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Original scientific paper

LARVICIDAL EFFICACY OF *Verbascum* spp. METHANOL EXTRACTS AGAINST *Plodia interpunctella* (HÜBNER, 1813) (LEPIDOPTERA: PYRALIDAE)

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ABSTRACT. *Verbascum* species (fam. Scrophulariaceae) contain a high concentration of rotenone and verbascoside and traditionally have long been used as insecticides. This study is aimed to investigate the larvicidal efficacy of *Verbascum thapsus* L. and *V. phlomoides* L. methanolic extracts in the suppression of the *Plodia interpunctella* (Hübner, 1813), under laboratory conditions. The experiment was set up in two separate blocks (for two different extracts), each as 3×3×3 factorial trial: three concentrations of extracts (1, 2 and 5%) were tested against the three larval age groups (14, 14-28 and 28 days old) and each treatment was repeated three times. Mortality was recorded after 24, 48, 72, and 96 h. Both tested extracts were the most effective 96 h after the exposure in 5% concentration applied on the youngest larvae. Extract of *V. thapsus* caused the mortality of 64.00%, while in treatment with *V. phlomoides* extract mortality was 48.00%. Tested *Verbascum* extracts have shown moderate potential for application as botanical larvicides.

Keywords: botanical insecticide, Indian meal moth, rotenone, plant extracts.

INTRODUCTION

Indian meal moth, *Plodia interpunctella* (Hübner, 1813) (Lepidoptera: Pyralidae) is a major cosmopolitan insect pest of a variety of stored food commodities (NAVARRO and NAVARRO, 2018; PREDOJEVIĆ *et al*., 2017; VUKAJLOVIĆ *et al*., 2017, 2019), particularly cereals, such as maize and wheat (JACOBS and CALVIN, 1990; PREDOJEVIĆ *et al*., 2017). Larvae of *P. interpunctella* are polyphagous and cause huge losses in the quality and quantity of infested cereals (PHILLIPS *et al*., 2000). Damages are reflected through the presence of larval feces, silken web, exuviae, and unpleasant odor of contaminated commodities.

To ensure the future of global food security, the major focus should be directed on reducing post-harvest losses (NAYAK and DAGLISH, 2018). Chemical control nowadays repre-

sents the main tool for controlling stored product pests. Many scientific studies are focused on finding alternative, safer, and more effective methods to protect stored cereals from *P. interpunctella* infestation (KHOSHNOUD and KHAYAMY, 2008; CAMPOS *et al*., 2018; VUKAJLOVIĆ *et al*., 2019). The application of chemical pesticides has led to many negative effects, such as the evolution of pest resistance and environmental pollution (SABER *et al*., 2004).

Plants synthesize many natural substances, primary and secondary metabolites as a chemical defense against herbivores, especially insects (HARTMANN, 1999). Some of those, particularly secondary metabolites, could be used as natural insecticides in fields and storages (JBILOU *et al*., 2006). The application of these compounds is a much safer and environmentally friendlier method for storage pest control, unlike the application of synthetic insecticides. Pesticidal plants are usually used in two ways: the raw or dried plant tissues or extracts are used directly; or the active compounds are isolated, identified, and synthesized and produced by the chemical industry (YANG and TANG, 1988).

