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The Toxic Effect of Greenhouse Insecticides on the Predatory Bugs *Nesidiocoris tenuis* Reuter and *Macrolophus pygmaeus* H.-S. (Heteroptera, Miridae)

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Abstract—The study reports the results of toxicity tests for several insecticides which are allowed to be used on greenhouse plants (Admiral, Mospilan, Spintor, and BT), as applied to different developmental stages of the predatory bugs *Nesidiocoris tenuis* Reuter and *Macrolophus pygmaeus* H.-S. (family Miridae), which feed on whiteflies, aphids, thrips, and spider mites. The juvenoid Admiral is found to be the least toxic to these species, whereas the spinosyn Spintor is most toxic. The neonicotinoid Mospilan and a microbiological insecticide BT are intermediate in their effects. The timing of application of insecticides most favorable to the predatory mirids when both pest management methods are used together is discussed.

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Monitoring of the species composition of greenhouse herbivores which has been regularly undertaken over the last two decades in Russia suggests that an established complex of arthropod pests has changed with an increasing range of cultivated plants and implementation of novel growing methods. This complex which comprises such common pest species as the red spider mite *Tetranychus urticae* Koch and carmine spider mite *T. cinnabarinus* Boisd., the melon aphid *Aphis gossypii* Glov., the green peach aphid *Myzus persicae* Sulz., the glasshouse potato aphid *Aulacorthum solani* Kalt., and the potato aphid *Macrosiphum euphorbiae* Thom., greenhouse whitefly *Trialeurodes vaporariorum* Westw., and the onion thrips *Thrips tabaci* Lind. has expanded to include a number of new species. The latter are represented not only by indigenous thrips (the chrysanthemum thrips *Th. nigropilosus* Uz., rose thrips *Th. fuscipennis* Hal., and the European flower thrips *Frankliniella intonsa* Tryb.), but also an invasive species, the western flower thrips *Frankliniella occidentalis* Perg. (Ivanova and Velikan', 1995). Besides, another invasive thrips, *Echinothrips americanus* Morgan, is known from the protected ground of the Northwestern region and Moscow Province, which has been introduced to Russia with ornamental plants (Drugova and Varfolomeeva, 2005; Velikan' and Klishina, 2007; Akhatov, 2015).

Due to the changes in the phytosanitary situation in the protected ground facilities of Russia, there are cases that call for improving and even revisiting previously developed systems of protection of cultivated vegetable and ornamental plants. These systems, in accordance with the current environmental safety requirements, should be based as much as possible on biological control measures (Pavlyushin et al., 2002; Ivanova et al., 2011; Belyakova and Pavlyushin, 2013). Emphasis on biological control in the systems of protection of greenhouse vegetables and ornamental plants is instrumental for reducing pesticide load in the greenhouse ecosystems and hampering the evolution of resistance to pesticides in the populations of economically important arthropods. All of this, in turn, will ameliorate the phytosanitary situation and improve the hygienic and environmental safety of plant protection measures (Ivanova et al., 2002; Sukhoruchenko et al., 2008).

There is a sizable array of predatory and parasitic arthropods with a different breadth of dietary specialization which is recommended for use against the insects and mites on greenhouse vegetables. For example, the management of the red spider mite, thrips, and aphids involves the use of such specialist mites as *Phytoseiulus* and *Amblyseius*; the greenhouse whitefly is controlled with *Encarsia*; various aphid species, with a predatory gall midge, coccinellids, and aphidiids. In addition to the release of specialized natural enemies of insects and mites, broadly polyphagous predatory bugs from the families Anthocoridae and Miridae are released against the same pest complex as a prophylactic measure or for mitigation of outbreaks. In such a manner, nursery managers are able to maneuver with the use of insect and mite control agents, depending on the pest abundance and the availability of a given predator or parasite (Asyakin et al., 2010).

