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SIDE EFFECTS OF SELECTED BIOPESTICIDES ON REDUVIID PREDATOR *RHYNOCORIS MARGINATUS* (FAB.)

Kitherian SAHAYARAJ, Jasmine ABITHA, and Pratchas SELVARAJ

Crop Protection Research Centre, Department of Zoology, St. Xavier's College, Palayamkottai, Tamil Nadu – 627 002, India. E-mail: ttn_ksraj@sanchart.in

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Laboratory trial was conducted to investigate the impact of two neem-based insecticides (nimbicidine and vijayneem) and a viral pathogen S-NPV on the incubation period, egg hatching, nymphal development and survival and predatory potential of a reduviid predator *Rhynocoris marginatus* (Fabricius). No significant impact was observed on the incubation period; however, neem based biopesticides significantly reduce the egg hatching, prolong the nymphal development and reduce the nymphal survival rate. Studies further revealed that dermal toxicity had more side effects on this reduviid than the contact toxicity. Biopesticides S-NPV proved to have the maximum impact on *R. marginatus*. Field level evaluation may give more information about the impact of these biopesticides on this reduviid.

Heteroptera, Reduviidae, reduviid predator, neem biopesticides, azadirachtin, viral pathogen S-NPV, incubation, egg hatching, nymphal development, nymphal mortality, India.

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Izvršena su laboratorijska istraživanja dva insekticida na osnovi neema (nimbicidin i vijayneem) i virusa S-NPV u cilju utvrđivanja utjecaja na inkubacijsko razdoblje, izlazak ličinaka iz jaja, razvoj nimfi, preživljavanje i predatorski potencijal grabežljive stjenice *Rhynocoris marginatus* (Fabricius). Nije utvrđen utjecaj na inkubacijsko razdoblje, no insekticidi na osnovi neema signifikantno su smanjili broj ličinki izašlih iz jaja, produljili razvoj nimfi i smanjili stupanj njihovog preživljavanja. Daljnja su istraživanja utvrdila da dermalna toksičnost ima više popratnih djelovanja od kontaktne. Biopesticid S-NPV imao je najveći učinak na predatora. Istraživanja u polju trebala bi dati više podataka o utjecajima ovih bioinsekticida na predatora.

Heteroptera, Reduviidae, grabežljive stjenice, neem biopesticidi, azadirachtin, virusni patogen S-NPV, inkubacija, izlaženje ličinaka, razvoj nimfi, mortalitet nimfi, Indija

Introduction

Natural enemies like reduviid predators play an important role in suppressing populations of many insect pests and is an essential component of integrated pest management (IPM) and integrated production of crops (SAHAYARAJ, 2003). In recent years entomopathogens and botanical insecticides are being used for the control of insect pests. It suggested that one must assess the impact of any form of pesticides on non-target beneficial insects, prior to the introduction of the products into the market. Neem based biopesticides such as neevar and nimbicidine and entomopathogen such as S-NPV are found to be effective for the control of *Spodoptera litura* (Fab.) in groundnut (SAJAP and YACOOB, 1994). *Rhynocoris marginatus* (Fab.) is an important reduviid predator of the groundnut ecosystems. Its nymphs and adults feed on many agricultural pests including *S. litura* (SAHAYARAJ, 1999). Earlier studies demonstrated that application of biopesticides does not reduce the biological control efficacy of *R. marginatus* (SAHAYARAJ, 2001). Impact of neem and their products on non-target beneficial organisms (JACOBSON, 1989; SCHMUTTERER, 1990; ASCHER, 1993) and also the impact of pathogens on predators has been documented (YONG and HAMM, 1985; SAJAP et.al., 1999) in literatures. The information about the side effects of S-NPV, nimbicidine and nivaar to *R. marginatus* is not available. The present study was conducted to assess the toxicity of these biopesticides against the life stages of *R. marginatus*. These products were chosen due to their increasing use in pest management.

Materials and methods

Nimbicidine (0.03%, Tstancs & Co Ltd., Coimbatore, India) (NC) and Vijayneem (VN) (0.03%, Shri Disha Biotec Pvt. Ltd., Hyderabad, India) were purchased from the local pesticide shops and the S-NPV was purchased from Government Agriculture office, Palayamkottai. *R. marginatus* was collected form groundnut agroecosystems and culture has been maintained in the laboratory on *S. litura*. Laboratory laid and emerged eggs and nymphal instars respectively were used for this experiment. Zero day old fresh and healthy egg masses having a minimum of 30 eggs of the predator were collected from the laboratory culture and their surface was sterilized with NaOCl (0.01%) for five seconds and washed thoroughly with double distilled water for 10 minutes and were placed on the filter paper kept inside the hatching vials (30 ml capacity). Biopesticides were sprayed on eggs by using hand sprayer (Amway product). Six batches of eggs collected from different pairs of reduviids were treated and

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maintained separately as replicates. The eggs sprayed with water were kept as control. The vials were incubated at 30 ± 1°C temperature, 70 ± 5 per cent relative humidity in the environmental chamber (Remi, Mumbai, India). Incubation period and percentage of hatching were recorded. Similar procedure was followed for one, two, three, four and five days old *R. marginatus* eggs.