Many studies are being conducted to find a new and efficient source of botanical insecticides (SABER *et al*., 2004; HARTMANN, 1999; JBILOU *et al*., 2006; VUKAJLOVIĆ *et al*., 2019; GRDIŠA and GRŠIĆ, 2013). Plant species with confirmed insecticidal effects are being traditionally used in crop protection. Species of the genus *Verbascum* (Scrophulariaceae) have been used for a long time as very effective traditional sources of insecticides (KHOSHNOUD and KHAYAMY, 2008; GROSS and WERNER, 1978; RIAZ *et al*., 2013). Phytochemical studies of *Verbascum* plants showed the presence of different classes of secondary metabolites, mainly phenolic compounds and terpenoids. Phenylethanoid glycosides, flavonoids, iridoids, and saponins are reported as the major constituents of these species (GEORGIEV *et al*., 2011). Their insecticidal properties are scientifically confirmed against different insects, such as stored product pests *Sitophilus oryzae* (Linnaeus, 1763) (Coleoptera: Curculionidae), *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) (KHOSHNOUD and KHAYAMY, 2008) and larvae of mosquito *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae) (SUPAVARN *et al*., 1974). Later has been discovered that *Verbascum* insecticidal efficacy is due to the high content of isoflavonoid rotenone in their leaves (FOSTER and DUKE, 2003). According to a research conducted by the US EPA in 1990, rotenone was found to be commonly used as a pesticide for insect control in home gardens, classified as a botanical insecticide (BRIGGS, 1992; GUPTA, 2019). It is a contact and, as well systemic insecticide (GUPTA, 2019). Rotenone can block the respiration of insects by inhibition of electron transport of the complex I in the mitochondrial respiratory chain (FANG and CASIDA, 1998; CABONI *et al*., 2004). It has been used as an insecticide for over a century (ISMAN, 2006). Although rotenone has been proven to be neurotoxicant, and in metabolic pathways converts in toxic metabolites in insects and fishes, while in nontoxic in mammals, it can be a component of an insecticide only if it is used in safe doses, nontoxic to vertebrates (GUPTA, 2019). A wide spectrum of industrial insecticides containing rotenone such as Bonide Rotenone 5 (DAYAN *et al*., 2009) or formulated in Barbasco, Chem-Fish, Cuberol, Rotacide, Tubatoxin, Green Cross Warble Powder, etc. (GUPTA, 2007).

Guided by the fact that bioactive components of *Verbascum* plants have a certain insecticidal effect, the aim of this study was to determine the larvicidal efficacy of *V. thapsus* and *V. phlomoides* methanol extracts, isolated from plants collected in Serbia, against different age groups of *P. interpunctella* larvae, on wheat, in laboratory conditions. Chemical profile and antioxidant activity of the extracts used in our research were investigated and published by MIHAILOVIĆ *et al*. (2016). As far as we know, *V. phlomoides* insecticidal potential as well as the insecticidal efficacy of *Verbascum* spp. extracts against any lepidopteran storage pest is investigated for the first time.

MATERIALS AND METHODS

Insect population

Plodia interpunctella larvae used in this research originated from the laboratory population, reared for ≈ 50 generations in the Laboratory of General and Applied Entomology, at the Faculty of Science, University of Kragujevac, Republic of Serbia. Larvae were reared in transparent, plastic containers (1.2 L in volume) for mass rearing, on standard laboratory diet for *P. interpunctella* (SILHACEK and MILLER, 1972), in the chamber set at 28 ±1°C, r. h. 60 ±10% and 14:10 (L:D) photoperiod.

Wheat

Grains of a winter wheat cultivar Takovčanka were used as a nutritive substrate. The grains were not treated with insecticides after the harvest or before setting up the experiment but were exposed to deep freezing (-80˚C) for two days, in order to eliminate the possible presence of storage insect pests and parasites.

The plant material and preparation of the extracts

Verbascum plants were collected during the flowering season in August 2010. The voucher specimens were deposited at the Department of Biology and Ecology, Faculty of Science, University of Kragujevac. Their voucher specimens and localities are *V. phlomoides*: Suva Planina, the mountain in southeastern Serbia (291 km from Belgrade), voucher no. 114/014 and for *V. thapsus*: Trgovište, south Serbia (295 km from Belgrade), voucher no. 115/014.

The above-ground parts of *V. phlomoides* and *V. thapsus* were air-dried, fine powdered and separately extracted with methanol by maceration (MIHAILOVIĆ *et al*., 2016). The extracts used for larvicidal studies were dissolved in deionized water and applied in experiments at concentrations of 1, 2, and 5%.

Experimental design and procedure

The experiment was set up in two separate blocks (for two different extracts), each one as 3×3×3 factorial trial. We used water solutions of methanolic extracts of two mentioned *Verbascum* species, in three different concentrations (1, 2, and 5%). Three different larval age groups were used (A1 < 14, A2 14-28, and A3 > 28 days old). Each treatment was repeated three times. The control contained 18 replicates. There were 72 replicates in total. Each assay, placed in a glass Petri dish (10 cm in diameter), contained 10 g of grains treated with 1 mL of adequate extract solution or 1 mL of distilled water for the control group and 10 larvae from one of the mentioned age group. Mortality was recorded after 24, 48, 72, and 96 h.