In order to immediately suppress a pest outbreak under the conditions of protected ground, microbiological and chemical insecticides and acaricides are still being used with the subsequent release of natural enemies. The efficacy of their simultaneous usage is determined by the level of hazard of a given pesticide to the biological agent, which in turn depends both on the natural species-specific sensitivity to the pesticide and on the mode of application of the latter (Sukhoruchenko et al., 2001; Dolzhenko et al., 2008; Belykh and Ivanova, 2010). Therefore, studies assessing the effects of chemical and microbiological pesticides on particular species of beneficial arthropods and choosing the least dangerous substances are of great practical importance.

This article presents the results of toxicity tests for several greenhouse insecticides as applied to the predatory bugs *Nesidiocoris tenuis* Reuter and *Macrolophus pygmaeus* H.-S. (family Miridae), which are efficient predators of whiteflies, aphids, thrips, and spider mites (Asyakin et al., 1999; Boyarin, 2000; Pazyuk, 2007, 2009; Pazyuk and Vasilyev, 2011). These studies continue the collaborative research at the laboratories for agroecotoxicology and biological plant protection (All-Russia Institute for Plant Protection) of the toxic effects of various chemical and microbial pesticides on beneficial arthropods for effective simultaneous usage of both techniques in modern greenhouse plant protection systems against harmful arthropods (Sukhoruchenko et al., 2001; Sukhoruchenko et al., 2001a, 2002).

MATERIALS AND METHODS

The studies were carried out in 2011 and 2012 on the laboratory colonies of the predatory bugs *N. tenuis* (Nonsan population, South Korea) and *M. pygmaeus* (Sochi population, Russia) which were reared at the Laboratory for biological plant protection, ARIPP.

Both bug species are polyphagous and feed on all sucking pests of protected agriculture (whiteflies, aphids, thrips, spider mites, etc.) with preference for whiteflies. Since both adults and larvae are active predators, the contact toxic effects of insecticides were tested on these two stages. The bugs lay eggs into plant tissues, and therefore the ovicidal effect of tested substances was determined as egg hatchability on treated and intact (control) plants.

The viability parameters of the developmental stages of experimental bugs of both species practically did not differ from those in the field, which allowed comparison of the results of toxicological studies with the control. However, *N. tenuis* and *M. pygmaeus* have substantially different biological characteristics including the mean daily number of larvae produced by the female. This parameter in *N. tenuis* (12–15 larvae) is significantly larger than in *M. pygmaeus* (2–3 larvae). Such relatively small fecundity of the latter species is compensated by greater longevity, which amounts to about 30 days versus 11 days in the former (Pazyuk and Belyakova, 2009).

The even-aged material for the study was obtained by using a technique developed at the Laboratory for biological plant protection, ARIPP (Pazyuk, 2010). According to this technique, the stock cultures of *N. tenuis* and *M. pygmaeus* were reared on *Nicotiana tabacum* L. plants in cages measuring 50×50×40 cm and covered with organza at a temperature of 25–27°C, relative air humidity of 80–90%, and photoperiod of 18 h of light per day. In order to obtain new larvae and adults, 4 plants at the stage of 6–8 expanded leaves were put in the cage and then 120 adults of *N. tenuis* or *M. pygmaeus* were released to lay eggs. Adults were fed *ad libitum* with eggs of the angoumois grain moth *Sitotroga cerealella* Olivier and flower pollen. Four days later, the tobacco plants were transferred to new cages where hatching began on the 7th–10th day of incubation and continued for several days. Part of the obtained even-aged larvae was used in the experiment, whereas the other part was fed on the same diets until the adult stage and then was used in the experiments or replenished the stock culture.

In the experiments, the following insecticides were tested which are allowed to be used on greenhouse plants.

Admiral, emulsion concentrate (100g/l pyriproxyfen), is a member of the chemical class of phenoxyphenyl ethers (a derivative of pyridine). This is a synthetic analogue of the insect juvenile hormone which regulates growth and development and causes morphogenetic abnormalities at different stages of ontogeny. This is a contact insecticide recommended for use against the greenhouse whitefly. The substance is low-hazardous to warm-blooded animals, humans, and bees (hazard category 3).