In another study all the nymphal instars and adults were used. The toxicity of these biopesticides was tested by topical toxicity (TT) and contact toxicity (CT). For contact toxicity groundnut leaves (Var. Tmv7) were sprayed with different biopesticides separately and were air-dried. After 15 minutes the leaves were placed in a plastic containers having 300 ml capacity. Then five first instar *R. marginatus* nymphs were introduced into the plastic container and reared on *S. litura*. In topical toxicity studies five first instar *R. marginatus* were introduced into the plastic container and sprayed the biopesticides with hand sprayer (Amway product). The test individuals were allowed to be in the same container for 96 hrs and the nymphs were provided with third instar larvae of *S. litura*. Control categories were treated with water. During this period mortality was recorded and corrected using Abbott's formula (ABBOTT, 1925). Similar procedures were followed for the remaining nymphal instars as well as the adults. Sixty predators were subjected to each treatment as well as the stage separately. After four days the survived predators from both experimental and control categories were then individually transferred to clean plastic containers and provided with *S. litura* larva. Observations like developmental period and number of prey consumed by a predator during its nymphal period were recorded.

Results and Discussion

Incubation and Hatching

Under in-vitro condition, maximum egg hatching (90.58%) was observed in VN (vijayneem) followed by S-NPV (90.10%) and NC (nimbicidine) (60.15%). They were statistically significant (P 0.05) when compared with control. In general both the experimental biopesticides and the control water treatment gradually decrease the hatch ability of the egg as the age of the egg increases. NPV-S did not affect the egg hatching up to 3 days. After 3 days, it significantly ($P < 0.05$) reduced the egg hatching. The VN and NC affect the egg hatching ($P < 0.05$) in all age groups except the VN treatment at zero and one day old eggs. Among the neem biopesticides, nimbicidine had more impact during the early stage of the embryonic development. In contrast, VOGT (1994) and CASTAGNOLI et al. (2002) reported that both the pyrethrins (Biopiren

plus) and azadirachtin (neemazal T/S) did not showed significant reduction in the egg hatching of a predatory mite *Amblyseius andersoni* (Chart) (CASTAGNOLI et al., 2002) and also in other beneficial organisms (VOGT, 1994). ASCHER (1993) suggested that one or more compounds present in neem cause oviposition repellency, egg inviability and inhibition of chitin biosynthesis. SAHAYARAJ and PAULRAJ (1999) also reported that plant extracts of *Azadirachta indica* Juss., *Vitex negundo* Linn., *Pongamia glabra* Vent. and *Calotropis gigantea* did not affect the incubation period of *R. marginatus*. However, *A. indica* and *C. gigantea* reduce the egg hatchability. These informations will be an indicative of the compatibility of neem based biopesticides and nuclear polyhedrosis virus and *R. marginatus* in an integrated pest management system.

Mortality

The results revealed that after 96 hrs of bio-pesticides exposure, the mortality of *R. marginatus* nymphs varied from 3.32 to 24.10 (TT) and 1.43 to 13.36 (CT) for vijayneem and nimbicidine treatments respectively and it was 1.66 to 23.21 (TT) and 1.35 to 10.08 percent (CT) in S-NPV treatment. All the biopesticides caused higher mortality in the first instar and gradually decreased when the nymphs grew older. Even though vijayneem did not cause any mortality in both fifth nymphal instar and adults of *R. marginatus*, it caused minimum mortality from the first to the fourth instars. Thus, the tested neem formulation vijayneem was safe to reduviid bug even after 96 hrs exposures. Application of neem seed oil to field plots of rice did not affect number of predatory spinners or mired bugs (SAXENA et. al., 1987). Similarly neem based biopesticides were also not toxic to the mirid predator *Cyrtorhimus lividipennis* Reuter (JHANSILAKSHMI et al., 1998). Irrespective of the biopesticides tested, TT caused more mortality than the CT. Since reduviids conceal themselves under the leaves and the stones in the field, there are fewer possibilities for reduviids exposed to direct spray of biopesticides. Hence, the integration of these biopesticides into the reduviid predators in IPM could not cause any detrimental effect on reduviids. In general the corrected mortality gradually diminished when the predator grew older (Table 2). Among the three biopesticides tested here, S-NPV caused more mortality in the first nymphal instars of *R. marginatus*, where as nimbicidine caused more mortality in other nymphal instars as well as in the adults.