Statistical analysis

Data were statistically analyzed using the IBM SPSS Statistics 21 software package (2012). The data were subjected to a Repeated Measures ANOVA to evaluate the effect of extracts concentration, time of exposure, larval age, and their interaction on larval mortality. One-way Anova was used for the analysis of the influence of one factor if it showed significance in the previous test. Dunnett T3 test was then used to assess the significance of differences between the treatments. All tests were performed at the level of significance of 95% ($p < 0.05$).

All results were corrected according to Schneider-Orelli's formula (PÜNTENER, 1981):

$$Mortality\ (corrected)\ \% = \frac{mortality\ (\%)\ in\ the\ treatment - mortality\ (\%)\ in\ the\ control}{100 - mortality\ (\%)\ in\ the\ control} * 100$$

RESULTS AND DISCUSSION

The results of the efficacy of *V. thapsus* methanol extract are presented in Table 1. Generally, this extract caused higher mortality when applied to the younger stage of larvae (A1), compared to the older (A2 and A3), as well as in the highest (5%) than in lower concentrations (1 and 2%). According to the results of Repeated Measures Anova, there was no significant interaction between time of exposure and concentration of *V. thapsus* extract (Wilk's Lambda = 0.826, F=0.486, P = 0.887, Partial Eta Square = 0.062) but was between the time of exposure and larval age (Wilk's Lambda = 0.478, F=3.273, P = 0.01, Partial Eta Square = 0.309). Interaction among all three tested factors was not significant (P > 0.05). However, time of exposure as separate factor had a significantly high effect on the mortality of all larval age groups (Wilk's Lambda = 0.117, F=55.41, P < 0.0005, Partial Eta Square = 0.883). According to the One-way Anova and Dunnett T3 test, concentration had a significant influence (P < 0.05) on larval mortality as presented in Table 1. According to the One-way Anova and Dunnett T3 test, there was statistically significant influence of concentration (P < 0.05) on mortality after 24 h, when we registered much higher mortality in the A1 age group of larvae when applied 5% extract (57.85%, P=0.031), then in two younger age groups of larvae (A1 and A2).

Table 1. The mortality (%) of larval age groups (A1, A2, and A3) of *Plodia interpunctella* larvae treated with *Verbascum thapsus* methanol extract applied at different concentrations (1, 2, and 5%).

Larval stage	Concentrations (%)	Exposure period			
		24 h	48 h	72 h	96 h
A1	1	14.28±18.55^{a}	22.22±11.11^{a}	20.00±13.86^{a}	24.00±18.33^{a}
	2	21.43±6.19^{a}	28.52±16.71^{a}	24.00±13.86^{a}	24.00±13.86^{a}
	5	57.85±19.2^{b}	52.96±14.75^{a}	48.00±24.98^{a}	64.00±20.78^{a}
	P; F value	0.031; 6.539	0.085; 3.824	0.21; 2.048	0.053; 5.000
A2	1	17.24±10.34^{a}	17.86±12.37^{a}	33.33±11.11^{a}	52.18±19.92^{a}
	2	18.39±18.39^{a}	28.57±16.37^{a}	37.04±23.13^{a}	52.18±19.92^{a}
	5	24.14±5.97^{a}	39.28±6.19^{a}	40.74±6.41 ^{a}	56.52±19.92^{a}
	P value	0.791; 0.244	0.187; 2.249	0.842; 0.176	0.954; 0.048
A3	1	0.00±0.00^{a}	11.49±9.95^{a}	11.49±9.95^{a}	11.11±11.11^{a}
	2	10.00±10.00^{a}	8.04±8.68^{a}	13.79±5.97^{a}	40.74±32.08^{a}
	5	6.67±5.77^{a}	10.35±5.97^{a}	20.69±15.80^{a}	22.22±19.24^{a}
	P value	0.252; 1.750	0.879; 0.132	0.611; 0.536	0.334; 1.324

The results represent mean values ± SD. Values with the same letter in the column (in superscript) are on the same level of significance, * - P < 0.05, ns – P > 0.05; A represents the age groups of *P. interpunctella* larvae: A1 < 14, A2 14-28, and A3 > 28 days old.

