010.04.070

Ricupero, M., Abbes, K., Haddi, K., Kurtulus, A., Desneux, N., Russo, A. ... Zappalà, L. (2020). Combined thermal and insecticidal stresses on the generalist predator *Macrolophus pygmaeus*. *Science of the Total Environment*, *729*, 138922. doi: 10.1016/j.scitotenv.2020.138922

Robertson, J.L., Russell, R.M., Preisler, H.K., & Savin, N.E. (Eds). (2007). *Bioassays with arthropods* (2nd ed). Boca Raton, FL, USA: CRC Press.

Robertson, J.L., & Worner, S.P. (1990). Population toxicology: suggestion for laboratory bioassays to predict pesticide efficacy. *Journal of Economic Entomology*, *83*(1), 8-12. doi.org/10.1093/jee/83.1.8

Şengonca, Ç., & Liu, B. (2001). Infuence of mixed biocide GCSC-BtA on the pupae and adult stages of *Apanteles plutellae* Kurd. (Hym., Braconidae) and its host. *Plutella xylostella* (L.) (Lep., Plutellidae). *Journal of Pesticide Science*, *74*(6), 145-149. doi.org/10.1046/j.1439-0280.2001.01033.x

Shankarganesh, K., Ricupero, M., & Sabtharishi S. (2022). Field evolved insecticide resistance in the cotton mealybug *Phenacoccus solenopsis* and its direct and indirect impacts on the endoparasitoid *Aenasius arizonensis*. *Scientific Reports 12*, 16764. doi: 10.1038/s41598-022-20779-3

Shimokawatoko Y., Sato, N., Yamaguchi, T., & Tanaka, H. (2012). *Development of the novel insecticide spinetoram (DIANA®)*. Retrieved from https://www.sumitomo-chem.co.jp/english/rd/report/files/docs/01_2012e.pdf

Srivastava, M., Bosco, L., Funderburk, J., & Olson, S., & Weiss, A. (2008). Spinetoram is compatible with the key natural enemy of *Frankliniella* species thrips in pepper. *Plant Health Progress*, *9*(1), 30. doi:10.1094/PHP-2008- 0118-02-RS

Stark, J.D., & Banken, J.A.O. (1999). Importance of population structure at the time of toxicant exposure. *Ecotoxicology and Environmental Safety*, *42*(3), 282-287.

Stark, J.D., Tanigoshi, L., Bounfour, M., & Antonelli, A. (1997). Reproductive potential: its influence on the susceptibility of a species to pesticides. *Ecotoxicology and Environmental Safety*, *37*(3), 273-279. doi.org/10.1006/eesa.1997.1552

Sugiyama, K., Katayama, H., & Saito, T. (2011). Effect of insecticides on the mortalities of three whitefly parasitoid species, *Eretmocerus mundus*, *Eretmocerus eremicus* and *Encarsia formosa* (Hymenoptera: Aphelinidae). *Applied Entomology and Zoology*, *46*(3), 311–317. doi.org/10.1007/s13355-011-0044-z

van Lenteren, J.C., & Martin, G. (1999). Biological control of whiteflies. In R. Albajes, M. Lodovica Gullino, J.C. van Lenteren, & Y. Elad (Eds.), *Integrated pest and disease management in greenhouse crops* (pp 202-215). Dordrecht, Netherlands: Kluwer.

Villaverde, J. J., Sevilla-Morán, B., Sandín-España, P., López-Goti, C., & Alonso-Prados, J. L. (2014). Biopesticides in the framework of the European pesticide regulation (EC) no. 1107/2009. *Pest Management Science*, *70*, 2-5. doi:10.1002/ps.3663

Williams, T., Valle, J., & Viñuela, E. (2003). Is the naturally derived insecticide Spinosad® compatible with insect natural enemies? *Biocontrol Science and Technology*, *13*(5), 459–475. doi.org/10.1080/0958315031000140956

Komparativna toksičnost spinetorama za *Trialeurodes vaporariorum* Westwood i parazitoida *Encarsia formosa* Gahan

REZIME

Uloga potencijalno selektivnih bioinsekticida u okviru integralnog koncepta zaštite biljaka, pored efikasnosti u suzbijanju štetočina, umnogome zavisi i od njihove bezbednosti po neciljane organizme. Spinetoram je polu-sintetski analog mikrobiološki dobijenog bioinsekticida spinosada koji se koristi širom sveta u suzbijanju raznih poljoprivrednih štetočina, ali je procena rizika primene spinetorama po korisne artropode veoma slabo istražena. Štaviše, toksičnost spinetorama prema štetočini - beloj leptirastoj vaši *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae), kao i/ili prema njenom efikasnom biološkom agensu, parazitoidu *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae), nikada nije dokumentovana. U laboratorijskim uslovima, utvrđivana je akutna toksičnost (letalni efekti) insekticida na bazi spinetorama (25% a.s.) po razvojne životne stadijume (adulte, nimfe i jaja) bele leptiraste vaši, kao i po adulte i lutke parazitoida. Svi ogledi su izvedeni na temperaturi 27±1°C i relativnoj vlažnosti vazduha od 60±10%, uz fotoperiod 16:8 h, u pet ponavljanja. U svim doza-odgovor biotestovima, insekticid na bazi spinetorama primenjen je pomoću Potter Spray Tower aparata na lišće duvana, smešteno na sloju 1% agara, u ventiliranim Petri šoljama. Adulti štetočine i parazitoida izlagani su dejstvu svežih rezidua spinetorama u period od 48 sati, dok su jaja i nimfe štetočine, kao i lutke parazitoida, direktno tretirani serijom koncentracija spinetorama, pokrivajući odgovore od 10-90% smrtnosti. Akutna toksičnost spinetorama prema *E. formosa* procenjena je kroz indekse selektivnosti, koji predstavljaju odnos između srednjih letalnih koncentracija dobijenih za parazitoida i štetočinu. U biotestovima akutne toksičnosti dobijene su sledeće srednje letalne koncentracije spinetorama: 4,593, 15,027 i 11,73 mg a.s./l za adulte, nimfe i jaja bele leptiraste vaši, respektivno, kao i 0,686 i 1,715 mg a.s./l za adulte i lutke parazitoida, respektivno. Analiza dobijenih rezultata kroz indekse selektivnosti koji su bili niži od 1, nagoveštava neselektivnu prirodu insekticida na bazi spinetorama, prema oba testirana životna stadijuma parazitoida. Sveobuhvatna determinacija rizika zajedničke primene spinetorama i *E. formosa* u okviru integralnog koncepta zaštite biljaka od bele leptiraste vaši zahteva i procenu subletalnih efekata, kao i dalje testiranje spinetorama u poljskim uslovima, sa naglaskom na populacione parametre parazitoida.

Ključne reči: bioinsekticid, *Encarsia formosa*, integralni koncept zaštite biljaka, indeks selektivnosti, bela leptirasta vaš

Pestic. Phytomed. (Belgrade), 38(2), 2023, 75–84
DOI: https://doi.org/10.2298/PIF2302075S

UDC 632.937:632.981.1:582.628:632.51
Original scientific paper

Antioxidant activity, phytotoxicity and allelopathic potential of green walnut (*Juglans regia* L.) fruit extract

Marija Sarić-Krsmanović[1]*, Jelena Gajić Umiljendić[1], Tijana Đorđević[1], Ljiljana Radivojević[1], Ljiljana Šantrić[1], Dragana Božić[2] and Sava Vrbničanin[2]

[1]Institute of Pesticides and Environmental Protection, Banatska 31b, 11080 Belgrade-Zemun
[2]University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080, Belgrade-Zemun
**Corresponding author: marijasaric.msaric@gmail.com*

Received: 16 June 2023
Accepted: 7 August 2023

SUMMARY

The potential allelopathic effect of a green walnut fruit extract on seed germination and early growth of three weed species (*Chenopodium album*, *Amaranthus retroflexus*, *Daucus carota*) was tested, as well as its phytotoxic effect on seed germination and early growth of maize (*Zea mays*). Another objective was to analyze the plant extract and assess its antioxidative activity.

Antioxidative activity of the plant extract was evaluated based on its ability to neutralize DPPH (2,2-diphenyl-1-picrylhydrazyl) radical and capacity for iron reduction using the FRAP method. Inhibition of the evaluated parameters (total germination and seedling length) decreased proportionally with decreasing concentrations of extract solution of green walnut fruit. The parameters of seedling growth were also found to show greater susceptibility than total seed germination of all three of the tested weed species. Although the seed bioassay results indicated a high inhibitory effect on germination and early growth of the tested weeds, they also revealed phytotoxic activity during early growth of the cultivated *Z. mays*.

Keywords: alleopathy, walnut, plant extract, weeds, maize

INTRODUCTION

Detrimental effects of herbicides on the environment and human health, increase in the number of weed species developing resistance to herbicides, slower development of novel herbicides, retraction of a significant number of herbicides from use, and increasing growth of organic agriculture have been the main factors over the past several decades for promoting environment-friendly approaches to weed control (Hatcher & Melander, 2003; Vilà et al., 2010; Villa et al., 2017; Hossard et al., 2017; Kanissery et al., 2019). Allelopathy is one such approach that refers to any direct or indirect, beneficial or detrimental impact of plants on the growth and development of other plants (Narwal, 2010). Plants may exude their products of metabolism by roots,

by evaporation from above-ground plant parts or by decomposition of plant matter in the soil (Wu et al., 2000; An et al., 2003; Gatti et al., 2010), which may cause inhibition of seed germination or its delay, inhibition of root system growth or radicle burst, lack of root hair, elongation of coleoptile, etc. (Gella et al., 2013). Natural products are sources of a variety of compounds that can be used as leading structures in the synthesis of new bioherbicides intended either for independent use or combinations with synthetic herbicides within the framework of integrated control strategies (Duke et al., 2000). Bioherbicides are made from naturally synthesized components having allelopathic properties that are readily degradable, which makes their application safer than synthetic herbicides (Belz, 2007).