Eventhough the S-NPV treated *R. marginatus* consumed more number of *S. litura* (SAHAYARAJ, 2001), population build up of *C. lividipennis* was less in plants sprayed with a solution of neem based biopesticide (SAXENA et.al., 1987). JHANSILAKSHMI et. al. (1998) pointed out that neem formulations caused less than 50%

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mortality in a mirid predator *C. lividipennis*. Who also reported that if the neem oil was blended with synthetic insecticide, then the combination is more toxic to *C. lividipennis*. SCHMUTTERER (1990) suggested that azadirachtin modifies the programme of insect's life by influencing hormonal systems especially that of ecdysone. More over, the extent of mortality from residual infection is strongly influenced by environmental conditions so this contribution itself will vary according to time and place of the application.

Table 1. Biopesticides on *R. marginatus* incubation period (in days) and hatching (in %)

Age of the Egg	Incubation				Hatching			
	Nimbicidine	Vijay-neem	S-NPV	Control	Nimbicidine	Vijay-neem	S-NPV	Control
0	8.0^{a}	5.5^{ab}	8.0^{a}	8.3^{a}	60.15^{d}	90.58^{abc}	90.10^{ab}	95.12^{a}
1	8.0^{a}	6.0^{b}	8.0^{a}	8.6^{a}	60.23^{d}	83.02^{ac}	90.01^{ab}	90.22^{a}
2	9.0^{a}	9.0^{a}	9.0^{a}	8.7^{a}	58.33^{d}	83.15^{abc}	90.13^{ab}	85.15^{a}
3	9.0^{a}	10.0^{b}	9.0^{a}	8.9^{a}	55.16^{cd}	50.56^{c}	85.10^{a}	75.02^{b}
4	9.0^{a}	10.0^{b}	9.0^{a}	9.0^{a}	50.11^{d}	30.11^{c}	76.22^{a}	60.01^{b}
5	10.0^{b}	10.0^{b}	9.0^{a}	9.0^{a}	45.22^{d}	30.33^{c}	70.22^{a}	55.12^{b}

Means followed by the same letter, with in a parameter separately (incubation/hatching) are not significantly different (Duncan test at 0.05 level).

Table 2. Biopesticides on the corrected mortality (in %) of *R. marginatus* life stages

Life stages	Nimbicidine		Vijayneem		S-NPV	
	DT	CT	DT	CT	DT	CT
First instar	24.10^{b}	13.36^{a}	6.64^{a}	6.64^{a}	23.21^{b}	10.08^{a}
Second instar	21.84^{b}	13.3^{a}	3.32^{b}	6.64^{a}	15.75^{b}	5.25^{a}
Third instar	14.35^{b}	3.32^{a}	3.32^{a}	3.32^{a}	12.01^{b}	2.50^{a}
Fourth instar	13.82^{b}	1.17^{a}	3.32^{a}	1.43^{a}	5.32^{b}	1.35^{a}
Fifth instar	13.02^{b}	0.58^{a}	0	0	1.66	0
Adult	9.98^{b}	0^{a}	0	0	0	0

DT - dermal toxicity and CT - contact toxicity. Means followed by the same letters (DT and CT separately), with in a column are not significantly different (Duncan test at 0.05 level)

Nymphal development and predatory potential

The virus shows detrimental effect on the development of the surviving nymphs (Table 3). Irrespective of the biopesticide treatment, the highest stadial period was recorded in TT than CT. Application of monocrotophos either increased or decreased the developmental period of *Rhynocoris fuscipes* (Fab.) (GEORGE and AMBROSE, 1999). The prolongation of the nymphal developmental period was a stage dependent factor. Among the three biopesticides, S-NPV in TT experiment showed significant prolongation in the nymphal developmental period (P) than other biopesticides as well as the CT bioassay.

Table 3. Impact of bio-pesticides on the nymphal developmental period (in days) and predatory rate (no. of prey) - predator/nymph of *R. marginatus*

Bio-pesticides	Type of treatment	Life stages					Predatory rate
		First	Second	Third	Fourth	Fifth	
Water	DT	38.5^{a}	38.6^{a}	38.8^{a}	39.0^{a}	39.6^{a}	31.46^{a}
	CT	38.6^{A}	38.5	38.9^{A}	38.9^{A}	39.0^{A}	31.92^{A}
Nimbicidine	DT	41.2^{b}	41.5^{b}	42.9^{b}	42.9^{b}	43.7^{b}	34.55^{b}
	CT	40.1^{AB}	40.7	41.3^{B}	41.3^{B}	41.6^{B}	33.67^{AB}
Vijay-neem	DT	39.7^{ac}	41.6^{bc}	42.7^{bc}	44.7^{bc}	46.5^{c}	35.63^{bc}
	CT	39.1^{ABC}	40.2	40.3^{BC}	41.6^{BC}	42.9^{BC}	33.82^{ABC}
S-NPV	DT	49.8^{d}	50.5^{d}	51.7^{d}	52.0^{d}	52.2^{d}	40.73^{d}
	CT	38.8^{ABC}	38.8	39.0^{AD}	39.0^{AD}	39.3^{AD}	32.45^{ABCD}

Means followed by the same letters (DT and CT separately) (small alphabets for water to NC, VN and S-NPV and caps. For DT's and CT's separately), with in a column, are not significantly different (Duncan test at 0.05 level).