Mospilan, soluble powder (200 g/kg acetamipride), is a member of the chemical class of neonicotinoids. This is a systemic insecticide that works as contact and digestive toxin with translaminar activity recommended for use against the greenhouse whitefly. The substance is low-hazardous to warm-blooded animals, humans, and bees (hazard category 3).

Spintor 240, suspension concentrate (240 g/l spinosad), is a member of the chemical class of spinosyns. The active substance spinosad is a mixture of natural bacterial metabolites (spinosyn A + spinosyn D) and is produced by fermentation of a biological substrate that is based on a soil actinomycete *Saccharopolyspora spinosa*. This is a contact and digestive insecticide recommended for use against the western flower thrips but it is highly toxic to bees when in direct contact (hazard category 1), which requires the isolation of bees during treatment.

Bitoxibacillin (BT), powder (biological activity 1500 units/mg, titer no less than 20 billion spores/g). This is a microbial product that contains spores, crystal endotoxin, and a temperature-stable exotoxin of the bacterium *Bacillus thuringiensis* var. *thuringiensis*. This is a contact and digestive insecticide and acaricide recommended for use against the red spider mite in greenhouses. It is low-hazardous to warm-blooded animals, humans, and bees (hazard category 3).

Contact toxicity of the insecticides to different developmental stages of the bugs was estimated by spraying adults, larvae, plants one day before oviposition, and plants containing eggs with routine concentrations of pesticides (Admiral: 0.03%, Mospilan: 0.02%, Spintor 0.05%, and BT: 0.5%). This is quite a harsh pesticide treatment that allows revealing under the laboratory conditions the maximal level of hazard of tested substances to pest control agents before their practical implementation. The experiments were made in 6–8 replicates.

In order to estimate contact toxicity of the insecticides to adult bugs, 15 adult individuals of *N. tenuis* and *M. pygmaeus* of the same age were placed onto tomato plants in 3.3 l cylinders sprayed with insecticide and covered with a gauze cap. In the control, plants with bugs were treated with water. The cylinders with experimental material were kept in the environmental chambers at a temperature of 24°C. The survivors were counted 1 and 4 days after the treatment.

The effect of the pesticides on female fecundity was tested as described above, but females and males were placed into the cylinders in the 1:1 ratio. One day after the treatment, the surviving females and males were collected with an aspirator and transferred in couples onto intact tomato plants so that there were 3 to 5 pairs of bugs per each experimental variant. After 3 days, the adults were removed with an aspirator and subsequently the number of emerged larvae was counted.

The ovicidal effect of the insecticides was determined by counting the number of emerged larvae on the plants treated after or one day prior to oviposition. In the first variant, tomato plants with two normal leaves were put singly in plastic 500 ml glasses into which 3 inseminated females of *N. tenuis* or *M. pygmaeus* were also placed for oviposition. It is not recommended to use more than 3 females per plant because a greater number of bugs may severely damage the plant and lead to its death. The day after, the females were removed with an aspirator, and the plants with eggs were treated with insecticide or water (control). At the end of the embryonic period (1 week in *N. tenuis* and 1.5–2 weeks in *M. pygmaeus*), larval emergence was checked daily with living and perished larvae counted separately. In the second variant of the experiment, tomato seedlings with two normal leaves were treated with insecticide or water (control). Once dry after treatment, the plants were put singly in plastic 500 ml glasses into which 3 inseminated bug females were placed for one day for oviposition. The day after, the females were removed with an aspirator. At the end of the embryonic period, larval emergence was checked daily and living and perished larvae counted. In both variants, the glasses with material were kept at a temperature of 25°C which is optimal for egg development, relative humidity of 60 ± 5%, and photoperiod of 18 h of light per day.

