



Evaluating the Biosafety of Newer and Conventional Insecticides against Parasitoids, Predators and Pollinators Prevailing in Agricultural Ecosystem: To achieve Agricultural Sustainability in Future

M. Shanmuga Prema*, S. Sridharan and S. Kuttalam

Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore – 641 003, India
prema.ms1990@gmail.com

Available online at: www.isca.in, www.isca.me

Received 30th April 2016, revised 29th July 2016, accepted 1st July 2016

Abstract

Promoting sustainable agriculture in developing countries will provide a direct and indirect impact on economic development. In the field of agriculture, sustainability is the ultimate goal. Taking this in consideration it is very important to access the biosafety of insecticides against natural enemies as chemical pesticides play a major role in our Indian agriculture. This study was conducted with Fipronil 80WG, chlorpyrifos 20EC, dimethoate 30EC and NSKE 5% to study their biosafety against *Trichogramma chilonis* Ishii, *Chrysoperla carnea* Stephens and *Apis cerana indica* Fabricus. Results indicated that, NSKE 5% was safe to *T. chilonis* with higher rate of parasitization (92.6%) and emergence (94.1%) followed by fipronil 80WG @ 40 and 50g a.i.ha⁻¹ which recorded 80% egg parasitization and adult emergence. NSKE 5% had maximum *Chrysoperla* egg hatchability of 88.75%. Fipronil 80WG @ 50 and 40g a.i.ha⁻¹ had minimal negative effect on eggs of *Chrysoperla*, 48HAT the hatchability was 80 and 82.5%, respectively. NSKE 5% recorded least toxicity to bees with a mortality of 10% at 24HAT. Fipronil 80WG @ 40 and 50g a.i.ha⁻¹ recorded the mortality of 50% over control. NSKE 5% and fipronil 80WG were superior in their safety over conventional insecticide, chlorpyrifos 20EC @ 200g a.i.ha⁻¹ and dimethoate 30EC @ 375g a.i.ha⁻¹.

Keywords: Biosafety, Rice, *Trichogramma chilonis*, *Apis cerana indica*, *Chrysoperla carnea*.

Introduction

Food crops are damaged by more than 10,000 species of insects¹. Sometimes the yield loss by insects reaches as high as 60-70%. Indian agriculture is currently suffering an annual loss of about Rs. 8, 63, 884 million due to insect pests². In spite of various control measures against pests farmers are mainly depend on chemical control which cause consistently increase in crop loss³. This is due to misuse and overuse of insecticides which cause resistance and increase the survival rate of insect pests. Therefore, toward heavy crop loss, farmers resort to use the pesticides in large quantity, under the adage “if little is good, a lot more will be better”⁴. This causes harmful effect on non-target living organism⁵.

Taking this in consideration it is very important to access the biosafety of insecticides against natural enemies as chemical pesticides play a major role in our Indian agriculture. Knowledge on use of these chemicals will be useful to integrate the natural enemies effectively with chemical pesticides there by helps in maintaining the sustainability of agro ecosystem.

Agricultural ecosystem which comprises these insect pests also harbours natural enemies which naturally control the pest population. Parasitoid like *Trichogramma chilonis* Ishii and predator like *Chrysoperla carnea* Stephens helps naturally in

controlling the pest population and pollinators like *Apis cerana indica* Fabricus helps in pollination process. The insecticides commonly used to control the pest population have indirect effect on these natural enemies. Constant disturbances to agricultural ecosystem using chemical pesticides upset the natural balance causing pest upsurge.

Hence, the study was conducted to assess the effect of different insecticides used in agricultural ecosystem like fipronil 80 WG, a newer molecule and other conventional molecules like chlorpyrifos 20 EC and dimethoate 30 EC along with a botanical insecticide, neem seed kernel extract (NSKE) 5 per cent on these natural enemies under laboratory condition.

Materials and Methods

The laboratory experiments were conducted at the Department of Agricultural entomology, TNAU, Coimbatore during 2013 – 2014. These treatments were imposed as per the treatment schedule given in Table-1. The biosafety of different insecticide formulation were evaluated against natural enemies of agricultural ecosystem.