Walnut is one of the plant species most abounding in antioxidants, and rich in lipids and oils (up to 70%), proteins, carbohydrates, vitamins (C and E), essential fatty acids, phenolic acids (p-coumarin, chlorogenic, vanillic, syringic), flavonoids (quercetin, catechin, myricetin) and chinones (juglandin and juglone), as well as saponins, tannins, alkaloids, glucosides, etc. (Cosmulescu et al., 2014; Rusu et al., 2020; Zurek et al., 2022). Walnut is a significant source of phytochemicals that may have allelopathic properties. The purpose of the present study was therefore to test the allelopathic potential of an unripe walnut fruit extract, more specifically to: (1) analyze the extract and estimate its antioxidative activity; (2) estimate *in vitro* the allelopathic effect of the extract of green (unripe) walnuts on seed germination and early growth of three weed species, namely *Amaranthus retroflexus*, *Chenopodium album,* and *Daucus carota*, as well as its phytotoxic effect on seed germination and early growth of maize (*Zea mays*).

MATERIAL AND METHODS

Plant material

Seed bioassays included: *A. retroflexus* (seed collected from Surčin), *C. album* (seed collected from New Belgrade), and *D. carota* (seed collected from New Belgrade). The collected seeds were cleansed and kept in paper bags in the laboratory at a room temperature of 20-22°C. Commercial maize seed (hybrid NS3022), a product of the Institute of Field and Vegetable Crops of Novi Sad, were used in the bioassay. Immature fruits of walnut (*J. regia*) were picked in fields around Krčedin on 5th May 2022.

Seed bioassay

Testing of allelopathic potential of the extract of *J. regia* green fruit on seed germination and early seedling growth of *A. retroflexus*, *C. album,* and *D. carota*, and its phytotoxic effect on seed germination and early seedling growth of *Z. mays,* was carried out in a controlled environment. Before the bioassay, seeds of all tested species were immersed in thiourea solution for 24 h and then sterilized with 3% hydrogen peroxide solution for 5 minutes before they were well rinsed with distilled water. The disinfected seeds of three weed species were transferred into sterile Petri dishes (25 seeds/Petri dish of 9 cm) and 5 ml of prepared green walnut extract, diluted in distilled water in the initial concentration of 1% and a series of dilutions of 0.75%, 0.50%, 0.25%, 0.10%, and 0.05%, was added, while 25 maize seeds were transferred to each Petri dish (14 cm Ø) and 10 ml of extract was added in corresponding concentrations. Petri dishes were sealed with parafilm and kept in an incubator (Velp Scientifica, Europe) at 27°C temperature in the dark. Distilled water was used for the control treatment. All treatments were done in four replicates and the bioassay was repeated twice. Germinated seeds were counted and seedling length was measured 7 days after treatment (DAT). Ungerminated seeds were subjected to a standard tetrazolium test (TTC) to distinguish dormant from dead seeds and determine seed viability for all test plants.

Sequential ultrasonic extraction from plant material

The extract was obtained from unripe walnuts by sequential ultrasonic extraction using solvents with different polarities to achieve a satisfactory yield of phenolic compounds, as well as other plant secondary metabolites of different polarities. The ratio of ground plant material and solvent was 1:1 (w/v). Extraction included homogenization in Erlenmeyer flasks in an ultrasonic bath (lasting 15 minutes at 30°C temperature at 37 kHz frequency and ultrasonic bath power of 168 W) recurring in seven successive extraction cycles with hexane, ethyl-acetate, acetone, acetonitrile, ethanol, methanol, and distilled water. Extracted aliquots from each extraction cycle were filtered through quantitative filter paper into evaporator flasks and evaporated on a rotary vacuum evaporator at 40°C to dryness, while plant remains were used in the next extraction step with respective solvents. All dry matter was washed with distilled water into one container. The extract was

then frozen at -80°C in a lyophilization container and dried by lyophilization. The resulting dry sample was kept in vials at -20°C until analyses.

Phytochemical analyses of extract

Total phenolic content

Total phenolic content in the extract was determined by a modified Folin-Ciocalteu method. The principle of the assay is the reduction of Folin-Ciocalteu reagent (mixture of phosphomolybdate and phosphotungstate) in the presence of phenolics, resulting in the production of molybdenum-tungsten blue that is measured spectrophotometrically at 760 nm, and the intensity increases linearly with concentrations of phenolics in the reaction medium. For the analysis, 0.25 g of dry green walnut extract was dissolved in distilled water (25 ml). A volume of 100 µl of extract solution was mixed with 0.5 ml of Folin-Ciocalteu reagent and 6 ml of distilled water in test tubes and vortexed well. A volume of 2 ml of 15% Na_2CO_3 was then added to the solution, and supplemented further with distilled water up to 10 ml, and the solution was then incubated for 60 min at room temperature. Absorbance was measured at 760 nm wavelength by spectrophotometer. A solution containing only the reagents, beside extract, was considered a blank. Gallic acid (GAE) was used as an equivalent for calibration curve preparation, and the results were expressed as mg of gallic acid per g of dry extract (mg GAE g^{-1} d.e.).

Total flavonoid content

Total flavonoid content in green walnut extract was determined using a modified aluminum chloride colorimetric assay. The principle involved in the aluminum chloride colorimetric method is that $AlCl_3$ forms stable acid complexes with C-4 keto groups and either C-3 or C-5 hydroxyl group of flavones and flavonols, resulting in the production of pink color, which is measured spectrophotometrically at 510 nm. For analysis, dry green walnut extract (0.125 g) was dissolved in methanol (25 ml). A volume of 375 µl of extract solution was mixed with 1.5 ml of 5% $NaNO_2$ solution in test tubes and incubated at room temperature for 6 min. A volume of 150 µl of 10% $AlCl_3$ solution was added, vortexed well, and left for another 5 min. A volume of 750 µl of 1M NaOH solution was added and further incubated for 15 min. Absorbance was measured at 510 nm wavelength by spectrophotometer. A solution containing only the reagents, besides extract, was considered a blank. Quercetin (QE) was used as an equivalent for calibration curve preparation, and the results were expressed as mg quercetin per g of dry extract (mg QE g^{-1} d.e.).

Evaluation of antioxidant activity – determination of DPPH scavenging activity

Antioxidant activity of the obtained extract was tested by determining its DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity. In a DPPH assay, the violet-colored DPPH solution is reduced to the yellow-colored product diphenylpicryl hydrazine by antioxidants present in the sample. For the analysis, 0.25 g of dry green walnut extract was dissolved in distilled water (25 ml), and a series of dilutions were prepared from the stock solution to obtain concentrations ranging from 0.01 to 0.2 mg/ml. A volume of 0.5 ml of each dilution was mixed with 1 ml of DPPH solution (0.2 mM in methanol) and 3 ml of methanol in test tubes and vortexed well. The volume was then augmented to 5 ml by adding methanol, and the solution was incubated for 30 minutes at 25°C in the dark. Absorbance was measured by a spectrophotometer at 517 nm. The solution containing only reagents, besides extract, was considered as a control, and antioxidant activity was calculated using the formula: % inhibition = $[(Abs_{control} - Abs_{sample})/(Abs_{control})] \times 100$. IC_{50} values (concentration of sample required to scavenge 50% of free radicals) were calculated. Ascorbic acid was used as a positive control.

Evaluation of antioxidant activity - determination of ferric ion reducing antioxidant power (FRAP assay)

Antioxidant activity of the obtained extract was tested also by measuring its ferric ion-reducing potential (FRAP). FRAP assay is based on rapid reduction in ferric-tripyridyltriazine (FeIII-TPTZ) by antioxidants present in the test sample, forming ferrous-tripyridyltriazine (FeII-TPTZ), a blue-colored product. Acetate buffer (pH 3.6) (300 mM), TPTZ solution (10 mM in 40 mM HCl), and $FeCl_3 \times 6H_2O$ solution (20 mM) were mixed (10:1:1 v/v) for the analysis into a FRAP solution. Dry green walnut extract (150 µl, concentration 0.5 mg/ml in methanol) and 150 µl of distilled water were mixed with 3 ml of FRAP solution and incubated in the dark at 37°C for 30 minutes. FRAP solution was used as a blank. Absorbance was measured at 593 nm by spectrophotometer. Ferrous sulphate heptahydrate

($FeSO_4 \times 7H_2O$) was used as the equivalent for calibration curve preparation, and the results were expressed as μmol of ferric ion (Fe^{2+}) per g of dry extract. Butylated hydroxytoluene (BHT) was used as a positive control.

Statistical analyses

The results are shown as means ± standard deviation. The data were analyzed by single factorial analysis of variance (ANOVA) using the STATISTICA 8.0. software. When F values were statistically significant ($p < 0.05$) treatments were arranged according to LSD test. Effective concentrations (EC_{50}) of the plant extract were calculated using the BIOASSAY 97 software.

