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Laboratory contact effect of some insecticides on predatory assasin bug, *Rhynocoris marginatus* Fabricius (Reduviidae: Hemiptera)

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Abstract
Twenty two insecticides were evaluated against 4th instar nymphs of predatory *Rhynocoris marginatus* in laboratory. Corrected mortality (E) was considered to group the treatments as harmless (E < 30%), slightly harmful (30 <E < 79%), moderately harmful (80 < E < 99%) and harmful (E > 99%) according to IOBC protocols. Result indicated no insecticide as harmful category. Moderately harmful with significantly at par mortality (85.74- 81.85 %) found in 3 organophosphate insecticides such as chlorpyriphos, acephate 95 SG and quinalphos. The effect was slightly harmful with E ranged from 76.48 – 51.30 % by 7 insecticides such as bifenthrin, tolfenpyrad, fipronil, acephate 75 SP, lambda cyhalothrin, cartap hydrochloride and ethion + cypermethrin. Remaining 12 insecticides behaved as harmless against assassin predator with mortality varied from 27.22 – 5.37 %. Here the greatest safety found in thiacloprid followed by azadirachtin, acetamiprid, spirotetramat, imidacloprid, chlorfenapyr, chlorantraniliprole, flubendiamide, flubendiamide + thiacloprid, emamectin benzoate, spirotetramat + imidacloprid and ethiprole + imidacloprid.

Keywords: Insecticides, *Rhynocoris marginatus*, Harmful, Effect, Assasin predator

Introduction
Assassin bugs or Reduviids (Hemiptera: Reduviidae) are predacious insects against many economically important pests. They are abundant in agro-ecosystems, semiarid zones, scrub jungles and tropical rainforest ecosystems (Ambrose, 1999; Sahayaraj, 2007) [2, 17]. They stalk and ambush the prey, finally inject the poison to kill it. The rostrum of these bugs is curved outwards from the head, a diagnostic feature of the insects belonging to this family (Sheikh *et al*., 2016) [19]. On the other hand, different insecticides also play major role for suppressing insect pests in crop field. But remarkable hazardous effect is also established due to more dependence on different chemical pesticides for crop pest management. There has been an intense requirement for environment-friendly and sustainable approaches through using as well as conserving naturally occurring bio-control agents, such as Reduviid predators. They occur in diverse habitats in agro-ecosystems and being exposed to insecticides that are used to control insect pests. Although insecticides are evaluated for their control potential against particular insect pests, their effects on biology and physiology of non-target beneficial insects like reduviid are neglected. In integrated pest management (IPM) programmes, incorporation of natural enemies is possible only when they were protected from insecticides used against insect pests. With the introduction of new agro-chemicals, the assessment of the potential effect of these chemicals on survival, dispersal and beneficial capacity of the natural enemies is essential to identify selective insecticides for incorporation in IPM programmes (Paul and Thyagarajan, 1992) [13]. Such an understanding of the mortality effect of some commonly used insecticides on one type of reduviid bug, *Rhynocoris marginatus* would enable the selection of soft insecticides to protect beneficials and thereby to improve IPM.

Materials and Methods
Location of experiment
The experiment was conducted in entomological laboratory of College of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Farm Gate 1, Kalna Road, Burdwan sadar, 713101, West Bengal, India during spring season in 2019.

Rearing of predatory Assassin bug

The adult reduviid (*Rhynocoris marginatus)* cultures including males and females (1:1) was outsourced from National Institute of Plant Health Management (NIPHM), Hyderabad, India. It was used for mass multiplication under laboratory condition having temperature at 25 ± 2^{0}C and relative humidity 75 ± 5 % using larva of factitious host, *Corcyra cephalonica*. The collected predators were released in a circular plastic tray (15 cm diameter and 6 cm height) with 2 cm thick sterilized sand at bottom for mating. The tray was covered with a perforated lid for proper aeration. A small folded paper was kept inside the box for egg laying. The batch of eggs was kept for hatching into individual petriplate (9 cm diameter) lined with slightly moist absorbent cotton and filter paper. After hatching first instars nymphs were transferred into another petriplate of same size and provided with small sized *Corcyra* larva for food. In this way, it was grown upto 2nd instars nymphs and during 3rd instars they were shifted to plastic tray as described earlier for adult rearing. They were grown upto 4th instars nyphal stage for ultimate use in the present experiment.

Rearing of host insect *Corcyra cephalonica*

This host insect *Corcyra cephalonica* was also multiplied on sterilized crushed maize grain using rearing box in laboratory at normal room temperature and humidity following the method as described by Wahengbam *et al*., 2018 [23].

