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Effects of Spiracle-blocking Insecticides and Microbial Insecticides on the Predator Mirid Bug, *Nesidiocoris tenuis* (Reuter) (Heteroptera: Miridae)

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Abstract: Spiracle-blocking insecticides and microbial insecticides are widely used for Integrated Pest Management (IPM) in Japan while *Nesidiocoris tenuis* is used for the control of thrips and whiteflies in Kochi Prefecture, Japan. However, the effects of the insecticides mentioned above on *N. tenuis* were unclear. This study investigated the effects of five spiracle-blocking insecticides and two microbial insecticides on the nymphs and adults of *N. tenuis*. Propylene glycol fatty acid monoester was slightly harmful to both the nymphs and adults. Hydroxypropyl starch was slightly harmful to the nymphs, while sodium oleate was slightly harmful to the adults. Decanoyloctanoylglycerol and hydrogenated starch hydrolysate were not harmful to either the nymphs or adults. *Beauveria bassiana* was extremely harmful to the adults and was moderately harmful to the nymphs. *Lecanicillium muscarium* was slightly harmful to the adults. Therefore, decanoyloctanoylglycerol and hydrogenated starch hydrolysate can be used in combination with *N. tenuis* to establish an IPM program.

Key words: Miridae, integrated pest management, spiracle-blocking insecticide, microbial insecticide, insecticidal activity

INTRODUCTION

Spiracle-blocking insecticides are highly effective against major pests, such as thrips, aphids, whiteflies and spider mites (Matsuda *et al*., 1995; Motegi, 2001; Hondo *et al*., 2001; Ota, 2008). Similarly, microbial insecticides are highly effective against thrips, aphids and whiteflies (Masuda and Kikuchi, 1992; Askary *et al*., 1998; Abe and Ikegami, 2005; Saito and Sugiyama, 2005). Moreover, these insecticides are safe for most vertebrates. Therefore, these insecticides are widely used in Integrated Pest Management (IPM) programs to control major greenhouse insect pests in Japan.

Nesidiocoris tenuis is known to be a predator of thrips, aphids, whiteflies and spider mites (Kajita, 1978; Yasunaga *et al*., 1993; Torreno, 1994; Urbaneja *et al*., 2005) and is commercially used to control whiteflies in tomato greenhouses on the Mediterranean coast (Sanchez and Lacasa, 2008; Calvo *et al*., 2009; Hughes *et al*., 2009). In recent years, *N. tenuis* is being used as an indigenous biological control agent for whiteflies and thrips in eggplant and bell pepper greenhouses on the eastern coast of Kochi Prefecture in Japan. However, the effects of spiracle-blocking insecticides and microbial insecticides on *N. tenuis* were unknown. Thus, it was important to study the effects of these insecticides on *N. tenuis* in order to allow IPM programs to be established. In this study, the effects of spiracle-blocking insecticides and microbial insecticides on the survival of *N. tenuis* nymphs and adults were investigated in the laboratory.

MATERIALS AND METHODS

Insects: Approximately 100 *Nesidiocoris tenuis* adults were collected from eggplants in greenhouses in Aki City, Kochi Prefecture, Japan, in early May 2005. They were reared on defrosted Mediterranean flour moths, *Ephestia kuehniella* Zeller eggs and the branches and leaves of the fleshy plant, *Crassula portulacea* Lam., in a container (23×17×9 cm). Old moth eggs were removed and new moth eggs were placed in the container every 2-4 days. The fleshy plant branches and leaves were also replaced every 2-4 days. When an adult female *N. tenuis* laid her eggs on the fleshy plant branches or leaves, the plant containing the mirid eggs was removed. The eggs were then maintained in another container (under the same conditions mentioned above) until adult emergence of the next generation. Culturing of *N. tenuis* was continued for 45 generations under a light regime of 16L8D at 25±1.5°C in an incubator.

