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Efficacy of biological formulations against *Neoglocianus maculaalba* and *Dasineura papaveris* in *Papaver somniferum*

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Abstract: The effectiveness of selected insecticides against capsule weevils (*Neoglocianus maculaalba*) and capsule midges (*Dasineura papaveris*), which are the main pests of breadseed poppy, was verified in a small-plot field experiment in 2015–2017. The effect of foliar application was evaluated according to boreholes on capsule surfaces caused by the feeding of capsule weevil adults and larvae numbers in capsules collected approximately 4 weeks after application. Biological efficacy of the tested biological formulations Spintor (active ingredient spinosad 240 g/l) in dosage 0.4 l/ha and NeemAzal T/S (active ingredient azadirachtin A 10.6 g/l) in dosage 3 l/ha against *N. maculaalba* larvae in individual years of monitoring ranged from 46.4% to 77.7% and from 67.7% to 82.9%, respectively. The effect of the formulation Prev B2 (boron ethanolamine corresponding to 2.1% of water-soluble boron) in 0.3% dosage was in the range of 59.5–81.9%. Their efficacy did not differ significantly compared to the registered chemical standards Biscaya 240 OD (active ingredient thiacloprid 240 g/l) and Decis Mega (active ingredient deltamethrin 50 g/l). Therefore, these biological insecticides are potentially useful for the effective control of *N. maculaalba* and *D. papaveris* population densities and reduction of damage they cause to breadseed poppy.

Keywords: plant protection; capsule weevils; capsule midges; thiacloprid; spinosad; azadirachtin; boron ethanolamine

Among the main pests of breadseed poppy, *Papaver somniferum* L. (Papaveraceae), are capsule weevils (*Neoglocianus maculaalba,* Herbst, 1795) and capsule midges (*Dasineura papaveris,* Winnertz, 1890) (Kůdela *et al.* 2012; Kolařík & Rotrekl2015). They are very frequently called capsule pests because their larval development is closely tied to development inside the capsules. The adults of capsule weevils appear at the time of blossoming and feed on leaves, stem, and flowers. The female lays the eggs in capsules where the larvae develop and come out after two to three weeks for pupation. Damage from the adults of *N. maculaalba* is characterised by the outgrowth of white milk from damaged parts, which after drying creates very unsightly dark spots (boreholes) on the capsule. *Neoglocianus maculaalba* boreholes cause substantial yield losses in terms of both quantity and quality and lead to fungal pathogens (such as *Helminthosporium papaveris*) developing inside the capsules (Rotrekl 2000; Rotrekl & Kolařík 2011). Insecticides with very high biological effectiveness have been registered to minimise their damage. These formulations are primarily pyrethroids and neonicotinoids (Rotrekl 2008). When pyrethroid active ingredients are used, there arises a risk of their decreased effectiveness due to unfavourable conditions on the application date (high temperature, sunlight) followed by a possibility of resistance in the insects (decrease of effectiveness) to develop due to the repeated application (Wegorek & Zamojska 2008). Neonicotinoid formulations are currently being studied for their negative effects mainly on pollinators (Gajger *et al.* 2016). A suitable biological method for protecting breadseed poppy against

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these pests is needed. Biological protection generally involves suppressing insect pests using their natural enemies and utilising materials as environmentally friendly as possible in order to minimise environmental burdens. In addition, biological protection should have highly specific effects so that it does not generally endanger such non-target organisms as beneficial entomofauna and pollinators (Kolařík & Kolaříková 2015). Spinosad insecticides act through a neural mechanism causing hyperexcitation of the insect's nervous system. Spinosad is very effective against numerous insect groups from the orders Lepidoptera, Diptera, Thysanoptera, Coleoptera, Orthoptera, Hymenoptera, and many others (Sparks *et al.* 1995; Bret *et al.* 1997). Among its advantages are low oral toxicity and a very advantageous ecotoxicological profile (Clevlend *et al.* 2001).

The main effective mechanism of NeemAzal T/S is the inhibition of development and moulting of the target pest (Schumutterer 1990). The formulation Prev B2, meanwhile, has a contact effect, meaning the pest must be thoroughly wetted, thereby causing the insect's respiratory tubes to be clogged (Pobožniak *et al.* 2016).