In order to assess the larvicidal effect of the insecticides, newly hatched I instar larvae of *N. tenuis* and *M. pygmaeus* were placed by three individuals per one tomato plant (two normal leaves). Each plant with larvae was kept in a 500 ml plastic glass covered with

Table 1. Contact toxicity of insecticides to adult predatory bugs of the family Miridae

Insecticide	Concentration of the active substance, %	Adult mortality rates after 1 or 4 days, %				Daily fecundity (larvae/female)	
		N. tenuis		*M. pygmaeus*		*N. tenuis*	*M. pygmaeus*
		1	4	1	4		
Control*	-	0	2	0	9,0	7.0 ± 2.8 a	1.14 ± 0.66 a
Admiral	0.03	0	24.0	0	36.0	10 ± 3.9 a	1.8 ± 1.25 a
Spintor	0.05	87.0	100	51.0	100	0	0
Mospilan	0.02	17.0	52.0	32.0	41.0	3.75 ± 1.25 b	0.15 ± 0.08 b
BT	0.5	1.0	36.0	3.0	42.0	7.25 ± 1.18 a	1.0 ± 0.68 a

*Sprayed with water. Different letters within one column denote significant differences at $p < 0.05$.

calico at a temperature of 25°C. When the larvae reached the II instar, the glasses with plants were treated with insecticide or water (control). Development time of the larvae that survived treatment and developed into adults and the number of emerged adults were used as proxies of insecticide toxicity.

The data obtained were analyzed by means of ANOVA in the respective module of SYSTAT 10.2 software package.

RESULTS

The imagicidal effect of insecticides. Contact toxicity of the insecticides to the adult predatory bugs was estimated from mortality values over the first 4 days following treatment, since prolonged monitoring was limited by the damage done to the young tomato plants by the experimental insects. Fecundity of the surviving adults was also taken into account because this is an important parameter for the usage of predatory bugs in pest management schemes.

The results of the experiments showed that there was practically no adult mortality of both bug species 24 h after spraying with Admiral and BT (Table 1). However, it was already during this period that the species differences in sensitivity to Mospilan and Spintor became evident, and these differences became even more pronounced after 4 days following the application of the two insecticides. It should be noted that the latter period ended with 100% mortality of both bug species from Spintor and 24–52% mortality from the other insecticides (Table 1). These data indicate that the maximal concentration of Spintor allowed for use against the quarantine pest, the western flower thrips, on flower crops grown in the protected ground is highly toxic to adults of both mirid species.

Further observations on the surviving experimental females of the two species showed that Admiral and BT did not reduce their fecundity, since the differences between treated and intact females in the daily emergence of larvae were non-significant (Table 1). However, there was a significant difference in this parameter between Mospilan-treated and intact females of both bug species. Furthermore, female sensitivity to this insecticide showed clear differences between the two species: while fecundity of *N. tenuis* decreased approximately twice relative to the control, that of *M. pygmaeus* was 7.6 times lower.

The ovicidal effect of insecticides. Since the predatory bugs of both species oviposit into plant tissues, two application modes were tried to test the effects of insecticides on this developmental stage: spraying the substrate (tomato leaves) with already laid eggs and treating the substrate one day prior to oviposition. The study showed that the tested substances, except Spintor, were relatively non-hazardous to newly hatched bug larvae, regardless of the application mode. In particular, for both modes of spraying the oviposition substrate with Admiral and BT, the number of hatched *N. tenuis* and *M. pygmaeus* larvae was similar to or greater than that in the control (Table 2). A slight (12.2–12.9%) decrease in the number of *N. tenuis* larvae after the use of these insecticides relative to the control was not statistically significant. No decrease in the number of hatched *M. pygmaeus* larvae was observed in these experimental variants.

However, when Mospilan was sprayed on the substrate with bug eggs, there was a significant difference in the number of surviving *N. tenuis* larvae; namely, their number was by 43.9% lower in the experiment relative to the control (Table 2). This difference was

Table 2. The effect of insecticides on the eggs of predatory bugs of the family Miridae