Verbascum thapsus extract applied in a concentration of 5% was the most effective in all assays, except against A3 larvae after 96 h, where 2% concentration was more effective (Table 1). This extract in 5% concentration showed the biggest success against the A1 group of *P. interpunctella* larvae, with the mortality rate of 57.85%, after only 24 h of exposure, and

64.00% after 96 h (which was the highest mortality recorded in this research). This extract was less efficient when applied against the older larvae (A2 or A3 group, then against the A1).

The oldest group of larvae (A3) was the most resilient to the extracts of *V. thapsus*. After 24 h of exposure, the mortality ranged from zero (1% concentration) to 10% (2% concentration). As the experiment progressed, the mortality in A3 was higher but still lower in comparison to A1 and A2 groups. The higher percent of mortality in A3 groups (40.74) was registered only 96 h from the exposure in the treatment where 2% extract concentration was applied but without statistically significant differences in comparison to replicates with 1 and 5% concentrations (11.11 and 22.22%, respectively, P =0.334).

According to the Repeated Measures Anova, there was no significant interaction between time of exposure and concentration of *V. phlomoides* extract (Wilk's Lambda = 0.659, F=1.521, P = 0.157, Partial Eta Square = 0.130). The only time of exposure significantly affected the mortality of larvae (Wilk's Lambda = 0.330, F=14.67, P < 0.0005, Partial Eta Square = 0.670), wherein A1 and A2 age groups the increase in mortality over time was registered (Table 2). One Way Anova was used to test the influence of concentration of extract for each larval age group, after a certain time of exposure, and all results are shown in Table 2. There was not a statistically significant influence of concentration (P > 0.05, in all comparisons) on mortality.

Verbascum phlomoides extract was also more effective when applied to the younger than the older *P. interpunctella* larvae (Table 2). In the A1 group larvicidal efficiency of the applied extract varied from 36.01% (1% concentration) to the highest 48.00% (2 and 5% concentration), 96 h of the exposure. In the A2 group, the highest mortality (15.94%) was registered after 96 h when treated with 5% extract. In the A3 group, this extract was less effective than in younger groups, after 48 h of exposure, where 2% extract caused the highest, but still very low, the mortality of 6.67% of the tested larvae (the same mortality was registered in the control).

Table 2. The mortality (%) of larval age groups (A1, A2 and A3) of *Plodia interpunctella* larvae treated with *Verbascum phlomoides* methanol extract applied at different concentrations (1, 2 and 5%).

Larval stage	Concentrations (%)	Exposure period			
		24 h	48 h	72 h	96 h
A1	1	3.57±0.00^{a}	00.00±0.00 ^{b}	12.82±16.01 ^{a}	36.01±13.85 ^{a}
	2	10.71±6.18^{a}	3.70±6.41 ^{ab}	8.98±9.68 ^{a}	48.00±30.19 ^{a}
	5	2.38±2.06^{a}	11.11±0.00 ^{a}	26.93±17.62 ^{a}	48.00±18.32 ^{a}
	P; F value	0.069; 4.300	0.027; 7.00	0.360; 1.217	0.751; 0.300
A2	1	2.30±3.98^{a}	1.19±2.06 ^{a}	11.11±19.24 ^{a}	7.25±12.55 ^{a}
	2	0.00±0.00^{a}	1.19±2.06 ^{a}	0.00±0.00^{a}	7.25±15.25 ^{a}
	5	8.05±8.68^{a}	10.71±6.18 ^{a}	14.81±16.97 ^{a}	15.94±27.61 ^{a}
	P; F value	0.261; 1.696	0.051; 5.818	0.487; 0.813	0.816; 0.211
A3	1	3.33±5.77^{a}	3.33±5.77 ^{a}	2.3±3.98 ^{a}	0.00±0.00 ^{a}
	2	3.33±5.77^{a}	6.67±5.77 ^{a}	5.75±9.95 ^{a}	3.70±6.41 ^{a}
	5	0.00±0.00^{a}	0.00±0.00 ^{a}	0.00±0.00 ^{a}	0.00±0.00 ^{a}
	P; F value	0.630; 0.500	0.553; 0.655	0.553; 0.655	0.422; 1.000

The results represent mean values ± SD. Values with the same letter in the column or number (in superscript) in a row are on the same level of significance, * - P < 0.05, ns – P > 0.05; A represents the age groups of *P. interpunctella* larvae: A1 < 14, A2 14-28, and A3 > 28 days old.