The aim of this study was to evaluate the efficiency of environmentally friendly insecticides Spinosad, NeemAzal T/S and Prev B2 in comparison with registered chemical standards for the regulation of capsule weevil and capsule midge in breadseed poppy.

MATERIAL AND METHODS

In the course of three seasons (2015–2017) small-plot experiments were established in Troubsko, Czech Republic, for evaluating the biological efficacy of biological insecticides intended for regulating the abundance of capsule weevil (*N. maculaalba*) and capsule midge (*D. papaveris*). Plots were 2.5 m wide and 10 m long (i.e. 25 m^2). The following insecticides were tested in 2015: biological insecticides Spintor (active ingredient spinosad 240 g/l) in 0.4 l/ha dosage and NeemAzal T/S (active ingredient azadirachtin A 10.6 g/l) in 3 l/ha dosage, synthetic insecticide Biscaya 240 OD (active ingredient thiacloprid 240 g/l) in 0.3 l/ha dosage, and Prev B2 (boron ethanolamine corresponding to 2.1% of water-soluble boron – 0.3%). In 2016 and 2017 we added the pyrethroid substance Decis Mega (active ingredient deltamethrin 50 g/l) in a dosage of 0.15 kg/ha to the group of tested insecticides. All insecticides were applied according to signalisation (just prior to flowering up to emergence of the first flowers). Before spraying (0 day after application – 0DAA), one (1DAA; only in 2017), and seven days (7DAA) thereafter, we assessed the quantity of damage on the newly developed capsules caused by adults of capsule weevil (= number of boreholes) and number (direct counting) of *N. maculaalba* adults, evaluating a total of 50 plants per plot. In the period of seven days after application, phytotoxicity of the tested insecticides potentially observable as negative effects on plants was also evaluated (BBCH 59–71). To evaluate the biological efficacy as indicated by the numbers of larvae inside the poppy capsules, 50 capsules were collected from each plot and the larvae inside were counted in the time of 3–4 weeks after application (DAA). To evaluate the biological efficacy on the capsule midge infestation, plants were divided into categories according to the number of larvae inside the capsule. The categories were as follows: 1 – no larvae, 2 – up to 10 larvae/capsule, and 3 – more than 10 larvae/capsule. The percentage of infested capsules was also determined. The plot yields were also evaluated.

The results were statistically processed using the single-factor analysis of variance ANOVA followed by Tukey's range test ($\alpha = 0.05$) using the UPAV GEP programme.

RESULTS

In 2015 the average capsule damage prior to application ranged between 1.34 and 1.68 boreholes/capsule, and very low abundance of adult capsule weevils was determined (Table 1). Seven days after application, the highest number of boreholes/capsule was determined on the untreated control (3.89 boreholes/capsule), and the lowest, 0.13 boreholes/capsule, in the treatment sprayed with Biscaya 240 OD, which means 96.7% effectiveness as compared to the untreated control. A statistically significant difference between sprayed treatments and the control was determined ($F = 18.364$). No significant difference was determined between the individual sprayed treatments.

In evaluating capsule infestation with weevil larvae, we determined the following: in the control, 50% of capsules were infested by the larvae of that pest, with the mean number of 1.82 larvae/capsule (30DAA). In the sprayed treatments, the infestation of capsules with capsule weevil larvae ranged from 7% (Biscaya

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Table 1. Efficacy of the tested insecticides on *Neoglocianus maculaalba* Herbst and *Dasineura papaveris* Winnertz in *Papaver somniferum* L. (Troubsko 2015)