Parasitoid, *Trichogramma chilonis*, Adult emergence of *Trichogramma chilonis*: The egg parasitoid, *Trichogramma chilonis* cultured in the biocontrol laboratory,

Department of Agricultural Entomology, TNAU, Coimbatore was used for conducting the bioassay. The biosafety of different insecticides were evaluated against *T. chilonis*, an effective egg parasitoid⁶. There were six treatments and four replications. In the bioassay experiment conducted, the parasitized egg cards were tagged with the leaves of rice seedlings and the parasitized egg cards were sprayed with insecticides using atomizer. Distilled water was sprayed in untreated check. The treated egg cards were shade dried for 10 min and then kept in polythene bags of size 30 x 20 cm. The number of adult parasitoids emerged 48 hours after treatment (HAT) was recorded and per cent adult emergence worked out using the formula,

$$\text{Adult emergence (\%)} = \frac{\text{Number of wasps emerged}}{\text{Total number of parasitized eggs in one cm}^2 \text{ card}} \times 100$$

Parasitization of *Trichogramma chilonis*: The effect of insecticides on the parasitization of *T. chilonis* was also tested. The fresh *Corcyra* eggs in cards were treated with different insecticides. There were six treatments and four replications. The sprayed cards were shade dried and kept in polythene bags (30 x 20cm size) along with parasitized egg card ready for emergence in 6:1 ratio. The newly emerged parasitoids were allowed for 48 hours to parasitise and then the adult parasitoids along with parasitoid card were removed. The number of parasitized eggs (eggs appearing black) was recorded after 4 days of parasitization. They were allowed to emerge and counted. Then per cent parasitization was worked out.

$$\text{Per cent Parasitisation} = \frac{\text{Number of parasitized eggs which showed adult emergence}}{\text{Total number of } \textit{Corcyra} \text{ eggs exposed for parasitization}} \times 100$$

Predator, Green lacewing, *Chrysoperla carnea* Stephens:
Ovicidal effect of fipronil 80 WG on the eggs of *C. carnea*: Eggs and grubs of *C. carnea* mass cultured in the Biocontrol laboratory, Department of Agricultural Entomology, TNAU, Coimbatore was utilized for the safety test in laboratory. To assess the effect of on the eggs of *C. carnea*, the brown paper strips containing stalked eggs were treated with insecticides using an atomizer⁷. Each treatment was replicated four times containing 20 eggs in each replication. The eggs sprayed with distilled water alone served as control. The number of grubs hatched from each treatment was recorded and per cent hatchability was worked out using the formula,

$$\text{Per cent Hatchability} = \frac{\text{Number of grubs Hatched}}{\text{Total number of eggs}} \times 100$$

Pollinators, Honey bees, *Apis cerana indica* Fabricius: Toxicity of fipronil 80 WG to honey bees: The worker bees of Indian bee (*Apis cerana indica* Fabricius) required for the safety study were obtained from Apiary, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore. The toxicity of fipronil to honey bees was assessed using contact toxicity method. The insecticide concentrations were prepared using distilled water.

Plastic containers were used for the bioassay and there were adequate perforations in the upper lid in order to provide proper aeration to the bees. The filter paper discs were cut according to the size of the plastic container. Then the filter paper disc was sprayed with one ml of the insecticide solution by means of atomizer. The wet filter paper discs were shade dried and then placed in the plastic containers. The honey bees kept in the refrigerator for one minute to calm them were transferred to plastic container with treated filter paper discs at the rate of 10 per container. After an exposure of one hour, the bees were transferred to nylon net bags of size 2 x 3 m and provided with 40 per cent sucrose solution soaked in cotton wool as feed. The mortality of bees was recorded at 12 and 24 hours after treatment. Per cent mortality of bees was worked out using the formula,

$$\text{Per cent mortality of bees} = \frac{\text{Number of dead bees}}{\text{Total number of bees}} \times 100$$

The per cent mortality in laboratory studies was corrected using Abbot's formula⁸,

$$\text{Per cent corrected mortality} = \frac{\text{Per cent test mortality} - \text{Per cent control mortality}}{100 - \text{Per cent control mortality}} \times 100$$

The data obtained from the laboratory experiments were analysed in completely randomized design⁹. The data on mortality per cent were transformed into arc sine values. Further, the treatment means were statistically differentiated by performing Least Square Means test (LSD) at $p < 0.05$ levels.