RESULTS AND DISCUSSION

Antioxidant activity of unripe walnut extract

The results of chemical analyses of the extract of unripe walnuts showed that total phenol content (TPC) was 173.7±7.9 mg GAE/g dry extract, while total flavonoid (TF) content was 870.9±16.3 mg QE/g dry extract. The antioxidative activity data, expressed in inhibition percentage, i.e. as neutralized free DPPH radicals against the control, are shown in Figure 1 by the dependence curve of plant extract and percentage of inhibition of DPPH radical. The IC_{50}, which is the concentration of green walnut extract needed to inhibit 50% of the initial amount of DPPH radicals, was 0.047 mg/ml. Comparing that value with the IC_{50} for ascorbic acid, which is used as a commercial antioxidant and rates 0.016 mg/ml, the obtained extract of green walnuts showed a significant antioxidative activity. The value received by analyzing ferric ion-reducing antioxidant power (FRAP) was 631.0 μmol Fe^{2+}/g dry extract. A comparison with the obtained FRAP value for BHT (6908 μmol Fe^{2+}/g) reveals that the reducing potential of green walnut extract was significantly weaker than the reducing potential of synthetic antioxidant but, having in mind that the extract contained only antioxidants of natural origin, such antioxidative activity is by no means insignificant.

Many studies have shown that the contents of total phenols and flavonoids, as well as antioxidative potential, change with ongoing maturity of plants and their parts (Jahanban-Esfahlan et al., 2019; Rusu et al., 2019, 2020). Besides, clear differences emerged between the values obtained from fresh, as opposed to dry materials, as well as different solvents, which ultimately resulted in varying results of these parameters reported from different studies (Zarghami Moghaddam et al., 2017).

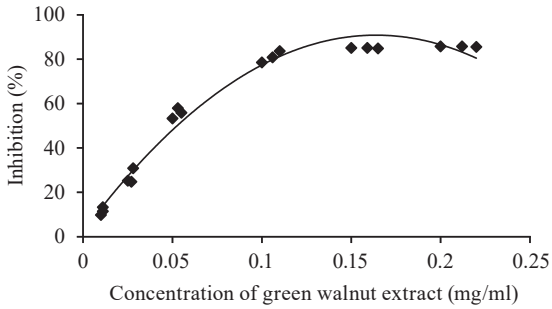


Figure 1. Dependence curve of green walnut extract concentrations (mg/ml) and DPPH radical inhibition (%)

Allelopathic effect of green walnut extract on seed germination and seedling growth of *A. retroflexus*, *C. album* and *D. carota*

The results of seed bioassays testing the allelopathic effects of green walnut extract on weed seeds are shown in Figures 2-4 and Table 2. The percentage of viable seeds after the TTC test for all weed species was over 80% for all concentrations, and 100% for maize seed (Table 1).

Table 1. Percentage of viable seeds of tested plants at different concentrations of extract

Test plant	Concentration of extract						
	Control	0.05%	0.1%	0.25%	0.5%	0.75%	1%
Amaranthus retroflexus	99.0	96.0	91.0	92.0	86.0	88.0	84.0
Chenopodium album	98.8	92.5	92.5	88.8	82.5	87.5	87.5
Daucus carota	98.8	96.3	100.0	85.0	85.0	83.8	87.5
Zea mays	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Seed germination of *A. retroflexus* ranged between 5% and 97%, and the highest germination was found in the control (97%) and treatment with the lowest concentration (0.05%) of extract (90%). Treatments with higher extract concentrations (1%, 0.75%, 0.50%, 0.25%, 0.10%) caused germination inhibition of 95%, 88%, 87%, 49% and 11%, respectively (Figure 2A), and statistical difference was significant between all extract concentrations and the control. The parameter of radicle length showed the highest susceptibility, compared to hypocotyls and overall seedling lengths, as all applied concentrations caused inhibition of 13-94% (Figure 2B). Statistically significant differences ($p<0.05$), noted between the control and all applied concentrations, confirm the finding. On the other hand, the extract of green walnuts had a stimulating effect (7-26%) on shoot length and total seedling length at the two lowest concentrations (0.05% and 0.1%) (Figure 2C and 2D). Higher extract concentrations (1%, 0.75% and 0.50%) resulted in shoot length inhibition of 70% to 100%, while total seedling length was inhibited by 8% to 97%. Statistically significant differences were missing only between the control and the two lowest concentrations (0.05% and 0.10%) in both parameters, while significant differences ($p<0.05$) were detected between 0.25%, 0.50%, 0.75% and 1% concentrations and the control (Figure 2C and 2D).

The bioassay results revealed that *C. album* was more susceptible than the other two weed species as inhibition was noted for all parameters even under the lowest concentration. On the other hand, the lowest concentration (0.05%) had a stimulating effect

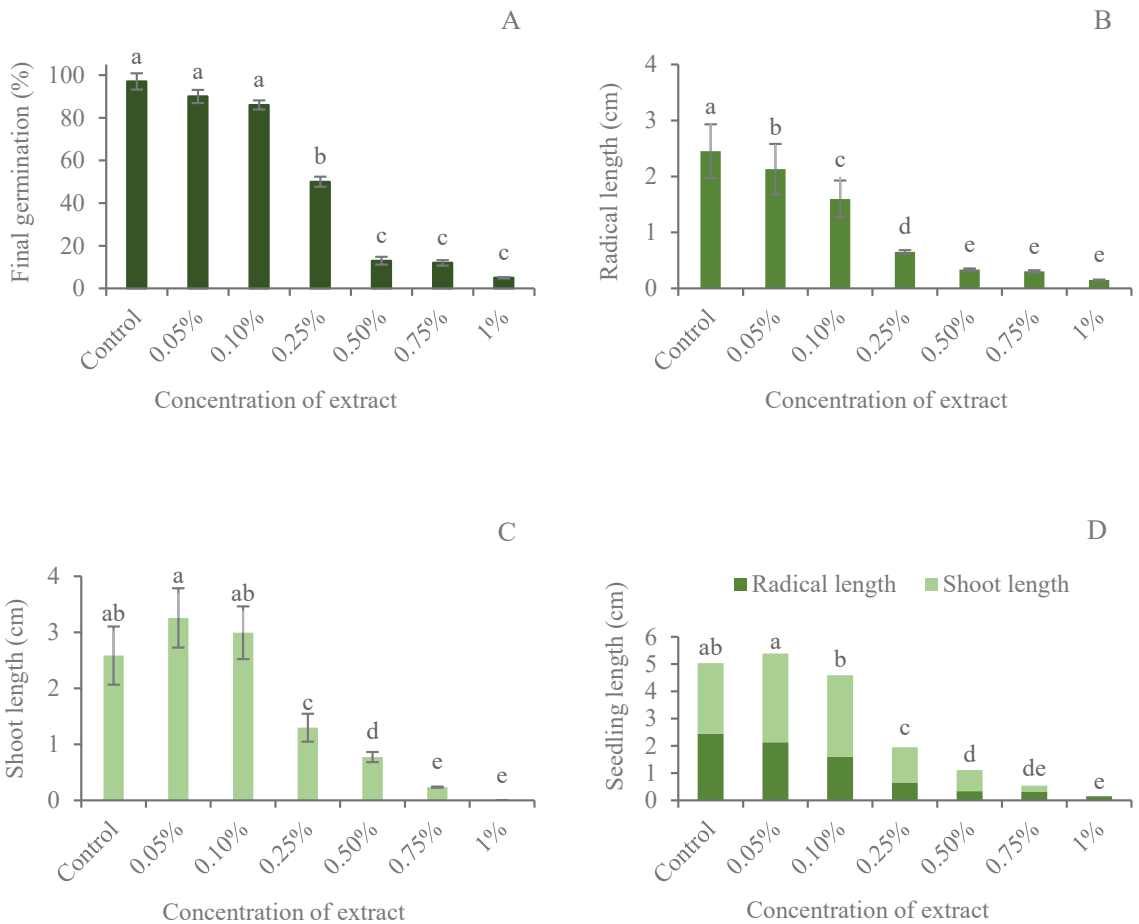


Figure 2. Effect of green walnut extract on seed germination (A), and seedling growth (B, C, D) of *A. retroflexus*. Differences were evaluated by one-way analysis of variance (ANOVA) completed with Fisher's least significant difference (LSD) test, $p<0.05$. Means marked by different letters (a, b, c, d, e) differ significantly ($p<0.05$) for all parameters at different concentrations

on *A. retroflexus* and *D. carota*. The inhibition of *C. album* germination under the lowest concentration was 33%, while higher concentrations caused 38% to 78% inhibition. Statistically significant differences ($p < 0.05$) were noted between the control and all extract concentrations (Figure 3A). Regarding the shoot length of *C. album* seed, no significant difference was detected between the control and the lowest extract concentration (0.05%), while inhibition of three lower concentrations did not exceed 33%. Conversely, higher concentrations caused significantly higher inhibition (92-94%) and a significant difference ($p < 0.05$) was confirmed between those concentra tions and the control (Figure 3C). Although inhibition of radicle length by the lowest extract concentration was 31%, it was lower than inhibition of shoot length, which ranged from 60% to 85%. The sensitivity of this parameter was confirmed by statistically significant differences ($p < 0.05$) found between the control and all tested concentrations (Figure 3B).

Inhibitory activity of the unripe (green) walnut extract was evidenced by all measured parameters of *D. carota*. Inhibition grew proportionally to rising extract concentrations, reaching 20-98%, 8-96%, 16-100%, and 12-98% based on seed germination, radical length, shoot length and total seedling length results, respectively (Figures 4A, B, C and D). However, a stimulative activity of the lowest extract concentration (0.05%) was also found for all measured parameters, ranging from 1% to 6%. Statistically significant differences ($p < 0.05$) were found between the control and concentrations of 0.25%, 0.50%, 0.75%, and 1% for all parameters, while the lowest concentration showed no significant difference from the control (Figure 4).