Evaluation and observation of insecticides against predator (Assassin bug)

Twenty two different types (conventional, novel and mixed) and widely used insecticides (Table 1) were dissolved separately in water at their field recommended dose to obtain individual insecticide solution. One untreated control using normal water was also maintained as check. Ten (10) individuals of 4th instars nymphs of *R. marginatus* were taken with three (3) replications for each of twenty three (23) treatments and they were dipped in respective solution approximately for 30 seconds to judge its mortality effect against these said treatments. Observations on survival of *R. marginatus* were taken at 24 hours interval upto 2 days after treatment. The collected data were used for calculation of corrected percent (%) mortality for each treatment using Abbott's formula (1925) [1] as follows.

$$\text{Corrected mortality (\%)} = \left(1 - \frac{\text{Number of insects in treatment}}{\text{Number of insects in untreated control}}\right) \times 100$$

Table 1: Insecticidal effect on *Rhynocoris marginatus* in laboratory

Insecticides	Concentration (%)	Percent mortality (Corrected) of *R. marginatus*			Toxicity class
		1 DAT	2 DAT	Average	
T1= Thiacloprid 21.7 SC	0.2	3.33 (9.01)^{hi}	7.41 (14.63)^{gh}	5.37 (12.57)g	1
T2 = Imidacloprid 17.8 SL	0.05	10.37 (16.96)^{fgh}	11.11 (19.92)^{fg}	10.74 (19.26)fg	1
T3 = Quinalphos 25 EC	0.2	71.11 (57.96)^{ab}	92.59 (77.33)^{ab}	81.85 (65.50)ab	3
T4 = Chlorpyriphos 20 EC	0.2	78.89 (63.44)^{a}	92.59 (77.33)^{ab}	85.74 (68.90)a	3
T5 = Azadirachtin 1 EC	0.2	6.67 (11.67)^{ghi}	7.41 (14.63)^{gh}	7.04 (13.97)g	1
T6 = Fipronil 5 SC	0.15	53.70 (47.42)^{c}	96.30 (83.66)^{a}	75.00 (60.40)b	2
T7 = Acephate 95 SG	0.2	74.81 (60.35)^{ab}	96.30 (83.66)^{a}	85.56 (68.21)a	3
T8 = Flubendiamide 19.92 + Thiacloprid 19.92 SC	0.1	3.33 (9.01)^{hi}	25.93 (30.84)^{ef}	14.63 (22.82)f	1
T9 = Spirotetramat 15 OD	0.1	7.04 (14.29)^{gh}	7.41 (14.63)^{gh}	7.22 (15.91)fg	1
T10 = Chlorfenapyr 10 SC	0.2	10.74 (19.58)^{efg}	11.11 (17.48)^{g}	10.93 (19.32)fg	1
T11 = Ethion 40 + Cypermethrin 5 EC	0.1	28.52 (32.52)^{d}	74.07 (59.83)^{cd}	51.30 (46.03)d	2
T12 = Bifenthrin 10 EC	0.2	71.48 (58.12)^{ab}	81.48 (65.14)^{bcd}	76.48 (61.47)ab	2
T13 = Chlorantraniliprole 18.5 SC	0.03	7.04 (14.29)^{gh}	14.81 (22.77)^{fg}	10.93 (19.41)fg	1
T14 = Spirotetramat 11.01 + Imidacloprid 11.01 SC	0.2	17.78 (25.10)^{def}	33.33 (35.38)^{e}	25.56 (30.49)e	1
T15 = Flubendiamide 20 WG	0.1	7.04 (14.29)^{gh}	14.81 (22.77)^{fg}	10.93 (19.38)fg	1
T16 = Acephate 75 SP	0.1	60.37 (51.35)^{bc}	85.19 (68.07)^{bc}	72.78 (58.98)bc	2
T17= Cartap Hydrocloride 50 SP	0.1	46.30 (43.11)^{c}	62.96 (52.85)^{d}	54.63 (47.97)d	2
T18 = Ethiprole 40 + Imidacloprid 40 WG	0.1	21.11 (27.30)^{de}	33.33 (35.38)^{e}	27.22 (31.53)e	1
T19 = Lambda Cyhalothrin 5 EC	0.1	53.33 (47.21)^{c}	70.37 (57.43)^{cd}	61.85 (52.15)cd	2
T20 = Emamectin Benzoate 5 SG	0.05	17.78 (25.10)^{def}	33.33 (35.38)^{e}	25.56 (30.49)e	1
T21 = Tolfenpyrad 15 EC	0.15	60.37 (51.35)^{bc}	92.59 (77.33)^{ab}	76.48 (61.55)ab	2
T22 = Actamiprid 20 SP	0.02	7.04 (14.29)^{gh}	7.41 (12.19)^{gh}	7.22 (14.03)g	1
T23 = Untreated Control (Water)	-	0.00 (4.05)^{i}	0.00 (4.05)^{i}	0.00 (4.05)	-
SEM (±)		3.34	4.40	2.68	-
CD at 5 %		9.53	12.55	7.63	-