Results and Discussion

Pesticidal effect on the emergence of *T. chilonis* adults: The effect of different insecticides on the emergence of *Trichogramma* adults was given in the Table-1. The results indicated that NSKE 5 per cent treated eggs had adult emergence of 90 per cent which was on par with untreated control (95.6%). Fipronil 80 WG at 40 and 50g a.i. ha⁻¹ recorded 83 and 82.5 per cent adult emergence, respectively. The conventional insecticides, chlorpyrifos 20 EC at 200g a.i. ha⁻¹ and dimethoate 30 EC at 375g a.i. ha⁻¹ recorded the least adult emergence of 56 and 58.5 per cent, respectively.

Egg parasitization by *T. chilonis*: The NSKE 5 per cent recorded maximum parasitization of 92.6 per cent and found on par with untreated check (97.5%). The new test chemical fipronil 80 WG at 40 and 50g a.i. ha⁻¹ effected 81.2 and 80.8 per cent parasitization as compared to untreated check (97.5%). The conventional insecticides, chlorpyrifos 20 EC at 200g a.i. ha⁻¹ and dimethoate 30 EC at 375g a.i. ha⁻¹ recorded the least parasitization of 47.7 and 49.7 per cent, respectively. The NSKE 5 per cent had least impact compared to other insecticides.

Fipronil had little impact on the egg parasitoid, *T. chilonis* compared to conventional insecticides. Fipronil 80 WG, at the recommended dose of 40 and 50 g a.i. ha⁻¹ recorded 83 and 82.5

per cent adult emergence and 81.2 and 80.8 per cent parasitization, respectively. The present finding is in accordance with the report which stated phenyl pyrazoles as the safest one to *Trichogrammasp*¹⁰. Similarly, fipronil at 64 and 75g a.i. ha⁻¹ were apparently toxic to *Trichogrammapretiosum* (Riley) under laboratory condition but exhibited very low toxicity in the field conditions¹¹. Chlorpyrifos and dimethoate recorded the least egg parasitization of 47.7 and 49.7 per cent, respectively. Studies revealed that the chlorpyrifos adversely affected the parasitization by *T. chilonis* at all the concentrations tested¹². Dimethoate at 25, 12.5 and 6.25 µl was found to be highly toxic with 100 percent reduction in parasitization as compared to untreated control¹³. The above findings are in consonance with the present results. The safety of neem seed kernel extract to *T. chilonis* was in line with the present study¹⁰.

Safety of insecticides to green lacewing, *Chrysoperlacarnea* :

The least egg mortality was recorded by NSKE 5 per cent (88.75 %). Recommended dose of fipronil 40 and 50 g a.i. ha⁻¹ had less impact on *C. carnea* with the egg hatchability of 82.5 and 80 per cent, respectively whereas the checks dimethoate and chlorpyrifos had lesser egg hatchability of 73.91 per cent (Table-2). The highest egg mortality was observed in dimethoate 30EC (68.75%) and chlorphyriphos 20EC (60.00%) respectively. The results were in line with the following reports. Intermediate safety nature of fipronil to the generalist predators like lady bird beetle, lace wing (*C. carnea*) and spiders¹⁴. The eggs and pupa of *C. carnea* when treated with fipronil 80 WG

has no deleterious effect¹⁵. This clearly supports the results obtained in the present study.

Safety of insecticides to Indian bees, *Apis cerana indica* F.:

The results on the toxicity of fipronil 80 WG to honey bees indicated that it was slightly harmful to Indian bees. The recommended dose of fipronil was slightly harmful to honey bees which are evident from the observations that the recommended dose of fipronil 40 and 50 g a.i. ha⁻¹ recorded 52.50 and 57.50 per cent mortality of worker bees 24 hours after treatment, respectively (Table 3). NSKE 5 per cent recorded a mortality of 10.00 per cent at 24 HAT and found to be the least toxic. The standard check chlorpyrifos 20 EC was highly toxic to honey bees and recorded 80 per cent mortality. However, fipronil was found relatively safer to honey bees than chlorpyrifos 20 EC. Application of fipronil to honey bees have intermediate effect on the bees¹⁶.