According to the literature data, species from the *Verbascum* genus were tested for insecticidal efficiency only against *S. oryzae*, *T. castaneum*, *Rhyzopertha dominica* (Fabricius, 1792) (Coleoptera: Bostrichidae) and *Callosobruchus maculatus* (Fabricius, 1775) (Coleoptera: Chrysomelidae), but not against any lepidopteran storage insect pest (KHOSHNOUD et al., 2008a, 2008b; KHOSHNOUD and KHAYAMY, 2008; Riaz *et al.*, 2013; DEMNATI and ALLACHE, 2014). Previously tested *Verbascum* spp. extracts were usually very efficient as insecticides against target insect species, causing from 64 to 100% mortality of adults and 100% suppersion of progeny production. *Verbascum thapsus* was less efficient against *S. oryzae* and *T. castaneum*, than *V. cheiranthifolium* and *V. speciosum* (KHOSHNOUD *et al*., 2008b; KHOSHNOUD and KHAYAMY, 2008; RIAZ *et al.*, 2013; DEMNATI and ALLACHE, 2014), but in our experiment, satisfactory larvicidal effect against *P. interpunctella* was recorded.

KHOSHNOUD and KHAYAMY (2008) suggested *V. cheiranthifolium* flowers' extract as a botanical material for the protection of stored wheat from infestations of stored product beetle pests. In their study, ethanolic extract of *V. cheiranthifolium* caused 100% mortality of adult *S. oryzae* 21 days after the exposure and complete suppression of the progeny production even at the lowest rate. In the study conducted by RIAZ *et al.* (2013), *V. thapsus* methanolic extract was investigated for potential insecticidal efficacy against adult coleopterans *S. oryzae* and *T. castaneum*. However, no significant insecticidal action was observed. The results of our study show that methanol extracts of *V. thapsus* against the youngest *P. interpunctella* larvae have larvicidal efficiency of 64.00%, 96 h after the treatment. In our experiment, the *V. thapsus* extract caused higher mortality of larvae then *V. phlomoides* extract, and the oldest larvae were especially resilient to the *V. phlomoides* extract.

According to previous research, the main phytochemical difference between two extracts tested in our study is in the concentration of verbascoside: *V. thapsus* contains 63.36, while *V. phlomoides* 50.03 mg of verbascoside per g of dry extract (MIHAILOVIĆ *et al*., 2016). It is already confirmed that crude plant extracts of *Calceolaria talcana* J. Grau and C. Ehrh (Calceolariaceae), which contain verbascoside as a major phenolic compound, cause high levels of mortality in *Drosophila melanogaster* (Meigen, 1830) (Diptera: Drosophilidae) and *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae) (MUÑOZ *et al*., 2013). Previous studies also provided evidence that purified verbascoside can be a very efficient insecticide against *Agrilus planipennis* (Fairmaire, 1888) (Coleoptera: Buprestidae) larvae (WHITEHILL *et al*., 2014). It has been discovered that verbascoside may disrupt ecdysteroid metabolism which results in an inhibition of emergence (HESTERLEE and MORTON, 1996).

Verbascum thapsus methanolic extract tested in our study show potential for application as a botanical larvicide since 64% efficacy was achieved against four days since the exposure against the youngest group of *P. interpunctella* larvae. *Verbascum* spp. are the undoubtedly important reservoir of verbascoside, rotenone, and other components that could be potentially harmful to the users of the treated crops. Hence, further investigations on the insecticidal efficacy and the safety of possible application of *V. thapsus* extract, dry or crude herbal forms in the protection of stored cereals against *P. interpunctella* for humans and domestic animals are needed. New studies about the insecticidal efficacy of different plant extracts against storage insect pests are in progress.

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