Experiments: The insecticides tested are listed in Table 1. The active ingredients in five spiracle-blocking insecticides tested were decanoyloctanoylglycerol (DOG) in San Crystal® (Sankei Chemical Co.), hydrogenated

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Starch Hydrolysate (HSH) in Ecopita® (Kyoyu Agri Co.), Hydroxypropyl Starch (HS) in Nenchaku-Kun® (Sumitomo Chemical Co.), propylene glycol fatty acid monoester (PGFM) in Acaritouch® (ISK Bioscience Co.) and Sodium Oleate (SO) in Oleate® (Otsuka Chemical Co.). The two microbial insecticides tested were *Beauveria bassiana* in BOTANIGARD® (Arysta LifeScience Co.) and *Lecanicillium muscarium* (synonym *Verticillium lecanii*) in MYCOTAL® (Arysta LifeScience Co.). These insecticides are generally used in Japan. The five spiracle-blocking insecticides tested were diluted with ion exchange water. The two microbial insecticides were also diluted with a small amount of ion exchange water. Then, one hour after dilution *B. bassiana* or *L. muscarium* were diluted to the concentration used for the experiments. Spreader solution was not used to dilute the insecticides and ion exchange water was used as a control.

The mirid eggs on the fleshy plant were transferred from the laboratory culture to new petri dishes (9×5 cm). Hatching of the mirids was observed daily and the first-instar nymphs were obtained within 48 h after hatching. The mirid adults were tested within 7 days of their emergence. Approximately 10-15 first-instar nymphs or adults were placed in an acrylic tube (2.5×2.5 cm) which was open at both ends and covered with nylon gauze. The tube was then dipped in one of the insecticide solutions or the control ion exchange water for five seconds. After the dipping treatment, the mirids were placed on the fleshy plant leaves inside a container (11.8×8.6×6 cm) which contained four holes (2 cm) covered with nylon gauze. The mirids were then reared on defrosted moths eggs under 16L8D at 25±0.5°C in an incubator. On the first, third and fifth days after the dipping treatment, we assessed the mortality of mirid nymphs and adults. The dipping test was replicated three times for each insecticide, for both nymphs and adults.

We analyzed mortality using a Tukey-type multiple comparisons for proportions test after arcsine transformation (Zar, 1999), In order to determine the harmfulness of each insecticide, mortality data was then corrected using Abbott's formula (Abbott, 1925) based on the control treatment. Harmfulness was assessed according to the criteria of Sterk *et al.* (1999).

RESULTS

The mortality of the mirid nymphs on the first day and the third day after the dipping treatment using spiracle-blocking insecticides was not significantly different between HSH and the control ($p>0.05$; Table 2), but for the other four insecticides, DGO, HS, PGFM and SO mortality was significantly higher than the control ($p<0.05$). Nymph mortality on the fifth day after treatment was not significantly different between DOG, HSH, SO and the control ($p>0.05$) but it was significantly higher using HS and PGFM versus the other three insecticides and the control ($p<0.05$). Adult mortality on the first, third and fifth day after treatment using PGFM and SO was significantly higher than for the other three insecticides and the control ($p<0.05$). Nymph mortality after treatments using microbial insecticides was not significantly different between *L. muscarium* and the control ($p>0.05$; Table 3), but it was significantly higher using *B. bassiana* than *L. muscarium* or the control ($p<0.05$). Adult mortality on the first day after treatment was not significantly different between the two microbial insecticides and the control ($p>0.05$). However, the mortality of nymphs and adults on the third and fifth day after treatment using *B. bassiana*

Table 1: Type, active ingredient and dilution factor of the insecticides used in this study

Type of agriculture chemical	Insecticides	Active ingredient content (%)	Dilution factor
Spiracle-blocking insecticides	Decanoyloctanoylgycerol EC	90	300
	Hydrogenated starch hydrolysate L	60	100
	Hydroxypropyl starch L	5	100
	Propyleneglycol fatty acid monoester EC	70	1.000
	Sodium oleate L	20	100
Microbial pesticides	*Beauveria bassiaua* EC	1.6×10^{10} spore mL^{-1}	500
	Lecauicillium lecauii WP	3.0×10^{9} spore g^{-1}	500