In Table-1 a column means followed by a common letter are not significantly different at P = 0.05 by LSD. Figures in parentheses are arcsine Per cent transformed values.

In Table-2 a column means followed by a common letter(s) are not significantly different at (P = 0.05) by LSD. Figures in parentheses are arcsine Per cent transformed values.

In Table-3 a column means followed by a common letter are not significantly different at P = 0.05 by LSD. Figures in parentheses are arcsine Per cent transformed values.

Table-1
Biosafety of insecticides to *Trichogrammachilonis* Ishii

S.No.	Treatments	Dose (ga.i.ha ⁻¹)	Adult emergence (%)	Corrected mortality (%)	Egg parasitisation (%)	Unparasitized egg (%)	Corrected mortality (%)
1.	Fipronil 80 WG	40	83.0 ^c (5.28)	15.82	81.2 ^b (5.16)	18.8	16.72
2.	Fipronil 80 WG	50	82.5 ^c (5.21)	16.32	80.8 ^b (5.16)	19.2	17.13
3.	Chlorpyrifos 20EC	200	56 ^d (4.29)	43.21	47.7 ^c (3.96)	52.3	51.08
4.	Dimethoate 30 EC	375	58.5 ^d (4.39)	40.67	49.7 ^c (4.04)	50.3	49.03
5.	NSKE 5 %	-	94.1 ^a (5.44)	4.56	92.6 ^a (5.52)	7.4	0.50
6.	Untreated control	-	98.6 ^a (5.61)	0	97.5 ^a (5.64)	2.5	0

Values in the column are mean of four replications.

Table-2
Toxicity of insecticides to eggs of *Chrysoperlacarnea*

S. No.	Treatments	Dose (g a.i. ha ⁻¹)	Egg hatchability (%)* (48 HAT)	Per cent mortality	Corrected mortality (%)
1.	Fipronil 80 WG	40	82.50 ^c (65.33)	17.50 ^c (24.68)	14.29
2.	Fipronil 80 WG	50	80.00 ^c (63.53)	20.00 ^c (39.23)	16.88
3.	Chlorpyriphos 20EC	200	60.00 ^c (50.77)	40.00 ^c (26.48)	37.66
4.	Dimethoate 30 EC	375	68.75 ^d (56.01)	31.25 ^d (33.96)	28.57
5.	Neem seed kernel extract 5 %	-	88.75 ^b (68.23)	11.25 ^b (19.53)	7.79
6.	Untreated control	-	96.50 ^a (90.00)	3.75 ^a (9.76)	0.00

*Mean of four replications

Table -3
Toxicity of insecticides to Indian bees-*Apis cerana indica*

S.No	Treatments	Dose (g a.i. ha ⁻¹)	6 HAT		12 HAT*		24 HAT*	
			Per cent mortality	Corrected mortality (%)	Per cent mortality	Corrected mortality (%)	Per cent mortality	Corrected mortality (%)
1.	Fipronil 80 WG	40	7.50 ^b (13.9)	7.5	22.5 ^b (28.23)	20.51	52.50 ^b (46.45)	48.65
2.	Fipronil 80 WG	50	10.00 ^b (18.4)	10.0	25.00 ^b (29.89)	23.08	57.50 ^b (49.39)	54.05
3.	Chlorpyriphos 20EC	200	25.00 ^c (29.89)	25.0	50.00 ^c (45.06)	48.72	85.00 ^c (70.01)	83.78
4.	Dimethoate 30 EC	375	22.5 ^c (28.23)	22.5	45.00 ^c (42.12)	43.59	77.50 ^c (61.78)	75.68
5.	NSKE 5 %	-	2.50 ^a (4.83)	2.50	7.50 ^a (13.9)	5.13	10.00 ^a (15.93)	2.70
6.	Untreated control	-	0.00 ^a	0	2.50 ^a (4.825)	0	7.50 ^a (13.9)	0.00

*Mean of three replication; HAT- Hours after treatment.