Figure in parenthesis indicates angular transformation, Mean followed by common letter are not significantly different at 5 % level by DMRT, Mortality Effect (E) (according to IOBC protocols) - Classes: 1 = Harmless (E < 30%), 2 = Slightly Harmful (30 <E < 79%), 3 = Moderately Harmful (80 < E < 99%), 4 = Harmful (E > 99%)

Mortality Effect (E) of each treatment on the said predator was commented according to IOBC protocols [Classes: 1 = Harmless (E < 30%), 2 = Slightly Harmful (30 <E < 79%), 3 = Moderately Harmful (80 < E < 99%), 4 = Harmful (E > 99%)] (Hassan *et al*., 1992).

Statistical analysis

Duncan's Multiple Range Test (DMRT) using MSTATC was followed to find out the statistical variations among the different treatments after getting necessary transformed (angular) value for each of corrected percent (%) mortality.

Results and Discussion

The effect of twenty two insecticides on mortality of 4th instars nymphs of *R. marginatus* is mentioned in table 1. Comparing with untreated control, the percent mortality of the test predator at 1 and 2 days after treatment (DAT) varied significantly in most of the insecticidal treatments.

Mortality (%) of *R. marginatus* at 1 DAT

Among the twenty two insecticides, Chlorpyriphos 20 EC caused the highest mortality (78.89 %) of *R. marginatus* at 1 DAT. It was statistically at par with Acephate 75 SG (74.81%), Bifenthrin 10 EC (71.48 %) and Quinalphos 25 EC (71.11 %). Thereafter, more or less statistically at par inferior insecticides were Acephate 75 SP (60.37 %), Tolfenpyrad 15 EC (60.37 %), Fipronil 5 SC (53.70 %), Lambda Cyhalothrin 5 EC (53.33 %) and Cartap Hydrocloride 50 SP (46.30 %). Whereas, the percent mortality in each case of Thiacloprid 21.7 SC (3.33), Flubendiamide 19.92 + Thiacloprid 19.92 SC (3.33) and Azadirachtin 1 EC (6.67) was significantly at par with untreated control. The same was almost 7.04% for each of four other insecticides like Spirotetramat 15 OD, Chlorantraniliprole 18.5 SC, Flubendiamide 20 WG and Actamiprid 20 SP followed by more or less at par result with Imidacloprid 17.8 SL (10.37 %), Chlorfenapyr 10 SC (10.74%), Spirotetramat 11.01 + Imidacloprid 11.01 SC (17.78%) and Emamectin Benzoate 5 SG (17.78 %). The next safer as well as significantly at par insecticides were Ethiprole 40 + Imidacloprid 40 WG (21.11 %) and Ethion 40 + Cypermethrin 5 EC (28.52 %).

Mortality (%) of *R. marginatus* at 2 DAT

The percent mortality of the tested predator against twenty two selected insecticides at 2 DAT varied significantly than untreated control. Comparing this mortality with 1 DAT, each insecticide resulted more fatality (%) to *R. marginatus* without considerable deviation its rank in most cases. The lowest at par mortality (7.41 %) was seen in Thiacloprid, Azadirachtin, Spirotetramat and Acetamiprid. The next safer and statistically at par treatments were Imidacloprid (11.11 %), Chlorfenapyr (11.11 %), Chlorantraniliprole (14.81 %) and Flubendiamide (14.81 %). The recorded mortality (25.93 %) in Flubendiamie + Thiacloprid was significantly at par (33.33 %) for each of Spirotetramat + Imidacloprid, Ethiprole + Imidacloprid and Emamectin benzoate. Whereas, both Fipronil and Acephate 95 SG gave highest mortality of 96.30 %, which was followed by statistically at par 92.59 % for all of Chlorpyriphos, Quinalphos and Tolfenpyrad. The next inferior and significantly at par treatments were Acephate 75 WP (85.19 %), Bifenthrin (81.48 %), Ethion + Cypermethrin (74.07 %) and Lambda cyhalothrin (70.37 %) followed by Cartap hydrochloride (62.96 %).

