



TOXICITY RESPONSE OF SOME INSECTICIDES ON INSECT EGG PARASITOID *TRICHOGRAMMA JAPONICUM* (ASHMEAD)

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ABSTRACT

Lethal role of some insecticides including one of recent introduction (broflanilide) on *Trichogramma japonicum* Ashmead (Hymenoptera: Trichogrammatidae) was assessed by exposing its juvenile stages (egg, larva and pupa) inside the host eggs and adults by residual contact. Transgenerational effect was also studied. The results revealed varied level of toxicity among these test methods. Treatment (insecticide) wise overall toxicity was also determined, and insecticides were grouped into different toxicity classes. Chlorantraniliprole and flubendiamide were harmless (< 30% mortality) followed by cyantraniliprole, emamectin benzoate, novaluron + indoxacarb and broflanilide which were harmless to slightly harmful (< 79% mortality). Slightly harmful was teflubenzuron, chlorfenapyr and azadirachtin. Spinosad was slightly harmful to moderately harmful (< 99% mortality).

Key words: Broflanilide, effect, emergence, harmful, lethal, mortality, parasitisation, slightly, toxicity, *Trichogramma*, Hymenoptera, Trichogrammatidae toxicity classes, harmful, safe

Parasitoids play a critical role in insect pests' management (Bompard et al., 2013). Significant achievement has been realised to manage lepidopteran pests by mass rearing and field releasing of egg parasitoid, *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) (Singh et al., 2018; Jiang et al., 2019). But its effectiveness in field largely depends on the use of soft insecticide. Most insecticides are unsafe to natural enemies, ecosystem and environment (Sabry et al., 2016; Kwizera and Susurluk, 2017). Eco-friendly pest reduction may be targeted by harmonious use of bio-agents with insecticides (Aliyu et al., 2017). This is due to demand for sustainable integrated pest management (IPM) using bio-agents and selective insecticides (Bastos et al., 2006; Brugger et al., 2010). One major purpose of IPM is sustainable use of chemical and biological control (Uma et al., 2014). Scientists have been searching for safer insecticides to minimize the use of broad-spectrum insecticides (Elbert et al., 2008). Some of them have questioned about insecticidal safety and revealed non selectivity for beneficial arthropods (Prabhaker et al., 2011). There is contradiction for use of *Trichogramma* spp. with insecticides. In this connection, it is indispensable to study the toxicity of novel insecticides against *Trichogramma* spp. In IPM, deleterious role of insecticides against *Trichogramma* spp. has been assessed enormously by testing acute toxicity on it. Their residual exposures on *Trichogramma* physiology and behaviour must also be considered for a complete analysis of insecticidal

impact. The objective of this study was to compare the lethal and sublethal impact of some novel insecticides to *Trichogramma japonicum* Ashmead (Hymenoptera: Trichogrammatidae) in laboratory condition.

MATERIALS AND METHODS

The experiment was conducted at 25±2°C and 70±5% RH at the Entomological Laboratory, College of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Burdwan, West Bengal during February-March 2020-21. The rice grain moth, *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) was used as host insect for laboratory rearing of *T. japonicum*. The nucleus culture of *C. cephalonica* (National Accession No. NBAII-MP-PYR-01) and *T. japonicum* (National Accession No. NBAII - MP - TRI -65) were collected from National Bureau of Agricultural Important Insect Resources (NBAIIR), ICAR, Bangalore. They were reared following the methodology by Wahengbam et al. (2018).

Ten insecticides viz. cyantraniliprole 10.26 OD (0.18%, Benevia, FMC India Pvt. Ltd.), chlorantraniliprole 18.5SC (0.04%, Coragen, DuPont), emamectin benzoate 5SG (0.05%, Proclaim, Syngenta India Pvt. Ltd.), spinosad 45SC (0.03 %, Spintor, Bayer Crop Science), flubendamide 20WG (0.03%, Takumi, Rallis India Ltd.), teflubenzuron 15SC (0.04%, Nomolt, BASF India Ltd.), novaluron 5.25 + indoxacarb 4.5SC (0.075%, Plethora, Adama India Pvt. Ltd.), broflanilide 30SC (0.012%, BASF India Ltd.), chlorfenapyr 24SC

(0.066%, Intrepid, BASF India Ltd.) and azadirachtin 0.15 EC (0.4%, Neem Baan, Pest Control India Ltd.) were evaluated along with untreated control using distilled water only. The solution of insecticide was prepared for each insecticide at recommended dose.