EC: Emulsifiable, L: liquid, WP: Water-dispersible

Table 2: Morality (%) of first inster nymphs and adults of *N. Tenuis* after spiracle-blocking insecticide application

		Tested nymphs				Tested adults		
Insecticides	N[a]	Day 1	Day 3	Day 5	N[a]	Day 1	Day 3	Day 5
Decanoyloctanoylglycerol	38	21.1[b]	21.1[bc]	28.9[a]	32	3.1[a]	3.1[a]	3.1[a]
Hydrogenated starch hydrolysate	33	6.1[ab]	6.1[ab]	9.1[a]	36	8.3[a]	11.1[a]	16.7[a]
Hydroxypropyl starch	47	66.0[c]	66.0[d]	68.1[b]	35	22.9[a]	22.9[a]	22.9[a]
Propyleneglycol fattyacid monoester	50	70.0[c]	72.0[d]	72.0[d]	39	69.2[b]	69.2[b]	69.2[b]
Sodium oleate	42	26.2[b]	33.3[c]	33.3[a]	37	70.3[b]	73.0[b]	78.4[b]
Ion exchange water (control)	39	0[a]	0[a]	10.3[a]	36	2.8[a]	13.9[a]	16.7[a]

[a]N indicates the number of tested, Means within a column followed by different letters are significantly different among the insecticides ($p<0.05$)

Table 3: Mortality (%) of *N. Tenuis* nymphs and adults after microbial pesticide application

Insecticide	N[a]	Tested nymphs Day 1	Day 3	Day 5	N[a]	Tested adults Day 1	Day 3	Day 5
Beauveria bassiana	38	60.5[b]	86.8[b]	97.4[b]	36	8.3[a]	100[c]	100[c]
Lecanicillium lecanii	48	4.2[a]	4.2[a]	14.6[a]	34	8.8[a]	29.4[b]	38.2[b]
Ion exchange water	41	4.9[a]	7.3[a]	7.3[a]	28	0[a]	3.6[a]	10.7[a]

[a]N indicates the number of tested, Means within a column followed by different letters are significantly different among the insecticides ($p<0.05$)

Table 4: Corrected morality (%) of *N. Tenuis* on the fifth day after insecticide application

Insecticides	Nymphs Corrected morality (%)[a]	Harmfulness[b]	Adults Corrected morality (%)	Harmfulness[b]
Decanoyloctanoylglycerol	20.8	-	0	-
Hydrogenated starch hydrolysate	0	-	0	-
Hydroxypropyl starch	64.4	±	7.4	-
Propyleneglycol fattyacid monoester	68.8	±	63.0	±
Sodium oleate	25.7	-	74.1	±
Beauveria bassiana	97.2	+	100	++
Lecanicillium lecanii	10.2	-	30.8	±

[a]Corrected according to the method of Abbot, 1925. [b]Toxicity was categorized based on morality as follows: -: Not harmful; ±: Slightly harmful; ++: Seriously harmful

was significantly higher than that using *L. muscarium* or the control ($p<0.05$). All the adults died on the third day after treatment with *B. bassiana*.

The corrected mortality of the nymphs on the fifth day after treatment using DOG, HSH, SO and *L. muscarium* was under 26% and thus these treatments were categorized as 'not harmful' to the nymphs (Table 4). The corrected mortality of nymphs exposed to HS and PFM were 64.4 and 68.8%, respectively and thus these insecticides were categorized a slightly harmful to nymphs (Table 4). The corrected mortality of the nymphs exposed to *B. bassiana* was 97.2% which was categorized as 'moderately harmful to nymphs (Table 4). The corrected mortality of the adults on the fifth day after treatment using DOG, HSH and HS was under 8% and therefore these insecticides were categorized as not harmful to adults (Table 4). The corrected mortality of the adults tested with PGFM, SO and *L. muscarium* was 30-75% and therefore these insecticides were categorized as 'slightly harmful' to adults (Table 4). The corrected mortality of adults exposed to *B. bassiana* was 100% and thus categorized seriously harmful to adults (Table 4).