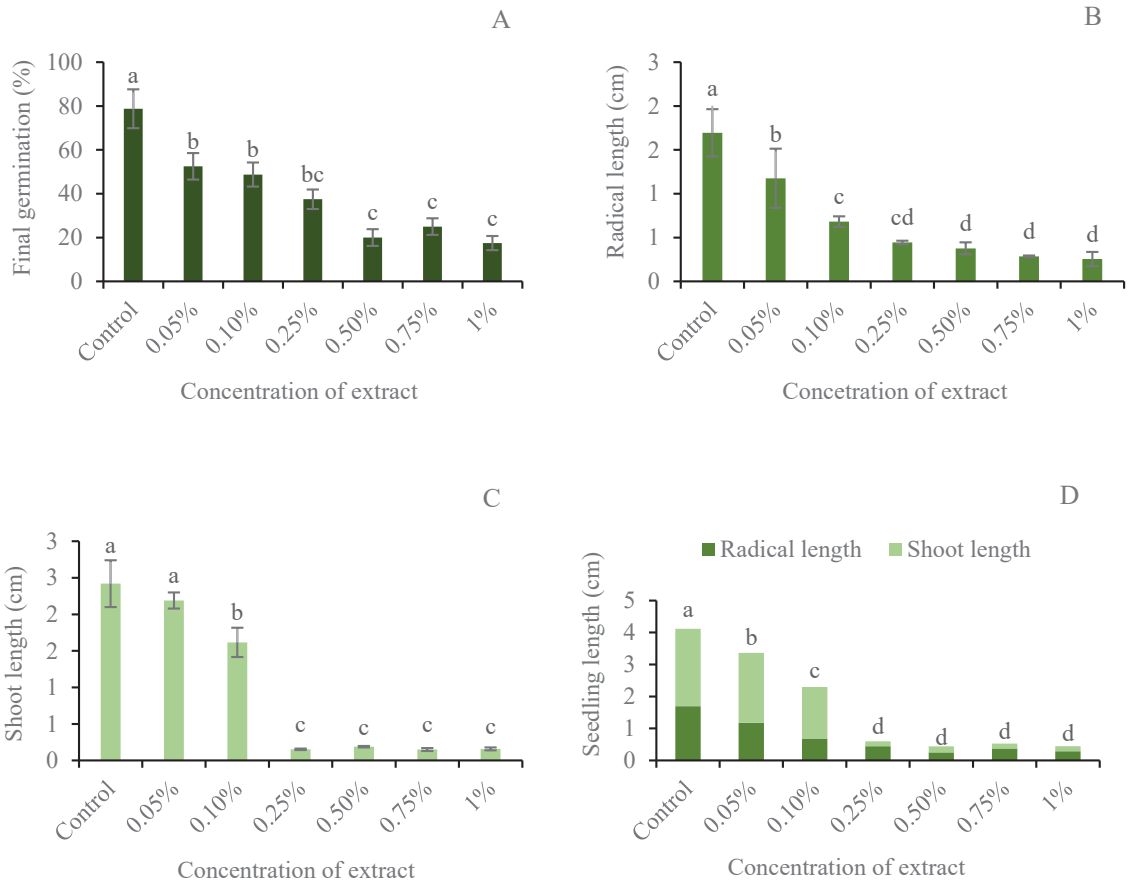


Figure 3. Effect of green walnut extract on seed germination (A) and seedling growth (B, C, D) of *C. album*. Differences were evaluated by one-way analysis of variance (ANOVA) completed with Fisher's least significant difference (LSD) test, $p<0.05$. Means marked by different letters (a, b, c, d) differ significantly ($p<0.05$) for all parameters at different concentrations

The described inhibition levels clearly showed that the parameters of seedling growth (radical and hypocotyl length, and total seedling length) were more sensitive in all three test weed species than total seed germination. Greater sensitivity of the growth parameters was further confirmed by lower EC_{50} values for those parameters in all tested plant species, *C. album* showing the lowest (Table 2).

Most literature data indicate that juglone has a predominantly toxic effect on plants of many species,

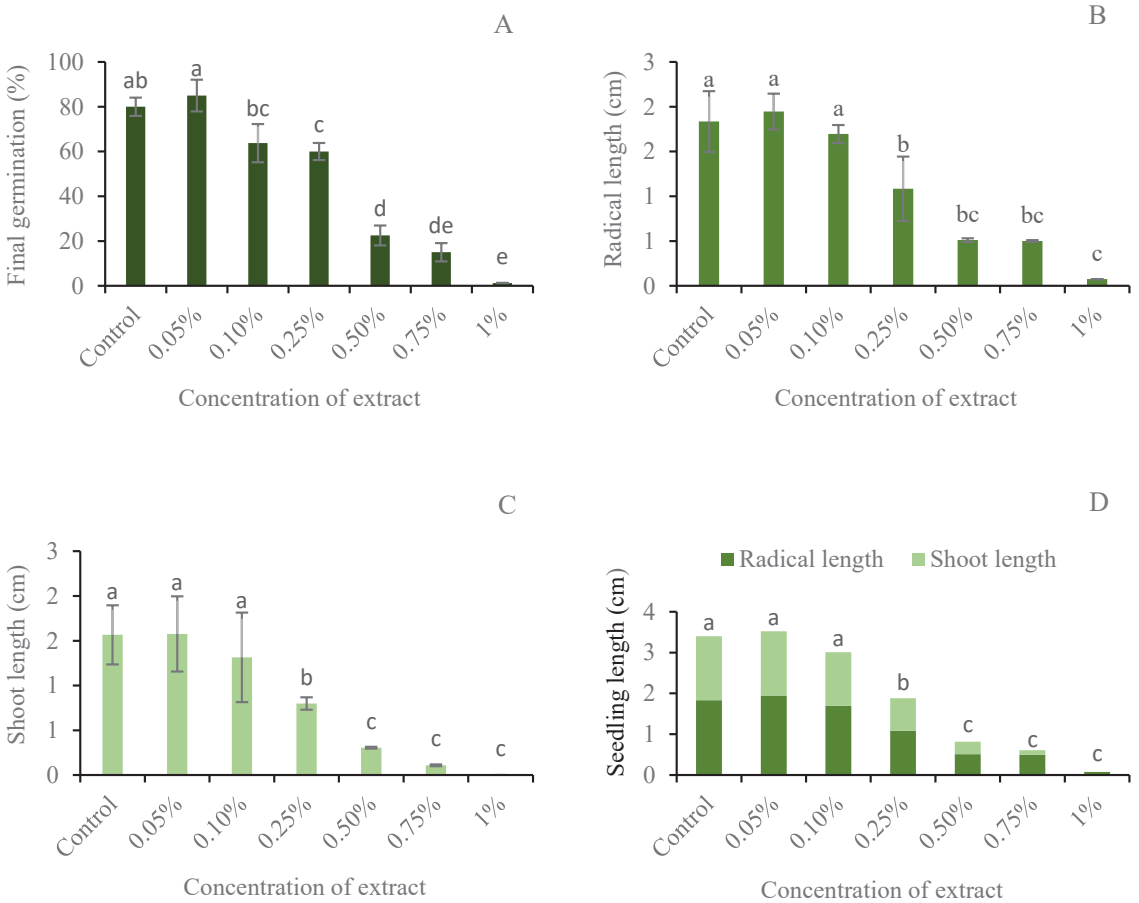


Figure 4. Effects of green walnut extract on seed germination (A) and seedling growth (B, C, D) of *D. carota*. Differences were evaluated by one-way analysis of variance (ANOVA) completed with Fisher's least significant difference (LSD) test, $p<0.05$. Means marked by different letters (a, b, c, d, e) differ significantly ($p<0.05$) for all parameters at different concentrations

Table 2. EC_{50} for seed germination and seedling growth of tested plants

Test plant	EC_{50}±SE			
	Final germination	Radical length	Shoot length	Seedling length
Amaranthus retroflexus	0.24 ± 0.02	0.13 ± 0.01	0.23 ± 0.05	0.21 ± 0.06
Chenopodium album	0.27 ± 0.09	0.06 ± 0.01	0.11 ± 0.01	0.14 ± 0.03
Daucus carota	0.59 ± 0.17	0.30 ± 0.09	0.28 ± 0.02	0.30 ± 0.09
Zea mays	–	0.70 ± 0.13	0.29 ± 0.17	0.70 ± 0.13

inhibiting their seed germination and early growth of young plants (Kocaçalikan & Terzi, 2001; Terzi, 2008; Maksimović & Hasanagić, 2020). Maksimović and Hasanagić (2020) confirmed the allelopathic activity of juglone on seed germination of tomato and green salad. Germination of tomato seed and early seedling growth were most intensively inhibited by concentrated juglone, while its lower concentrations resulted in a weak stimulative effect. On the other hand, compared to tomato, all applied juglone concentrations had a significant inhibitory effect on germination and early seedling growth of green salad. Some other studies (Kocaçalikan & Terzi, 2001; Terzi, 2008) also confirmed the inhibitory activity of juglone on seed germination of specific plant species, such as cabbage, beetroot, green salad, cucumber, alfalfa, etc. Inhibition of seed germination was shown to weaken proportionally to decreasing extract concentrations, which indicates that the allelopathic effect of a specific extract depends significantly on its concentration.