Insecticide	Concentration of the active substance, %	Mean number of surviving larvae, ind./female/day		Decrease in the number of larvae relative to the control, %	
		N. tenuis	*M. pygmaeus*	*N. tenuis*	*M. pygmaeus*
Insecticide application after oviposition					
Control*	–	11.6 ± 1.1 a	1.4 ± 0.4 a	–	–
Admiral	0.03	10.1 ± 0.7 a	3.6 ±1.2 a	12.9	0
Spintor	0.05	6.3 ± 1.3 b	0.1 ± 0.01 b	45.7	92.9
Mospilan	0.02	6.5 ± 1.1 b	2.4 ± 0.7 a	43.9	0
BT	0.5	12.7 ± 0.6 a	2.3 ± 1.0 a	0	0
Insecticide application one day prior to oviposition					
Control*	–	10.4 ± 1.4 a	2.1 ± 0.3 a	–	–
Admiral	0.03	11.0 ± 1.1 a	3.1 ± 0.5 a	0	0
Spintor	0.05	0.03 ± 0.03 b	0	99.7	100
Mospilan	0.02	8.9 ± 1.5 a	2.0 ± 0.2 a	14.4	4.8
BT	0.5	8.4 ± 0.9 a	2.8 ± 0.5 a	12.2	0

For designations, see Table 1.

Table 3. Contact toxicity of insecticides to larval predatory bugs of the family Miridae

Insecticide	Concentration of the active substance, %	Development time to adult, days		Number of adults formed, %		Decrease in the number of adults relative to the control, %	
		N. tenuis	*M. pygmaeus*	*N. tenuis*	*M. pygmaeus*	*N. tenuis*	*M. pygmaeus*
Control*	–	12.3 ± 0.15 a	16.1 ± 0.38 a	90.0	82.0	–	–
Admiral	0.03	13.2 ± 0.32 a	15.9 ± 0.38 a	80.0	71.8	28.0	12.4
Spintor	0.05	0	0	0	0	100	100
Mospilan	0.02	18 ± 0.0 b	0	6.7	0	90.3	100
BT	0.5	12.5 ± 0.50 a	17.0 a	15.4	2.9	80.3	97.7

For designations, see Table 1.

not significant when the second application mode was tested. Mospilan was practically non-toxic to newly emerged *M. pygmaeus* larvae, regardless of the application mode, i.e., before or after oviposition. Spintor significantly reduced the number of hatched larvae of both *N. tenuis* and *M. pygmaeus* relative to the control in both application modes, especially when spraying was done prior to oviposition (Table 2). Also, in contrast to Mospilan, it appeared more toxic to *M. pygmaeus* than to *N. tenuis*.

The larvicidal effect. Contact toxicity of the insecticides to the larval stage of the predatory bugs was studied on the II instar larvae and characterized by means of development time to the adult stage and the number of adults emerged.

The data obtained suggest that Admiral and BT did not exert any substantial effect on larval development time of both bug species, since this parameter showed no significant differences between the experiment and control in the case of application of these insecticides (Table 3). *N. tenuis* larvae that were treated with Mospilan developed 6 days longer than in the control, which was a significant difference. However, it should be noted that the increase in development time in this experimental variant negatively affected larval viability, so that only 6.7% of them reached the adult stage. Still more hazardous was the effect of Mospilan on *M. pygmaeus* larvae, as none of them were able to complete development. The most toxic effect on the larvae of both species was exerted by Spintor, and there was not a single treated larva in these experimental variants that survived till adult emergence (Table 3).

The comparisons of the estimates of contact action of several insecticides on the larvae of two species of predatory mirid bugs suggest that Admiral was the least toxic of all the tested substances. In the experimental variant with this insecticide, there was only a 12.4–28.0% decrease in the number of emerged adult bugs relative to the control. In the experimental variants with other insecticides, including BT, mortality rates of the treated larvae were significant, and the number of adults emerged was 80.3–100% lower than that in the control (Table 3).

DISCUSSION

Analysis of the results of toxicity tests for three modern chemical insecticides and a microbial one (BT), which are allowed to be used on greenhouse plants, as applied to different developmental stages of the predatory bugs *N. tenuis* and *M. pygmaeus* released in greenhouses against a complex of sucking pests, suggests the following.