Four methods were used for determination of instant and persistent toxicity as follows. i) Fresh sterilized host (*C. cephalonica*) egg card was dipped in insecticide solution and then after drying it was allowed for parasitization by *T. japonicum* @ 10:1. The percent (%) mortality in egg parasitization (dark colour of host eggs) and adult emergence was worked out. ii) Mostly *T. japonicum* larva inside host eggs (24 hr after exposure of sterilized *Corcyra* egg card with adult parasitoid) was dipped in insecticide solution and then it was kept to see percent (%) mortality in egg parasitization as pupal stage of *T. japonicum* and adult emergence in the first generation. The emerged adults were exposed again with sterilized host eggs to observe percent (%) mortality in egg parasitization and subsequent adult emergence in second generation iii) Mostly *T. japonicum* pupa inside host eggs (at dark colour of host eggs which is generally occurred 72 hr after exposure of *Corcyra* egg card with *T. japonicum*) was dipped in insecticide solution and then it was kept to see percent (%) mortality in adult emergence in first generation and subsequent (%) mortality in egg parasitization and adult emergence in next generation following same process as mentioned in second method iv) Inner side of test tubes were washed with insecticide solution and dried up in room temperature to produce a thin dry film of insecticides. Then trichocard ready to adult emergence and fresh sterilized *Corcyra* egg card was kept into the test tube to observe the contact effect of insecticides on emerged adult *T. japonicum* for percent (%) mortality in egg parasitization and subsequent adult emergence.

Completely randomized design (CRD) having 4 replications with 11 treatments including untreated control with distilled water was used for each of four methods. The (%) mortality in egg parasitization and adult emergence were calculated. Mortality data (E) were corrected using Abbott's formula (Abbott, 1925). Insecticides were grouped according to International Organization for Biological Control (IOBC) protocols (Classes: 1 = Harmless ($E < 30\%$), 2 = slightly harmful ($30 < E < 79\%$), 3 = moderately harmful ($80 < E < 99\%$), 4 = Harmful ($E > 99\%$) (Hassan, 1992). The data was analyzed by using the MSTATC program. Duncan's Multiple Range Test (DMRT) was followed to find out the statistical variations.

RESULTS AND DISCUSSION

Table 1 denotes data in parasitism and adult emergence of *T. japonicum* after application of insecticides at different developmental stages including transgenerational effect in some cases. Significant variations were observed, when applied on host eggs. Moderately harmful azadirachtin exhibited highest mortality (97.25%) in parasitism followed by slightly harmful spinosad, chlorfenapyr, cyantraniliprole and novaluron+indoxacarb with respective mortality ranged from 78.16-39.7%. It was lowest (0.00%) by harmless broflanilide followed by flubendiamide, chlorantraniliprole, emamectin benzoate and teflubenzuron having respective mortality varied from 5.46 –25.97%. Varied persistent toxicity prevailed for adult emergence except with harmless chlorantraniliprole, flubendiamide and broflanilide. Highest mortality (96.81%) depicted in moderately harmful spinosad was followed by azadirachtin, and emamectin benzoate (93.78 – 86.38%), others insecticides were found to be slightly harmful resulting mortality varied from 36.84 – 56.93%.

Treated trichocard at larval stage resulted harmless parasitism in chlorantraniliprole, emamectin benzoate, spinosad and flubendiamide having 9.90 to 23.01% mortality, respectively. It was slightly harmful in broflanilide, novaluron + indoxacarb, cyantraniliprole, teflubenzuron, chlorfenapyr and azadirachtin ranging respective mortality 37.25 to 77.44%. Harmless residual toxicity exhibited in cyantraniliprole, chlorantraniliprole and flubendiamide. Emamectin benzoate was also harmless, but it behaved as moderately harmful for parasitism in subsequent generation. Harmless to slightly harmful effect was observed in teflubenzuron, novaluron + indoxacarb and broflanilide followed by slightly harmful to moderately harmful in chlorfenapyr, azadirachtin and spinosad. Adult emergence from insecticide treated trichocard at pupal stage was harmless in cyantraniliprole, flubendiamide, chlorantraniliprole, novaluron + indoxacarb, broflanilide and emamectinbenzoate with respective mortality being 2.17 to 28.54%. It was followed by slightly harmful chlorfenapyr and azadirachtin (36.56 – 74.67%); spinosad was moderately harmful exhibiting 86.77% mortality. Considering sublethality in subsequent generation, most of the treatments were harmless both for parasitism and adult emergence. Emamectin benzoate and azadirachtin were slightly harmful during parasitism. Whereas, spinosad performed as moderately harmful for parasitism and slightly harmful for adult emergence.