Phytotoxic effect of green walnut extract on seed germination and seedling growth of *Zea mays*

The results of bioassay testing aimed at clarifying the phytotoxic effects on seed germination and early seedling growth of a cultivated species (*Z. mays*) are shown in Figure 5. Consistent with the results of weed species seed bioassays, where a small inhibitory effect on seed germination was found, compared to the parameters of early seedling growth (radical and hypocotyls length and total seedling length), similar trends were revealed for *Z. mays*. Phytotoxic effect on maize seed germination was

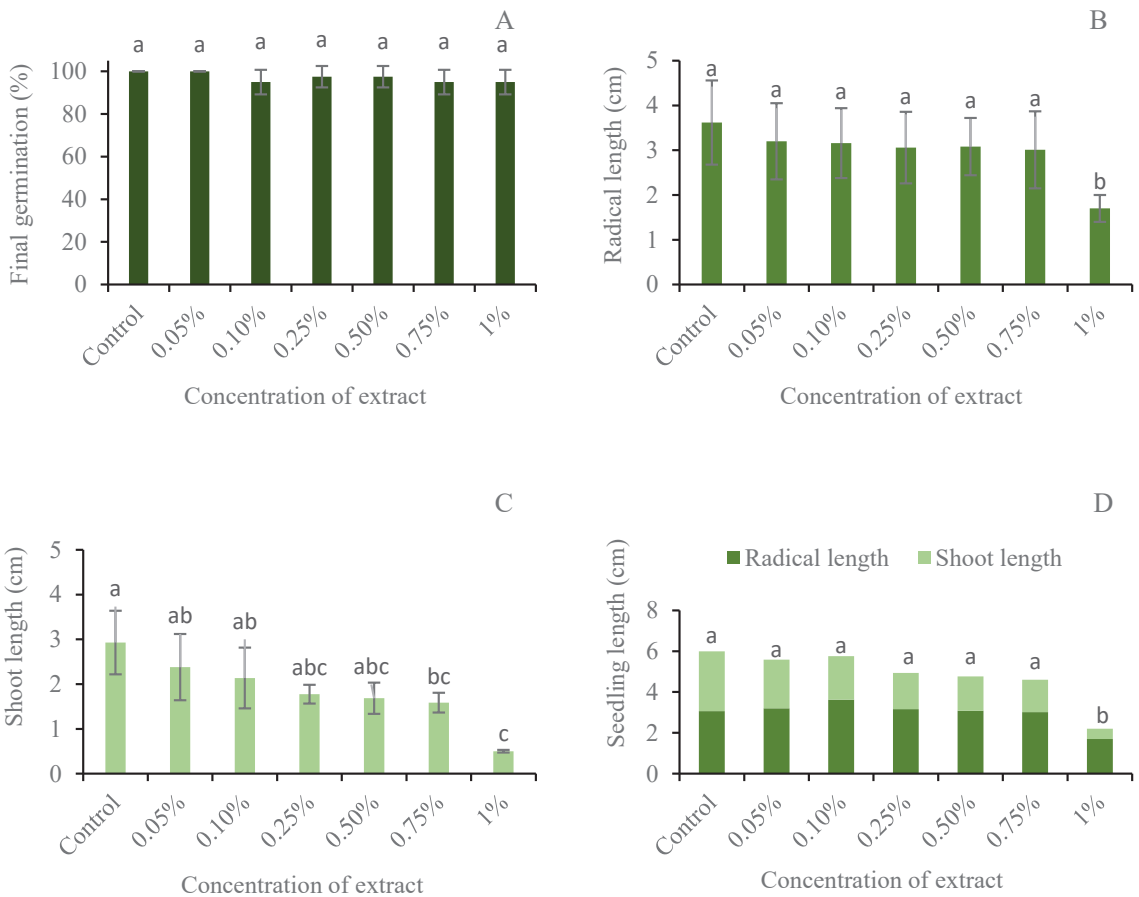


Figure 5. Effect of green walnut extract on seed germination (A), and seedling growth (B, C, D) of *Z. mays*. Differences were evaluated by one-way analysis of variance (ANOVA) completed with Fisher's least significant difference (LSD) test, $p<0.05$. Means marked by different letters (a, b, c, d, e) differ significantly ($p<0.05$) for all parameters at different concentrations

evident from growing inhibition caused by increasing extract concentrations, which ranged from 2.5 to 5% (Figure 5A). Inhibition of radical length and total seedling length was similar, ranging from 12 to 66% (Figures 5B and 5D), while greater inhibition percentages were found for hypocotyl length (19-83%) (Figure 5C).

Statistical data analysis revealed no significant difference ($p=0.485$) in maize seed germination, while seedling growth parameters showed significant differences ($p < 0.05$) between the control and the highest concentration (1%). Significant difference was also found for shoot length between control and concentrations of 0.75% and 1% (Figure 5).

CONCLUSION

Promising results of the initial screening of content (total phenols and flavonoids) and antioxidative properties of the obtained extract of green walnuts inspire further research steps which should include a complete HPLC analysis of that plant extract. The seed bioassay results indicate an inhibitory effect of different concentrations of green walnut extract on seed germination of the weed species *A. retroflexus*, *C. album,* and *D. carota*. Inhibitory activity of a series of extract concentrations on early seedling growth parameters (radical length, shoot length, and total seedling length) was revealed, which showed their greater sensitivity than seed germination. Although the seed bioassay results show a high inhibitory effect on germination and early growth of all three weed species, they also reveal phytotoxic effects on early seed growth of the cultivated species *Z. mays*. Future research needs to include pot trials with different plants, as well as field micro-trials, to determine bioherbicidal effectiveness on weeds, and phytotoxic effects on cultivated species.

ACKNOWLEDGEMENTS

This research was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contracts No. 451-03-47/2023-01/200214 and 451-03-47/2023-01/200116).

REFERENCES

An, M., Liu, D.L., Johnson, I.R., & Lovett, J.V. (2003). Mathematical modeling of allelopathy: II. The dynamics of allelochemicals from living plants in the environment. *Ecological Modelling, 161*, 53-66. Doi: https://doi.org/10.1016/S0304-3800(02)00289-2

Belz, R.G. (2007). Allelopathy in crop/weed interactions – An update. *Pest Management Science*, *63*, 308-326. Doi: 10.1002/ps.1320

Cosmulescu, S., Trandafir, I., Nour, V., Ionica, M., & Tutulescu, F. (2014). Phenolics content, antioxidant activity and color of green walnut extracts for preparing walnut liquor, *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *42*, 551-555. Doi: https://doi.org/10.15835/nbha4229649

Duke, S.O., Romagni, J.G., & Dayan, F.E. (2000). Natural products as sources for new mechanisms of herbicidal action. *Crop Protection*, *19*, 583-589.

Gatti, A. B., Ferreirall, A. G., Arduin, M., & Andrade Perez, S. C. G. (2010). Allelopathic effects of aqueous extracts of *Artistolochia esperanzae* O. Kuntze on development of *Sesamum indicum* L. seedlings. *Acta Botanica Brasilica*, *24*, 454-461.

Gella, D., Ashagre, H., & Negewo, T. (2013). Allelopathic effect of aqueous extracts of major weed species plant parts on germination and growth of wheat. *Journal of Agricultural and Crop Research*, *1*(3), 30-35.

Hatcher, P.E., & Melander, B. (2003). Combining physical, cultural, and biological methods: Prospects for integrated non-chemical weed management strategies. *Weed Research*, *43*, 303-322. Doi: 10.1046/j.1365-3180.2003.00352.x

Hossard, L., Guichard, L., Pelosi, C., & Makowski, D. (2017). Lack of evidence for a decrease in synthetic pesticide use on the main arable crops in France. *Science of the Total Environment*, *575*, 152-161.

Jahanban-Esfahlan A., Ostadrahimi A., Tabibiazar M., & Amarowicz R. (2019). A comparative review on the extraction, antioxidant content and antioxidant potential of different parts of walnut (*Juglans regia* L.) fruit and tree. *Molecules*, *24*, 2133. Doi: 10.3390/molecules24112133

Kanissery, R., Gairhe, B., Kadyampakeni, D., Batuman, O., & Alferez, F. (2019). Glyphosate: Its environmental persistence and impact on crop health and nutrition. *Plants*, *8*, 499. doi: 10.3390/plants8110499

Kocaçalikan, I., & Terzi, I. (2001). Allelopathic effects of walnut leaf extracts and juglone on seed germination and seedling growth. *Journal of Horticultura Science & Biotechnology*, *76 (4)*, 436-440.

Maksimović, T., & Hasanagić, D. (2020). Allelopathic influence of *Juglans regia* L. aqueous extract on the germination and growth of lettuce and tomato. *Journal of Agricultural, Food and Environmental Sciences*, *74(2)*, 5-12.

Narwal, S.S. (2010). Allelopathy in ecological sustainable organic agriculture. *Allelopathy Journal*, *25*, 51-72.

Rusu, M.E., Fizesan, I., Pop, A., Mocan, A., Gheldiu, A., Babota M. ... Popa, D. S. (2020). Walnut (*Juglans regia* L.) septum: assessment of bioactive molecules and *in vitro* biological effects. *Molecules, 25,* 2187.

Rusu, M.E., Mocan, A., Ferreira, I.C.F.R., & Popa, D.S. (2019). Health benefits of nut consumption in middle-aged and elderly population. *Antioxidants, 8*(8), 302.

Terzi, I. (2008). Allelopathic effects of juglone and decomposed walnut leaf juice on muskmelon and cucumber seed germination and seedling growth. *African Journal of Biotechnology*, *7(12),* 1870-1874.