Treatments	15 June* (0DAA) boreholes/capsule	22 June (7DAA)		15 June (0DAA) adult (*N. macula alba*)/plant	22 June (7DAA)		15 July (30DAA)			15 July (30DAA)		12 Aug (58DAA)	
		boreholes/capsule	efficacy (%)		adult (*N. macula alba*)/plant	efficacy (%)	larvae (*N. macula alba*)/capsule)	efficacy (%)	% infested capsules (*N. maculaalba*)	% infested capsules (*D. papaveris*)	efficacy (%)	yield (t/ha)	% REL
Control	1.34^{a}	3.89^{a}		0.12^{a}	0.05^{a}		1.82^{a}		50	5		1.01^{a}	100.0
Spintor	1.5^{a}	0.87^{b}	77.63	0.08^{a}	0.00^{a}	100.0	0.33^{b}	81.87	13	1	81.4^{b}	0.93^{a}	92.1
Biscaya 240 OD	1.44^{a}	0.13^{b}	96.67	0.08^{a}	0.01^{a}	80.0	0.17^{b}	90.66	7	2	90.4^{b}	0.88^{a}	87.2
NeemAzal T/S	1.44^{a}	0.86^{b}	77.89	0.12^{a}	0.03^{a}	40.0	0.31^{b}	82.97	10	2	82.5^{b}	0.89^{a}	88.1
Prev B2	1.68^{a}	1.07^{b}	72.49	0.16^{a}	0.01^{a}	80.0	0.33^{b}	81.87	12	1	81.4^{b}	1.05^{a}	103.4

*before treatment; $^{a-b}$data marked by different letters in a column indicate significant difference

240 OD) to 13% (Spintor 0.4 l/ha), with the mean number of 0.17 to 0.33 larvae/capsule. Biological efficacy of the tested formulations ranged from 81.4% (Spintor and Prev B2) to 82.5% (NeemAzal T/S). In evaluating the number of capsule weevil larvae, no significant difference between the sprayed treatments was determined. A significant difference from the untreated control was found out (F = 17.849). The

Table 2. Efficacy of the tested insecticides on *Neoglocianus maculaalba* Herbst and *Dasineura papaveris* Winnertz in *Papaver somniferum* L. and impact on the yield (Troubsko 2016)

Treatments	21 June (0DAA) boreholes/capsule	28 June (7DAA)		21 June (0DAA) adult (*N. macula alba*)/plant	28 June (7DAA)		14 July (23DAA)			14 July (23DAA)		16 Aug (56DAA)	
		boreholes/capsule	efficacy (%)		adult (*N. maculaalba*)/plant	efficacy (%)	larvae (*N. maculaalba*)/capsule	efficacy (%)	% infested capsules (*N. maculaalba*)	% infested capsules (*D. papaveris*)	efficacy (%)	yield (t/ha)	% REL
Control	0.40^{b}	3.02^{a}		0.08^{a}	0.16^{a}		0.59^{a}		31	9		0.404^{a}	100.00
Spintor	1.42^{a}	1.64^{ab}	45.70	0.00^{a}	0.04^{a}	75.0	0.13^{b}	77.97	3	1	85.71^{ab}	0.499^{a}	123.61
Biscaya 240 OD	0.45^{ab}	0.42^{b}	86.09	0.04^{a}	0.00^{a}	100.0	0.05^{b}	91.53	1	2	71.43^{ab}	0.409^{a}	101.25
NeemAzal T/S	1.11^{ab}	1.63^{ab}	46.03	0.08^{a}	0.00^{a}	100.0	0.12^{b}	79.66	5	0	100.00^{b}	0.452^{a}	111.91
Prev B2	0.43^{b}	1.62^{ab}	46.36	0.00^{a}	0.00^{a}	100.0	0.17^{b}	71.19	7	4	42.86^{ab}	0.389^{a}	96.30
Decis Mega	0.19^{b}	0.66^{b}	75.15	0.00^{a}	0.00^{a}	100.0	0.20^{b}	66.1	4	2	71.13^{ab}	0.415^{a}	102.83

$^{a-b}$data marked by different letters in a column indicate significant difference

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occurrence of capsule midge 30 DAA was very low in 2015, with the infestation rate ranging between 1% and 5%. The highest number of capsules infested with capsule midge was observed in the untreated control, and there was no significant difference between the individual sprayed treatments (F = 0.853). Poppy seed yield ranged between 0.88 and 1.05 t/ha with no statistically significant difference (F = 2.973).