Conclusion

Insecticides will continue to serve as an essential component in Integrated Pest Management (IPM). However, their use should be based on sound economical principles. It is justifiable to use insecticides only when benefit-risk ratios are in favour of insecticides use. The adverse effects on non-target organisms are to be understood in greater depth in order to use insecticides most effectively in pest management. Thus, the insecticides are double edged swords and using them in an unscientific manner may bring catastrophic results. It is imperative therefore, to develop appropriate insecticide management strategies to contain the pest with protection of predators, parasitoids as well as the environment. So this helps in maintaining a sustainable environment to the future world.

References

1. Dhaliwal G.S., Dhawan A.K. and Singh R. (2007). Biodiversity and Ecological Agriculture: Issues and perspectives. *Indian J. Ecol.*,34(2), 100-109.
2. Dhaliwal G.S., Jindal V. and Dhawan A.K. (2010). Insect Pest Problems and Crop Losses: Changing Trends. *Indian J. Ecol.*,37(1), 1-7.
3. Dhaliwal G.S. and Koul O. (2010). Quest for Pest Management: From Green Revolution to Gene Revolution. Kalyani Publishers, New Delhi.

4. Aktar M.W., Sengupta D. and Chowdhury A. (2009). Impact of pesticide use in Indian agriculture - Their benefits and hazards. *Interdisciplinary Toxicology*, 2(1), 1-12.
5. Cork A., Kamal N.Q., Alam S.N., Choudhury J.C.S. and Talekar N.S. (2003). Pheromone and their applications to insect pest control. *Bangladesh journal of Entomology*, 13,1-13.
6. Pandiyan I.G., Gunasekaran K., Selvaraj P., Rangasmy A., Kim G.H., Chung K.Y. and Tongamin S. (2005). Labouratoury evaluation of relative toxicities of some insecticides against *Trichogrammachilonis* (Hymenoptera: Trichogrammatidae) and *Chrysoperlacarnea* (Neuropteran: Chrysopidae). *J. Asian-Pacific Entomol.*, 8(4), 381-386.
7. Krishnamoorthy A. (1985). Effect of several pesticides on eggs, larvae and adults of the green lacewing, *Chrysopascelestes* Banks. *Entomon.*, 10(1), 21-28.
8. Abbott W.S. (1925). A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.*, 18, 265-267.
9. Gomez K.A. and Gomez A.A. (1984). Statistical Procedures for Agricultural Research. A Wiley International Science Publication, John Wiley and Sons, New York, 680.
10. Xueping Z., Wu C., Wang Y., Cang T., Chen L., Yu R. and Wang Q. (2012). Assessment of toxicity risk of insecticides used in rice ecosystem on *Trichogrammajaponicum*, an egg parasitoid of rice lepidopterans. *J. Econ. Entomol.*, 105(1), 92-101.
11. Hamon N.M., Gamboa H. and Garcia J.E.M. (1996). Fipronil: A major advance for the control of boll weevil in Columbia. Proceedings Beltwide Cotton Conferences (Herzog, G.A., D.A. Hardee, R.J. Ottens, C.S. Ireland, and J.V. Nelms. eds.), USA. 2, 990-994.
12. Tiwari S. and Khan M.A. (2002). Effect of fenobucarb and chlorpyrifos-methyl on parasitization by *Trichogrammachilonis* Ishii. *Pestology*, 26(3), 40-42.
13. Kakakhel S.A. and Hassan S.A. (2000). The side-effects of pesticides on *Trichogrammacoeciae* Marchal (Hymenoptera: Trichogrammatidae), an egg parasite. *Pak. J. Biol. Sci.*, 3(6), 1011-1013.
14. Rishi K., Kranthi S., Nitharwal M., Jat S.L. and Monnga D. (2012). Influence of pesticides and application methods on pest and predatory arthropods associated with cotton. *Phytoparasitica*, 35, 280-285.
15. Medina P., Budia F., Tirry L., Smaghe G. and Vinuela E. (2004). Compatibility of spinosad, tebufenozide and azadiractin with eggs and pupae of the predator, *Chrysoperlacarnea* (Stephens) under laboratory conditions. *Biocon. Sci. Tech.*, 11(5), 597-610.
16. Mayer F. and Lunden C. (1999). Field and laboratory tests of the effects of fipronil on adult female bees of *Apismellifera*, *Megachilerotundata* and *Nomiamelanderi*. Australian Pesticides and Veterinary Medicines Authority. www.apvma.gov.au, 24-27.