Vilà, M., Basnou, C., Pyšek, P., Josefsson, M., Genovesi, P., Gollasch, S. ... Hulme, P. E. (2010). How well do we understand the impacts of alien species on ecosystem services? A pan-European, cross-taxa assessment. *Frontiers in Ecology and the Environment, 8*(3), 135-144.

Villa, F., Cappitelli, F., Cortesi, P., & Kunova, A. (2017). Fungal biofilms: Targets for the development of novel strategies in plant disease management. *Frontiers in Microbiology, 8,* 654.

Wu, H., Haig, T., Pratley, J., Lemerle, D., & An, M. (2000). Allelochemicals in wheat (*Triticum aestivum* L.): variation of phenolic acids in root tissues. *Journal of Agricultural and Food Chemistry, 48,* 5321-5325.

Zarghami Moghaddam, P., Mohammadi, A., Feyzi, P., & Alesheikh, P. (2017). *In vitro* antioxidant and antibacterial activity of various extracts from exocarps and endocarps of walnut. *Pakistan Journal of Pharmaceutical Sciences, 30,* 1725-1731.

Zurek, N., Pawłowska, A., Pycia, K., Grabek-Lejko, D., & Kapusta, T. I. (2022). Phenolic profile and antioxidant, antibacterial, and antiproliferative activity of *Juglans regia* L. male flowers. *Molecules, 27,* 2762. Doi: 10.3390/molecules27092762

Antioksidativno delovanje, fitotoksičnost i alelopatski potencijal ekstrakta zelenog ploda oraha (*Juglans regia* L.)

REZIME

Cilj ovog rada je bio da se ispita alelopatski potencijal ekstrakta zelenih nezrelih plodova oraha na klijanje i rani porast semena tri korovske vrste (*Chenopodium album*, *Amaranthus retroflexus*, *Daucus carota*), kao i fitotoksični efekat na klijanje i rani porast semena kukuruza *Zea mays*. Pored toga, cilj je bio i da se uradi analiza biljnog ekstrakta i procena njegove antioksidativne aktivnosti.

Antioksidativna aktivnost biljnog ekstrakta određena je kroz sposobnost neutralizacije DPPH (2,2-difenil-1-pikrilhidrazil) radikala i ispitivanjem sposobnosti redukcije gvožđa FRAP metodom. Inhibicija merenih parametara (ukupna klijavost i dužina klijanaca) se proporcionalno smanjivala sa smanjenjem koncentracije rastvora ekstrakta zelenih nezrelih plodova oraha. Takođe, parametri rasta klijanaca su ispoljili veću osetljivost u odnosu na parametar ukupne klijavosti semena kod sve tri korovske vrste. Iako dobijeni rezultati iz biotesta sa semenima pokazuju značajan inhibitorni efekat na klijanje i rani porast testiranih korovskih vrsta, ujedno ukazuju i na fitotoksične efekte kod ranog porasta semena gajene vrste *Z. mays*.

Ključne reči: alelopatija, orah, biljni ekstrakt, korovi, kukuruz

Instructions for Authors

About Journal

Pesticidi i fitomedicina (Pesticides and Phytomedicine) is dedicated to the following research fields: toxicology and ecotoxicology of pesticides; phytopathology; applied entomology and zoology; weed science; plant and food products protection; use of pesticides in agriculture, sanitation and public health.

The journal continues the title *Pesticidi*, which was published over the period 1986-2003.

Pesticidi i fitomedicina (Pesticides and Phytomedicine) publishes original scientific papers and review papers that have not been published previously.

Pesticidi i fitomedicina (Pesticides and Phytomedicine) is an Open Access journal.

Contributions to the journal must be submitted in English, with summaries in English and Serbian (Serbian-speaking authors only).

As of 2020, *Pesticidi i fitomedicina (Pesticides and Phytomedicine)* is issued triannually (three issues annually).

As of 2021, Pesticides and Phytomedicine (Pesticidi i fitomedicina) will be published **online only,** and paper copies of future issues will no longer be available. The primary platforms for journal publication will continue to be: Scindeks (http://scindeks.ceon.rs/journaldetails.aspx?issn=1820-3949) and the publisher's official web site (http://www.pesting.org.rs/).

The journal is indexed in: Chemical Abstracts, CAB International; DOAJ, EBSCO, AGRIS, Scindeks.

In 2011, the journal converted to an electronic online journal management system on the SCIndeks Assistant portal at **http://scindeks.ceon.rs/journaldetails.aspx?issn=1820-3949.** The system enables easy article submission and communication among the editorial staff, reviewers and authors. It also includes several quality control services: *CrossRef* for DOI assignment, *CrossChek* for plagiarism prevention and *KWASS* for equipping articles with keywords extracted from a dictionary/thesaurus. Electronic editing is in compliance with the Journal Editing Act of the Ministry of Education, Science and Technological Development of the Republic of Serbia, and provides record-keeping stipulated in the Act.

Manuscript submission

To be published in *Pesticidi i fitomedicina (Pesticides and Phytomedicine),* an article must be based on original scientific results that have not been previously published and are not under consideration for publication elsewhere. Review articles should contain a comprehensive survey of a particular subject based on referenced literature and published results of the author(s) own research. All contributions are peer reviewed in a double blind process.

A click on "submit a manuscript" on the left-hand side of the journal home page in SCIndeks Assistant will lead users to a registration page and further on into a guided process of electronic manuscript submission. Serbian authors are requested to fill out the application form in both English and Serbian. Each visual or graphic item (table, chart, diagram or photo) should be submitted as a separate (supplementary) file.

Authors need NOT specify keywords in their articles. They will be extracted and selected by the Editor-in-Chief from the *KWASS* thesaurus (dictionary), which will significantly improve article visibility. Authors are entitled to accept or change some of the keywords.

Manuscript preparation

The manuscript should be prepared in Microsoft Word (A4 format, all margins 25 mm, font Times New Roman 12 pt). Articles have to be written in the English language, and only the title and abstract in both English and Serbian (Serbian summary will be furnished by the copyeditor for foreign authors'manuscripts).

Title should be concise and refer to the subject. Full names and surnames of all authors, details of their respective affiliations and emails should be indicated below the title. If discrepancy in such data occurs between the textual document and submission metadata in Assistant, the former will be given precedence.

Abstract (not exceeding 300 words) should briefly state the main results and conclusions.

Articles should contain the following sections: Introduction, Material and Methods, Results, Discussion, Acknowledgement and References.

Introduction should present the state-of-the-art in a particular research field, as well as research intent.

Material and Methods should provide sufficient detail to allow the work to be reproduced. Conventional methods should only be referenced.

Results should be presented in a logical order, clearly and concisely, using adequate tables and graphics. Avoid repetition of the results in tables and graphics, or in the text.

Discussion should emphasize the importance of the results, as well as their place within the context of previous research. Wherever possible, Results and Discussion should be separate sections.

Acknowledgement should be collated at the end of the manuscript before References.

References cited in the text need to include the author's/authors' surname(s) and year of publication:

- author, year;
- first & second author, year;
- first author et al., year.

References mentioned in the manuscript must be listed in the References section at its end, in alphabetic order and using the **APA citation style** (see description at e.g. https://owl.english.purdue.edu/owl/resource/560/01/).

Journal references are required to contain the following information: name(s) of author(s), year of publication, title of article, title of journal, volume, issue number (unless pagination is continuous), pages (from-to) and DOI if available.

Dedić, B. (2012). Testing sunflower inbred lines for tolerance to phoma black stem. *Pesticides & Phytomedicine*, *27*(4), 299-303. doi:10.2298/PIF1204299D

Abbaspoor, M. & Streibig, J.C. (2005). Clodinafop changes the chlorophyll fluorescence induction curve. *Weed Science, 53*(1), 1-9. doi:10.1614/WS-04-131R

Abbaspoor, M., Teicher, H.B. & Streibig, J.C. (2006). The effect of root-absorbed PSII inhibitors on Kautsky curve parameters in sugar beet. *Weed Research, 46*(3), 226-235. doi:10.1111/j.1365-3180.2006.00498.x

Books: name(s) of author(s) or editor(s), year of publication, title, place of publication and name of publisher.

Timbrell, J. (2000). *Principles of biochemical toxicology* (3rd ed). London, UK: Taylor and Francis Ltd.

Frank, R. H. & Bernanke, B. (2007). *Principles of macroeconomics* (3rd ed.). Boston, MA: McGraw-Hill/Irwin.

Saari L.L. & Thill, D.C. (Eds.). (1994). *Resistance to acetolactate synthase inhibiting herbicides: Herbicide resistance in plants*. Boca Raton, FL, USA: CRC Press.

Dissertations: author's name, year of presentation, title, full name of the institution at which dissertation was defended.

Stepanović, M. (2012). *Osetljivost izolata Alternaria solani (Sorauer) iz različitih krajeva Srbije na fungicide i rizik rezistentnosti*. (Doktorska disertacija). Univerzitet u Beogradu, Poljoprivredni fakultet, Beograd.

Book chapters and articles in conference proceedings: author(s), year of publication, title of chapter/article/abstract, source title (with editors names), pages, place of publication and publisher.

Hammond, K. R. & Adelman, L. (1986). Science, values, and human judgment. In H. R. Arkes & K. R. Hammond (Eds.), *Judgement and decision making: An interdisciplinary reader* (pp. 127-143). Cambridge, England: Cambridge University Press.

Edwards, J.P., Fitches, E.C., Audsley, N. & Gatehouse, J.A. (2002). Insect neuropeptide fusion proteins – A new generation of orally active insect control agents. In T. Margini (Ed.), *Proceedings of the BCPC – Pests and diseases* (pp. 237-242). Brighton, UK: University of Brighton Press.