The least toxic to the predatory bugs of both species was Admiral, which is an analogue of insect juvenile hormone. It was moderately toxic to adults (24.0% mortality in *N. tenuis* and 36.0% in *M. pygmaeus*) when directly sprayed at a routine concentration under laboratory conditions and did not reduce fecundity in the individuals that survived the treatment. Admiral appeared to be low-hazardous to bug eggs, regardless of the time of oviposition (either before or after the treatment), and to treated larvae. Thus, the number of adults emerged following the treatment was no more than 12.4% lower than in the control for *M. pygmaeus* and 28.0% lower in *N. tenuis*. The safety of Admiral usage with respect to biological control agents, including predatory bugs from a different family Anthocoridae [*Anthocoris antevolens* White, *A. nemoralis* (F.), and *Orius laevigatus* Fieber], is also shown by some authors (McMullen, 1990; Burov et al., 2002).

Based on these results, we believe that the optimal regime of simultaneous use of predatory mirids and Admiral against the greenhouse whitefly is to release adult bugs several days before spraying or one day after; however, the insecticide may be applied in the presence of bug larvae on the plants as well.

The study of BT toxicity to predatory mirids revealed ambiguity of its effects on different developmental stages. Similarly to Admiral, it is moderately toxic to adults (36.0% mortality in *N. tenuis* and 42.0% in *M. pygmaeus*) and did not cause a decrease in fecundity of surviving individuals. BT was practically non-toxic to larvae emerging from the eggs laid into treated substrates, regardless of the mode of insecticide application, since the eggs were embedded into plant tissues, which prevented their contact with the insecticide, and took 8–12 days to develop, by which time the insecticide would have lost its high initial toxicity. At the same time, BT was highly toxic to larvae when applied directly, and the number of adults emerged from treated individuals was 80.3–97.7% lower than that in the control.

Therefore, when it is necessary to combine BT with *N. tenuis* or *M. pygmaeus*, the bugs should be released for oviposition precisely one day before or one day after the insecticide treatment. This would guarantee survival of a fraction of the released adults and of a considerable number of newly hatched larvae.

Mospilan is more toxic than Admiral and BT to the adult predatory bugs (52.0% mortality in *N. tenuis* and 41.0% in *M. pygmaeus*). In addition, it decreased fecundity of *N. tenuis* by 1.8 times and that of *M. pygmaeus*, by 7.6 times. This insecticide appeared to be especially toxic to larvae of both species after direct spraying: the number of individuals surviving to the adult stage was 93.0–100% lower than in the control. However, it showed moderate toxicity to hatching *N. tenuis* larvae and low toxicity to *M. pygmaeus* hatchlings. These results suggest that the release of predatory bugs, when situation requires its combination with Mospilan, should be made either one day before insecticide application or one day after.

At the same tine, the results of the studies showed that the most toxic insecticide to the studied mirids was Spintor, which had a deleterious effect on all of their developmental stages. In our experiments, this insecticide was tested at a maximum allowed concentration for use on flower crops (0.05%) against such a harmful quarantine pest as the western flower thrips. In joint use of Spintor at this concentration against the western flower thrips and the mirid predatory bugs against a complex of flower pests their release may only be accomplished after a period of 7–10 days during which the high initial toxicity of this chemical to arthropods decreases. When Spintor is used against the western flower thrips on vegetables at a concentration of 0.03%, the hazard level for predatory mirids will decrease since there is evidence that at a concentration of 0.02% it was not dangerous to *N. tenuis* and *M. pygmaeus* (Arnó and Gabarra, 2011).

The insecticides tested by us have no truly ovicidal effect on the predatory bugs. In all the experimental variants, spraying the substrate that contained bug eggs resulted in the emergence on the plant surface of bug larvae that died during the first days after hatching. This testifies to the presence in the tested substances of the so-called "pseudo-ovicidal" activity, i.e., high contact toxicity to newly hatched larvae which quickly die on the insecticide-treated substrate surface. The greatest extent of such activity was observed for Spintor and the least extent, for Admiral. Taking into account the long period of embryonic development of *N. tenuis* (7 days) and *M. pygmaeus* (10–14 days), during which the initial acute toxicity of the tested insecticides under the conditions of protected ground decreases, the safety of these substances for newly emerged bug larvae is provided by strict adherence to the regime of their simultaneous use recommended above.

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