In 2016, the average capsule damage ranged from 0.19 to 1.11 boreholes/plant (Table 2). In this evaluation, very low numbers of adult capsule weevils were found in the stand. On the next evaluation date (7 days after application), the greatest damage was determined on the untreated control (3.02 boreholes/capsule). The lowest damage was observed in the treatments with the formulations Decis Mega (0.66 boreholes/capsule) and Biscaya 240 OD (0.42 boreholes/capsule), when there was a highly significant difference from the untreated control (F = 4.216). In analysing the presence of capsule weevil larvae in capsules, the highest number of infested capsules was determined on the untreated control, which reached 30% in 2016. In comparison with sprayed treatments, a highly significant difference was determined (F = 8.336). The lowest number of infested capsules was determined in the treatment with Biscaya 240 OD (1%). The efficacy of that treatment in comparison with the untreated control was 91.5%. Relatively high efficacy was determined also in the other sprayed treatments, ranging from 66.1% to 79.7%. No statistically significant difference was determined between the sprayed treatments. Capsule infestation with capsule midge ranged between 0 and 7%, with a highly significant difference detected between the control and the treatment with NeemAzal T/S (F = 2.660). No significant difference between the treatments was determined in evaluating the yield (F = 0.544).

In 2017, the average capsule damage ranged between 2.25 and 2.84 boreholes/capsule (Table 3). Already on the first evaluation date (0DAA), adult capsule weevils were found out on the individual experimental treatments (0.22–0.30 beetles/plant). On the subsequent evaluation date (1 day after application), the greatest capsule damage was determined on the untreated control (3.15 boreholes/capsule). The lowest damage was observed in the treatment with Biscaya 240 OD (0.05 boreholes/ capsule) and Spintor (0.08 boreholes/capsule), and there was a highly significant difference between all the treatments relative to the untreated control (F = 14.495). Until the evaluation date seven days after application the extent of damage increased across all treatments, ranging between 2.12 (NeemAzal T/S) and 8.03 boreholes/capsule (untreated control). A highly significant difference was determined to exist between the untreated control and the sprayed treatments (F = 9.333).

In analysing the presence of capsule weevil larvae in capsules (Table 4), the highest percentage of infested capsules was determined on the untreated control, reaching 60% in 2017. On the untreated control 5.82 larvae/capsule were recorded on average. In comparison with the sprayed treatments (except for treatment 2 – Spintor), a highly significant difference in the number of larvae (F = 9.341) was determined statistically. The lowest numbers of *N. maculaalba* larvae were recorded in the treatments with Biscaya

Table 3. Efficacy of the tested insecticides on *Neoglocianus maculaalba* Herbst adults and on the level of damage in *Papaver somniferum* L. (Troubsko 2017)

Treatments	19 June (–1DAA) boreholes/capsule	21 June (1DAA)		26 June (7DAA)		19 June (–1DAA) adult (*N. maculaalba*)/plant	21 June (1DAA)		26 June (7DAA)	
		boreholes/capsule	efficacy (%)	boreholes/capsule	efficacy (%)		adult (*N. maculaalba*)/plant	efficacy (%)	adult (*N. maculaalba*)/plant	efficacy (%)
Control	2.84[a]	3.15[a]		8.03[a]		0.27[a]	0.23[a]		0.15[a]	
Spintor	2.56[a]	0.08[b]	97.46	3.57[b]	55.54	0.22[a]	0.06[ab]	73.91	0.12[a]	20.00
Biscaya 240 OD	2.60[a]	0.05[b]	98.41	2.23[b]	72.23	0.28[a]	0.02[b]	91.3	0.04[a]	73.33
NeemAzal T/S	2.68[a]	0.43[b]	83.35	2.12[b]	73.60	0.31[a]	0.16[ab]	30.43	0.05[a]	66.67
Prev B2	2.25[a]	0.57[b]	91.90	2.88[b]	64.13	0.27[a]	0.16[ab]	30.43	0.04[a]	73.33
Decis Mega	2.65[a]	0.20[b]	93.65	2.74[b]	65.88	0.30[a]	0.14[ab]	39.13	0.11[a]	26.67

[a–b]data marked by different letters in a column indicate significant difference

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Table 4. Efficacy of some insecticides on occurrence and damage caused by larvae of *Neoglocianus maculaalba* Herbst and *Dasineura papaveris* Winnertz, and impact on the yield in *Papaver somniferum* L. (Troubsko 2017)