Internet references: author(s), year of publication, title, source title, link.

Graora, D., & Spasić, R. (2008). Prirodni neprijatelji *Pseudaulacaspis pentagona* Targioni-Tozzetti u Srbiji. *Pesticidi i fitomedicina*, *23*(1) 11-16. Retrieved from http://www.pesting.org.rs/media/casopis/2008/no.1/23_1_11-16.pdf

Radunović, D., Gavrilović, V., Gašić, K., Krstić, M. (2015). Monitoring of *Erwinia amylovora* in Montenegro. *Pesticides and Phytomedicine*, 30(3), 179-185. doi 10.2298/PIF1503179R or http://www.pesting.org.rs/media/casopis/2015/no.3/30-3_179-185.pdf

Kerruish, R.M. & Unger, P.W. (2010). *Plant protection 1 – Pests, diseases and weeds*. Retrieved from APPS at http://www.appsnet.org/Publications/Kerruish/PP1.pdf

Tables need to be numbered in Arabic numerals consecutively as they appear in text. Tables should be made exclusively in Word for Windows using the toolbar menu Table-Insert-Table, Times New Roman font, 12 pt, and single line spacing. Footnotes immediately below the table body should be given priority over other explanation in table header or in table cells, and text should be in Times New Roman font, 10 pt. Each table must have a header. Tables should be submitted as supplementary (separate) files, and their approximate location in the text marked.

Graphs should be processed in Microsoft Excel and all data in Times New Roman font. Explanations should be provided in captions, consecutively and marked with Arabic numerals. Graphs should be submitted as supplementary files, and their approximate location in the text marked.

Diagrams should be processed in Corel Draw (version 9 or later) or in Adobe Illustrator (version 9 or later) and all data written in Times New Roman font. Diagrams should be submitted as supplementary files and their approximate locations in the text marked.

Photos need to be taken by digital camera (resolution at least 150 dpi, photo dimension A4, file format JPG or TIFF). If authors are unable to submit original photos, those should be scanned in RGB mode (colour) or as Grayscale (black and white), with 300 dpi resolution in original size. Photos need to be marked with Arabic numerals in consecutive order. Provide each photo with a caption, mark its approximate location in the text and submit it as a supplementary file.

Authors are expected to use the accepted International System of Units (SI). Abbreviations should be defined in brackets at their first in-text mention. Provide full Latin names along with common names of organisms, and italicize only Latin names of genera and species, e.g. Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). After first mention, the Latin name can be abbreviated (e.g. *L. decemlineata*).

Review articles need to contain an introduction, appropriate subtitles and a reference list.

Publishing in *Pesticidi i fitomedicina (Pesticides and Phytomedicine)* is free of charge.

The editorial staff practice a policy of plagiarism prevention.

Uputstvo autorima

O časopisu

Časopis *Pesticidi i fitomedicina (Pesticides and Phytomedicine)* objavljuje naučne radove iz oblasti: toksikologije i ekotoksikologije pesticida; fitopatologije; primenjene entomologije i zoologije; herbologije; zaštite bilja i prehrambenih proizvoda; primene pesticida u poljoprivredi, komunalnoj higijeni i javnom zdravstvu.

Časopis *Pesticidi i fitomedicina (Pesticides and Phytomedicine)* predstavlja nastavak publikacije *Pesticidi*, koja je pod tim imenom izlazila u periodu 1986-2003.

Časopis *Pesticidi i fitomedicina (Pesticides and Phytomedicine)* objavljuje originalne i pregledne, prethodno neobjavljene radove.

Časopis *Pesticidi i fitomedicina (Pesticides and Phytomedicine)* je dostupan u režimu otvorenog pristupa.

Radovi koji se prilažu moraju biti napisani na engleskom jeziku, sa rezimeom na engleskom i srpskom jeziku.

Od 2020. godine, časopis izlazi četvoromesečno (tri broja godišnje).

Od 2021. godine, časopis Pesticidi i fitomedicina (Pesticides and Phytomedicine) objavljuje sveske samo u elektronskom obliku, bez štampane verzije. Osnovne platforme na kojima se postavljaju sadržaji časopisa su: Scindeks (http://scindeks.ceon.rs/journaldetails.aspx?issn=1820-3949) i zvanični veb sajt izdavača (http://www.pesting.org.rs/).

Časopis se indeksira u sledećim bazama: Chemical Abstracts, CAB International; DOAJ, EBSCO, AGRIS, Scindeks.

Tokom 2011. godine, časopis je prešao na sistem onlajn uređivanja (Elektronsko uređivanje – e-Ur) na portalu SCIndeks Asistent **http://scindeks.ceon.rs/journaldetails.aspx?issn=1820-3949**. Ovaj sistem uređivanja omogućava funkcionalniju prijavu radova i komunikaciju uredništva sa recenzentima i autorima, a obuhvata i servise za kontrolu kvaliteta radova: *CrossRef* za dodelu DOI, *CrossChek* za prevenciju plagijarizma i *KWASS* za opremanje radova ključnim rečima ekstrahovanim iz rečnika/tezaurusa. Elektronsko uređivanje je usaglašeno sa Aktom o uređivanju časopisa Ministarstva prosvete, nauke i tehnološkog razvoja RS i obezbeđuje vođenje evidencije koje ovaj akt nalaže.

Prijavljivanje radova

Publikovanje u časopisu *Pesticidi i fitomedicina (Pesticides and Phytomedicine)* podrazumeva da rad sadrži rezultate originalnih istraživanja koji nisu objavljeni, odnosno nisu dostavljeni nekom drugom časopisu za objavljivanje. Pregledni radovi treba da sadrže sveobuhvatan prikaz određene teme zasnovan na referentnoj literaturi i publikovanim rezultatima sopstvenih istraživanja. Svi radovi se recenziraju, a recenzija je obostrano anonimna.

Klikom na "submit a manuscript" na levoj polovini početne stranice u SCIndeks Asistentu, dolazi se do opcije za registraciju i prijavu rukopisa i ulazi u vođeni postupak elektronske prijave rada. Obaveza srpskih korisnika je da prijavu popune na oba jezika (srpskom i engleskom). Svaki likovno-grafički prilog (tabela, grafikon, dijagram, slika) se prilaže kao zasebna (dopunska) datoteka.

Autori u radu NE NAVODE ključne reči. Njih će glavni urednik ekstrahovati iz *KWASS* tezaurusa (rečnika), što će značajno poboljšati vidljivost rada. Autori imaju pravo da dodeljene ključne reči prihvate ili da neke od njih zamene.

Priprema rada

Rad treba pripremiti u programu za obradu teksta Word (format A4, margine 25 mm, font Times New Roman 12 pt). Radovi treba da budu isključivo na engleskom jeziku sa naslovom i rezimeom na oba jezika (engleskom i srpskom).

Naslov treba da bude kratak i da upućuje na temu. Puna imena i prezimena svih autora, puni nazivi i adrese institucija svih autora i njihove email adrese treba navesti ispod naslova rada. U slučaju neslaganja ovih podataka u samom tekstu rada i u prijavi na platformi za uređivanje, prioritet će se dati podacima u samom tekstu rada.

Rezime (obima do 300 reči) treba da predstavi ono što je za rad najznačajnije.

Rad treba, po pravilu, da sadrži sledeća poglavlja: Uvod, Materijal i metode, Rezultati, Diskusija, Zahvalnica i Literatura.

Uvod treba da sadrži najnužniji pregled istraživanja u datoj oblasti i ciljeve istraživanja.

Materijal i metode treba opisati dovoljno detaljno da omogućе ponavljanje ispitivanja. Poznate metode i tehnike označiti samo odrednicom iz literature.

Rezultate predstaviti logičnim redosledom, jasno i precizno, koristeći prigodne tabele i grafičke prikaze. Izbegavati ponavljanje rezultata u tabelama i grafikonima, ali i u tekstu rada.

Diskusija treba da istakne značaj dobijenih rezultata, kao i njihovo mesto u kontekstu prethodnih istraživanja. Kad god je to moguće, diskusiju treba odvojiti od rezultata.

Zahvalnica se navodi na kraju teksta rada, pre literature.

Literatura se u tekstu rada citira navođenjem prezimena autora i godine:

- autor, godina;
- prvi & drugi autor, godina;
- prvi autor et al., godina.

Literatura citirana u radu se navodi na kraju rada, abecednim redom prema pravilima **APA citatnog stila** (pis videti npr. na https://owl.english.purdue.edu/owl/resource/560/01/).

Reference u časopisima treba da sadrže sledeće podatke: autor(i), godina publikovanja, naslov rada, naslov časopisa, volumen, broj (ako se paginacija ponavlja), brojeve stranica (od – do) i doi broj (ukoliko postoji).

Dedić, B. (2012). Testing sunflower inbred lines for tolerance to phoma black stem. *Pesticides & Phytomedicine*, *27*(4), 299-303. doi:10.2298/PIF1204299D

Abbaspoor, M., & Streibig, J.C. (2005). Clodinafop changes the chlorophyll fluorescence induction curve. *Weed Science, 53*(1), 1-9. doi:10.1614/WS-04-131R

Abbaspoor, M., Teicher, H.B., & Streibig, J.C. (2006). The effect of root-absorbed PSII inhibitors on Kautsky curve parameters in sugar beet. *Weed Research, 46*(3), 226-235. doi:10.1111/j.1365-3180.2006.00498.x

Knjige: autor(i) ili editor(i), godina publikovanja, naslov, mesto publikovanja i naziv izdavača.