Since the dermal toxicity of S-NPV prolonged the developmental period, the mortality may be reduced when compared to the nimbicidine. The S - NPV did not showed detrimental effect on the developmental time of the surviving *Sycanus leucomesus* Walk (Hemiptera: Reduviidae) nymphs (SAJAP et al., 1999). However, the virus impaired the development of the predator. Such a kind of impairment was not observed in this reduviid. It shows the resistant capacity of this reduviid to virus. In order to maintain the normal development, the predator consumed more amount of food than predators reared with or without neem based biopesticides. Furthermore the predator apparently failed to accumulate enough protein resource during the pre-imaginal stages that are required for growth. THOMAS et al. (1998), suggested that *Metarhizium flavoviride* Gams and Rozsypal (Deuteromycotina: Hyphomycetes) spores infect the *Zonocerus variegatus* via penetration of the external cuticle. The results of the present study also in accordance with the view that S-NPV infect the predator via cuticle within the vegetation canopy of the plant (LEGER, 1993). *R. marginatus* was significantly consumed more number of *S. litura* in TT than CT bioassay. No deviation was observed among the neem biopesticide treatments. Among the biopesticides tested, S-NPV group consumed more number of *S. litura* (P 0.05) than neem based insecticides. Insect pathogens used as biological control agents will have effects on the beneficial insect community, not only by reducing the prey resource but, perhaps, by directly infecting the beneficial insects themselves (VINSON, 1990). This study supports the importance of considering pesticides effects including the biopesticide in field situation. However, further studies in semi-field and field conditions are necessary to know the impact of biopesticides (both plant based and microorganisms based) on reduviids.

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MOLECULAR TYPING OF TUNISIAN CLONES OF *Myzus persicae* (HEMIPTERA APHIDIDAE) USING MICROSATELLITE MARKERS

Fattouma DJILANI KHOUADJA[1], Joelle ROUZE-JOUAN[2], Jean Christophe SIMON[2], Nathalie LETERME[2], Sonia BOU HACHEM[3], Mohamed MARRAKCHI[1], Hatem FAKHFAKH[1]

[1]Laboratoire de Genetique Moleculaire, Immunologie et Biotechnologie, Faculte des Sciences de Tunis, Campus Universitaire El Manar, 2092 Tunis, Tunisie; [2]Laboratoire de Zoologie UMR BiO3P, INRA Le Rheu, Domaine de la Motte, BP 35327-35653, Rennes Cedex, France; [3]Institut National de la Recherche Agronomique de Tunisie; E-mail: hafakhfakh@voila.fr or Hatem.fakhfakh@fsb.rnu.tn

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In order to assess the genetic differentiation among Tunisian clones belonging to the *Myzus persicae* complex *(M. persicae* (Sulzer), *M. antirrhinii* (Macchiati) and *M. nicotianea* Blackman), the molecular technique of microsatellites was used in this study. These markers offer sensitivity and are useful in population genetic studies of parthenogenetic organisms. Here, nine polymorphic microsatellite loci were amplified to distinguish between six parthenogenetic clones belonging to *M. persicae* complex collected from two different Tunisian areas. Interestingly, this technique allowed discrimination between five different genotypic classes among the six clones. Furthermore analysis of genetic relatedness between the genotypic classes revealed that two Tunisian clones did not cluster either in *M. persicae* or in *M. antirrhinii* taxa, whereas, the four other Tunisian clones clustered into the *M. persicae* Sulzer taxa.

Hemiptera, Aphididae, *Myzus persicae* complex, clones, molecular typing, parthenogenesis, microsatellite markers, Tunisie

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U cilju utvrdivanja genetičkih razlika izmedu tuniskih klonova kompleksa *Myzus persicae M. persicae* (Sulzer), *M. antirrhinii* (Macchiati) i *M nicotianea* (Blackman) upotrijebljena je molekularna mikrosatelitska tehnika. Ovi su biljezi vrlo osjetljivi i korisni u takvim istraživanjima partenogenetskih organizama. Umnoženo je devet polimorfnih lokusa mikrosatelita kako bi se razlikovalo šest partenogenetskih