Treatments	11 July (22DAA)					8 Aug (50DAA)	
	larvae (*N. maculaalba*)/ capsule	efficacy (%)	% infested capsules (*N. maculaalba*)	% infested capsules (*D. papaveris*)	efficacy (%)	yield (t/ha)	% REL
Control	5.82[a]		60	6		0.408[a]	100.00
Spintor	3.12[ab]	46.39	57	4	32.75	0.436[a]	106.87
Biscaya 240 OD	1.65[b]	71.65	38	2	66.37	0.469[a]	114.96
NeemAzal T/S	1.88[b]	67.70	48	1	77.58	0.461[a]	112.97
Prev B2	2.36[b]	59.45	54	0	100.00	0.457[a]	111.94
Decis Mega	2.25[b]	61.34	49	2	55.16	0.444[a]	108.69

[a–b]data marked by different letters in a column indicate significant difference

240 OD (1.65 larvae/capsule; 71.65% biological efficacy in comparison with untreated control) and NeemAzal T/S (1.88 larvae/capsule; 67.7% biological efficacy in comparison with untreated control). Capsule infestation with capsule midge ranged between 0% and 6%, and no significant difference was determined between the individual monitored treatments (F = 1.421). In evaluating the yield, a highly significant difference was determined between the control and treatments 3–5 (F = 5.057).

DISCUSSION

Capsule weevil and capsule midge are among the main pests of poppy (Kolařík & Rotrekl 2014). Long-term monitoring of their occurrence and damage shows that the percentage of capsules infested by capsule weevil larvae in untreated stands amounts up to 51%, whereas infestation with capsule midge is usually less than 11% in the Czech Republic (Kolařík & Rotrekl 2015). The targeted application of chemical formulations is carried out just prior to flowering and until emergence of the first flowers. Stanca-Moise (2016) stated that the targeted application of insecticides was effective at the end of adult migration into the stand and when 70% of the stand was in flower. On the later application date, the efficacy of applied insecticides is very low to none. The biological efficacy of pyrethroid formulations may be influenced by weather conditions at the time of application. High temperatures together with UV radiation significantly influence the effect of pyrethroid formulations (Ma *et al.* 2012). Those treatments can thereby become entirely ineffective and insect pests thus have ideal conditions for causing further damage. Recently, the negative effects of neonicotinoid formulations, especially in relation to pollinators, have been confirmed (Gajger *et al.* 2016). Rotrekl (2008) reported high biological efficacy against capsule weevil and capsule midge for formulations Decis EW 50 (active ingredient deltamethrin) at 61.5%, and Biscaya 240 OD (active ingredient thiacloprid), ranging between 67.1% and 100%. Similar biological efficacy of those two insecticides, which were also used in our study, was determined also in our field experiments. Spintor and NeemAzal T/S are registered in the Czech Republic for a number of field crops (Anonymous 2018). At the same time, they can be used for protection as part of integrated pest management systems, particularly in fruit orchards and perennial crops. Biological formulations can also be used in cases when standard registered compounds are of limited usefulness due to bee flight or because there is an assumption of their diminishing biological effect under adverse weather conditions. The main advantage of spinosad and azadirachtin applications was that they did not exert any side-effects on useful insects, predators, and parasitoids commonly occurring in crops (Nawrocka 2008). The tested biological formulations showed very high biological efficacy in the field experiments against capsule weevil and capsule midge without significant difference in comparison with registered standard products in all monitored years. The biological efficacy of Spintor on the occurrence of capsule weevil larvae in the individual years ranged between 46.4 and 77.7%, and for NeemAzal T/S it ranged between 67.7 and 82.9%. Pobožniak *et al.* (2016) reported the very good efficacy of Prev B2

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on *Thrips tabaci* in garlic, with significantly higher occurrence of the thrips on an untreated control in comparison with Prev B2 treatments. The formulation we applied demonstrated high efficacy against the larvae of capsule weevil in field experiments ranging between 59.5 and 81.9%.

CONCLUSION

The obtained results indicate that the application of insecticide formulations against poppy pests prior to flowering significantly limits damage to plants by adult feeding and subsequently also the infestation rates by capsule weevil and capsule midge are significantly lower as compared to the untreated control. Very good biological effectiveness was determined for Spintor (active ingredient spinosad 240 g/l) in 0.4 l/ha dosage and NeemAzal T/S (active ingredient azadirachtin A 10.6 g/l) in comparison with the registered formulations in use. Therefore, these biological insecticides are potentially useful for the effective control of *N. maculaalba* and *D. papaveris* population densities and reduction of damage they cause to breadseed poppy.

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