Average mortality (%) of *R. marginatus* and toxicity class of insecticides

Considering IOBC protocol apropos average mortality, Chlorpyriphos 20 EC (85.74 %), Acephate 95 SG (85.56 %) and Quinalphos 25 EC (81.85 %) were grouped in toxicity class 3. Whereas, the descending sequence of insecticides belonging to toxicity class 2 was Bifenthrin 10 EC/Tolfenpyrad 15 EC (76.48 %) > Fipronil 5 SC (75.00 %) > Acephate 75 SP (72.78 %) > Lambda cyhalothrin 5 EC (68.85 %) > Cartap Hydrocloride 50 SP (54.63 %) > Ethion 40 + Cypermethrin 5 EC (51.30 %). However, toxicity class 1 included all other tested insecticides such as Thiacloprid 21.7 SC, Azadirachtin 1 EC, Spirotetramat 15 OD/Actamiprid 20 SP, Imidacloprid 17.8 SL, Chlorfenapyr 10 SC/Chlorantraniliprole 18.5 SC/Flubendiamide 20 WG, Flubendiamide 19.92 + Thiacloprid 19.92 SC, Spirotetramat 11.01 + Imidacloprid 11.01 SC/ Emamectin Benzoate 5 SG and Ethiprole 40 + Imidacloprid 40 WG with ascending sequence of percent (%) average mortality follows as 5.37 < 7.04 < 7.22 < 10.74 < 10.93 < 14.63 < 25.56 < 27.22, respectively.

A very few information is available apropos insecticidal impact on predatory assassin bug. But it is sufficient against other predators such as lady beetles, Chrysoperla spp., spiders etc. Stadial body weight, fecundity and longevity of *Rhynocoris marginatus* was negatively affected by quinalphos and other organophosphate insecticides (George *et al*., 1998) [4]. Cypermethrin reduced the predatory efficiency in another species of assassin bug, Acanthaspis pedestris (Claver *et al*., 2003) [3]. Nimbicidine (Azadirachtin 0.03 %) showed only 1.17 % nyphal (4th instar) mortality of *Rhynocoris marginatus* through contact toxicity (Sahayaraj and Selvaraj, 2003) [16]. The side effect of several organophosphate insecticides against coccinellid predators were proved by Staubli *et al*., 1984. In general, spiders were more sensitive to synthetic pyrethroids and organophosphates (Wakeil *et al*., 2013) [9]. The negligible detrimental field effects of natural enemies (lady beetles and spiders) were recorded in flubendiamide + thiacloprid (Patel, 2018) [11] and in spirotetramat + imidacloprid (Patel and Sarkar, 2019) [12]. Compatability was noticed in chlorantraniliprole with biocontrol agents and considered it as less toxic insecticide (Larson *et al*., 2012) [8]. Slightly harmful effect of chlorantaraniliprole was noticed in laboratory on larva of *Chrysoperla carnea* (Sabry *et al*., 2014) [15]. The impact was moderately harmful on adult and larva of *Cryptolaemus montruzeiri* with neonicotenoids imidacloprid and acetamiprid (Halappa *et al*., 2013) [5]. Fipronil was harmful against larva of *Chrysopa lacciperda* under laboratory condition (Singh *et al*., 2010) [20]. Direct laboratory application of spirotetramat on larvae and adults of *Cryptolaemus montrouzieri* did not affect its survival, longevity, fecundity, egg hatching, and offspring survival (Planes *et al*., 2013) [14]. Coccinellids and spiders were unsafe during field application of bifenthrin, cartap hydrochloride and emamectin benzoate (Karthick *et al*., 2014) [7]. The broad-spectrum insecticide tolfenpyrad demonstrated to reduce populations of the key predator of thrips in pepper, *Orius insidiosus* (Srivastava *et al*., 2014) [21]. Acephate suppressed the population of coccinellids in rice ecosystem (Sharanappa *et al*., 2019) [18]. Whereas safety of chlorfenapyr was observed against coccinellids in onion crop (Yadav *et al*., 2020) [24]. Ethiprole resulted average toxicity against Cyrtorrhinus lividipennis, a predator of brown plant hopper in rice (Nagalingam *et al*., 2009) [10]. All these previous findings are more or less in agreement with result as reported in this present research manuscript.

Conclusion

This may be the first report about slightly harmful effect caused by most of the taken new generation insecticides on the survival of one economically important insect predator *Rhynocoris marginatus*. Such findings must help to make decision for compatible use of insecticides with *R. marginatus* or other natural enemies in integrated pest management.

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