DISCUSSION

Although, we used DOG on eggplant in the greenhouse, DOG had no effect on *Orius* spp. and HSH has little effect on *Orius* sp. (Ota, 2008). In contrast, HS has high insecticidal activity against *Orius* sp. (Hondo *et al*., 2001). The density of *Orius* sp. also fell after sprinkling SO twice, consecutively in a cucumber greenhouse (Matsuda *et al*., 1995). In this study, DOG and HSH were not harmful to either the nymphs or adults of *N. tenuis*. In contrast, HS was slightly harmful to the nymphs and SO was slightly harmful to the adults (Table 4). Although the target insects differed, this result is almost the same result as that mentioned above. Finally, PGFM was slightly harmful to both the nymphs and adults (Table 4). Therefore, three spiracle-blocking insecticides, HS, PGFM and SO were slightly harmful to the nymphs and/or adults and continuous use of these insecticides could be seriously harmful to *N. tenuis*. It is known that spiracle-blocking insecticides function by closing the spiracle of an insect. In this study, the difference in insecticidal harmfulness to *N. tenuis* between the tested spiracle-blocking insecticides was determined by degree of insecticide's adhesive activity.

Beauveria bassiana is highly effective against stink bugs, *Plautia crossota stali* Scott (Tsuda *et al*., 1996, 1997; Ishimoto and Nagase, 2010), rice weevil, *Sitophilus oryzae* (Linnaeus) (Sheeba *et al*., 2001), sweet potato weevil, *Cylas formicarius* (Fabricius) (Yasuda *et al*., 1997), five thrip species (Abe and Ikegami, 2005) and sweet potato whitefly, *Bemisia tabaci* Gennadius (Saito and Sugiyama, 2005). *L. muscarium* is highly effective against greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Hall, 1982; Masuda and Kikuchi, 1992), cotton aphid, *Aphis gossypii* Glover (Masuda and Kikuchi, 1992), and potato aphid, *Macrosiphum euphorbiae* (Thomas) (Askary *et al*., 1998). The insecticidal activity of *B. bassiana* and *L. muscarium* on the target insect differs according to the microbe strain (Abe and Ikegami, 2005; Saito and Sugiyama, 2005; Hall and Burges, 1979; Hall, 1982; Masuda and Kikuchi, 1992). This study shows that the *B. bassiana* strain from commercial products (BOTANIGARD®) used in this study has highly insecticidal activity against both the nymphs and adults of *N. tenuis* (Table 3, 4). Whereas, the *L. muscarium* strain from commercial products (MYCOTAL®) used in this study has no insecticidal activity against the nymphs, it is slightly harmful to the adults (Table 3, 4). However, the overall insecticidal activity of *L. muscarium* on *N. tenuis*, is considered to be low. These results demonstrate that *B. bassiana* cannot be used effectively with *N. tenuis* for biological control in greenhouses.

Finally, two of the five spiracle-blocking insecticides that we investigated showed no insecticidal activity against *N. tenuis*. Therefore, the two spiracle-blocking insecticides, DOG and HSH can be used in combination with *N. tenuis* to establish IPM programs.

CONCLUSION

We investigated the effects of five spiracle-blocking insecticides and two microbial insecticides on the nymphs and adults of *N. tenuis*. The results show that two spiracle-blocking insecticides, DOG and HSH, do not have any insecticidal activity against *N. tenuis*. Therefore, those two spiracle-blocking insecticides can be used in combination with *N. tenuis* to establish IPM programs.

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