Timbrell, J. (2000). *Principles of biochemical toxicology* (3rd ed). London, UK: Taylor and Francis Ltd.

Frank, R. H., & Bernanke, B. (2007). *Principles of macroeconomics* (3rd ed.). Boston, MA: McGraw-Hill/Irwin.

Saari L.L., & Thill, D.C. (Eds.). (1994). *Resistance to acetolactate synthase inhibiting herbicides: Herbicide resistance in plants*. Boca Raton, FL, USA: CRC Press.

Disertacije: autor, godina odbrane, naslov, i puni naziv institucije u kojoj je disertacija odbranjena.

Stepanović, M. (2012). *Osetljivost izolata Alternaria solani (Sorauer) iz različitih krajeva Srbije na fungicide i rizik rezistentnosti*. (Doktorska disertacija). Univerzitet u Beogradu, Poljoprivredni fakultet, Beograd.

Poglavlja u knjigama i radovi u zbornicima: autor(i), godina publikovanja, naslov poglavlja/rada/apstrakta, naslov izvornika sa imenom (imenima) urednika, strane priloga, mesto publikovanja i naziv izdavača.

Hammond, K. R., & Adelman, L. (1986). Science, values, and human judgment. In H. R. Arkes & K. R. Hammond (Eds.), *Judgement and decision making: An interdisciplinary reader* (pp 127-143). Cambridge, UK: Cambridge University Press.

Edwards, J.P., Fitches, E.C., Audsley, N. & Gatehouse, J.A. (2002). Insect neuropeptide fusion proteins – A new generation of orally active insect control agents. In T. Margini (Ed.), *Proceedings of the BCPC – Pests and diseases* (pp 237-242). Brighton, UK: University of Brighton Press.

Internet reference: autor(i), godina publikovanja, naslov, naziv izvornika, link.

Graora, D., & Spasić, R. (2008). Prirodni neprijatelji *Pseudaulacaspis pentagona* Targioni-Tozzetti u Srbiji. *Pesticidi i fitomedicina*, *23*(1) 11-16. Retrieved from http://www.pesting.org.rs/media/casopis/2008/no.1/23_1_11-16.pdf

Radunović, D., Gavrilović, V., Gašić, K., Krstić, M. (2015). Monitoring of *Erwinia amylovora* in Montenegro. *Pesticides and Phytomedicine*, 30(3), 179-185. doi 10.2298/PIF1503179R or http://www.pesting.org.rs/media/casopis/2015/no.3/30-3_179-185.pdf

Kerruish, R.M. & Unger, P.W. (2010). *Plant protection 1 – Pests, diseases and weeds*. Retrieved from APPS at http://www.appsnet.org/Publications/Kerruish/PP1.pdf

Tabele se obeležavaju arapskim brojevima prema predviđenom redosledu. Tabele se izrađuju isključivo u programu Word for Windows, kroz meni Table-Insert-Table, koristeći font Times New Roman, 12 pt i osnovni prored. Fusnotama neposredno ispod tabela treba dati prednost nad drugim objašnjenima u zaglavlju tabela ili u samim tabelama, a tekst se daje u fontu Times New Roman, 10 pt. Svaka tabela mora imati zaglavlje. Tabele se prilažu kao dopunske (zasebne) datoteke, a u samom tekstu se obeležava njihovo približno mesto.

Grafikoni treba da budu urađeni i dostavljeni u programu Excel, sa podacima u fontu Times New Roman. Potrebna objašnjenja daju se u legendama obeleženim arapskim brojevima prema redosledu. Grafikoni se prilažu kao zasebne (dopunske) datoteke, a u samom tekstu se obeležava njihovo približno mesto.

Dijagrami treba da budu urađeni i dostavljeni u programu Corel Draw (verzija 9 ili novija), ili u programu Adobe Illustrator (verzija 9 ili novija). Za unos podataka treba koristiti font Times New Roman. Grafikoni se prilažu kao zasebne (dopunske) datoteke, a u samom tekstu se obeležava njihovo približno mesto.

Fotografije treba da budu snimljene digitalnim fotoaparatom (rezolucija najmanje 150 dpi, dimenzija fotografije A4, a format zapisa JPG ili TIFF). Ukoliko autori nisu u mogućnosti da dostave originalne fotografije, treba ih skenirati u RGB modelu (ukoliko su u boji), odnosno kao Grayscale (ukoliko su crno-bele), sa rezolucijom 300 dpi u originalnoj veličini. Fotografije je potrebno obeležiti arapskim brojevima prema predviđenom redosledu. Za svaku fotografiju se daje legenda i obeležava njeno približno mesto pojavljivanja u tekstu. Svaka fotografija se prilaže kao zasebna (dopunska) datoteka.

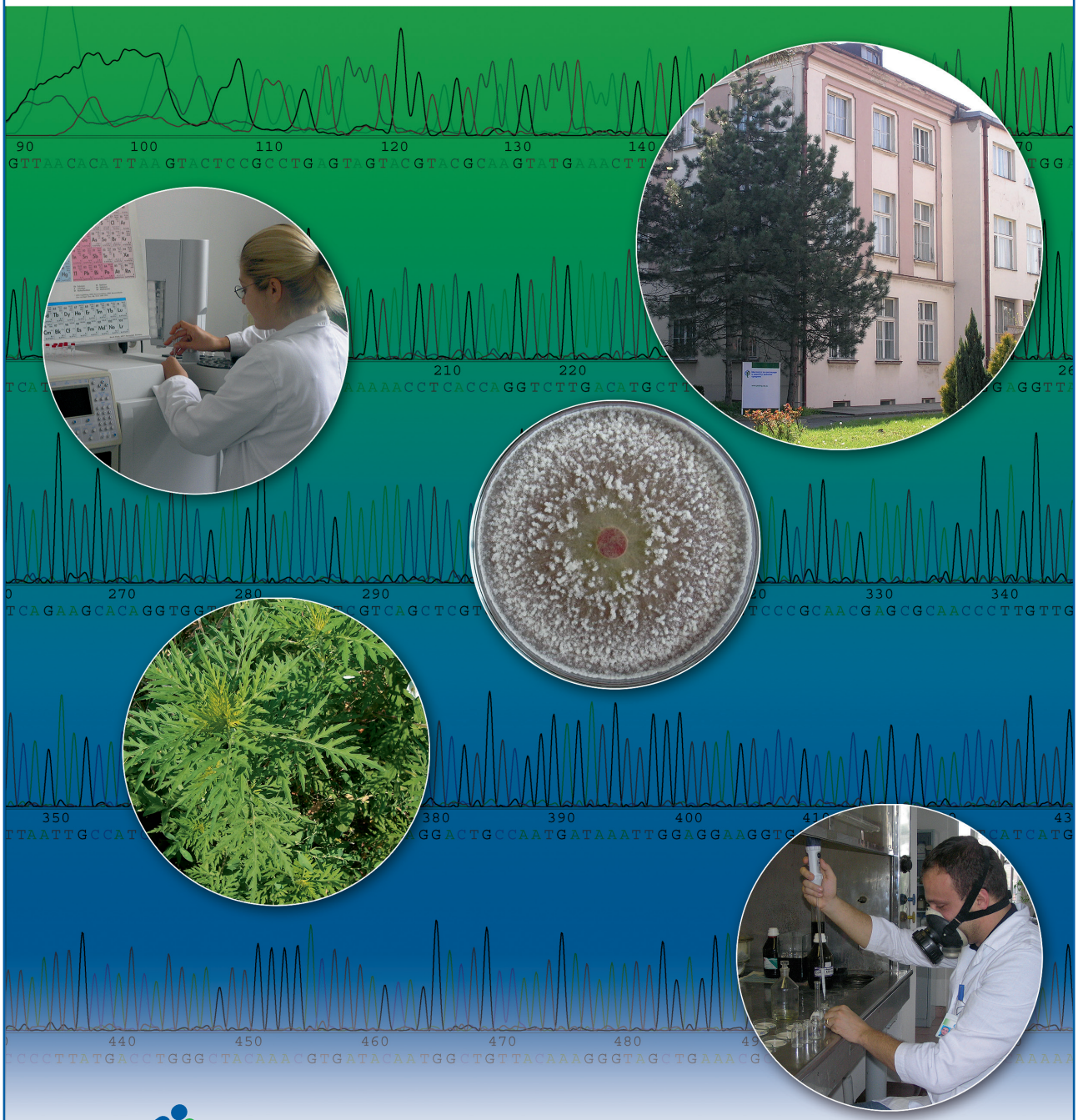
Od autora se očekuje da koriste preporučene jedinice međunarodnog sistema (SI). Skraćenice je potrebno definisati u zagradama nakon prvog pominjanja u tekstu. Narodni nazivi organizama se daju uz pun latinski naziv, a kurzivom se obeležavaju samo latinski nazivi rodova i vrsta, npr. krompirova zlatica, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). Nakon prvog pojavljivanja, latinsko ime dalje treba pisati skraćeno (npr. *L. decemlineata*).

Pregledni radovi treba da sadrže uvod, odgovarajuće podnaslove i spisak literature.

Objavljivanje radova u časopisu *Pesticidi i fitomedicina (Pesticides and Phytomedicine)* je besplatno.

Uredništvo preduzima mere protiv grubog kršenja etičkih normi u vezi sa plagijarizmom.

Nauka u službi zdravlja bilja, čoveka i prirode



Institut za pesticide i zaštitu životne sredine

11080 Beograd - Zemun, Banatska 31b
Tel/fax: (011) 3076-133, 3076-136
www.pesting.org.rs