Table 1. Instant and residual toxicity of insecticides evaluated on developmental stages of *T. japonicum*

Treatment	Corrected mortality (%) in parasitism (a) and adult emergence (b) of <i>T. japonicum</i> from insecticide treated host eggs		Corrected mortality (%) in parasitism (a) and adult emergence (b) of <i>T. japonicum</i> upto next generation from insecticide treated Trichocard at larval stage		Corrected mortality (%) in parasitism (a) and adult emergence (b) of <i>T. japonicum</i> upto next generation from insecticide treated Trichocard at pupal stage		Corrected mortality (%) in parasitism in parasitism (a) and adult emergence (b) of <i>T. japonicum</i> from contact exposure of its adults to insecticides				
	Current generation		Next generation		Current generation		Next generation				
	a	b	a	b	a	b	a	b			
Cytraniliprole	53.49	36.84	52.93	14.65	18.97	17.97	2.17	6.92	7.65	7.49	3.85
10.26OD @ 0.18 %	(47.30) ^d	(37.67) ^f	(56.97) ^b	(22.90) ^f	(26.15) ^f	(25.41) ^e	(9.07) ^h	(15.65) ^e	(16.20) ^{de}	(16.14) ^h	(11.94) ^h
Chlorantraniliprole	8.73	0.00	9.90	12.60	6.19	26.36	11.57	7.50	4.33	23.02	1.92
18.55C @ 0.04 %	(17.44) ^g	(4.05) ^g	(18.81) ^f	(21.16) ^f	(14.93) ^g	(31.21) ^{cd}	(20.06) ^g	(16.14) ^e	(12.23) ^{de}	(28.99) ^e	(8.90) ⁱ
Emamectin benzoate	22.13	86.34	12.39	19.05	96.21	7.89	28.54	30.85	4.94	62.23	3.27
5SG @ 0.05 %	(28.16) ^f	(68.74) ^c	(20.94) ^f	(26.24) ^e	(79.77) ^a	(16.28) ^f	(32.50) ^d	(34.04) ^b	(13.46) ^{de}	(52.38) ^c	(11.07) ^h
Spinosad	78.16	96.81	18.20	96.45	91.45	39.57	86.77	94.40	31.03	70.47	23.72
45SC @ 0.03 %	(62.49) ^b	(82.15) ^a	(25.55) ^e	(80.56) ^a	(73.56) ^b	(39.27) ^b	(69.16) ^a	(76.96) ^a	(34.04) ^a	(57.40) ^b	(29.47) ^d
Flubendiamide	5.46	0.00	23.01	4.93	5.20	23.86	10.63	2.57	13.35	18.74	42.91
20WG @ 0.03 %	(13.36) ^g	(4.05) ^g	(28.96) ^d	(13.48) ^g	(13.64) ^g	(39.50) ^{de}	(19.21) ^g	(9.77) ^f	(21.75) ^{bc}	(26.00) ^f	(41.21) ^a
Teflubenzuron	25.97	47.44	53.77	74.97	54.70	5.65	22.80	17.17	3.74	91.11	18.42
15SC @ 0.04 %	(30.92) ^f	(43.82) ^e	(47.45) ^b	(60.32) ^b	(47.99) ^d	(17.39) ^f	(28.83) ^e	(24.80) ^e	(11.55) ^e	(73.19) ^a	(25.78) ^e
Novaluron 5.25 + indoxacarb 4.55C @ 0.075 %	39.87	56.83	38.99	3.02	25.26	35.11	16.02	12.62	9.67	17.27	11.54
	(39.43) ^e	(49.22) ^d	(38.92) ^e	(10.66) ^g	(30.49) ^e	(36.64) ^b	(23.90) ^f	(21.11) ^d	(17.25) ^{cd}	(24.84) ^f	(20.22) ^f
Broflamide	0.00	0.00	37.25	2.77	73.28	49.79	25.15	12.88	16.25	46.50	37.61
30SC @ 0.012 %	(4.05) ^h	(4.05) ^g	(37.90) ^e	(10.33) ^g	(59.22) ^c	(45.16) ^a	(30.32) ^{de}	(21.42) ^d	(24.08) ^b	(43.28) ^d	(38.11) ^b
Chlorfenapyr	68.89	56.93	56.69	36.54	90.98	33.08	36.56	15.05	3.95	68.40	8.62
24SC @ 0.066 %	(56.42) ^c	(49.28) ^d	(49.13) ^b	(37.47) ^d	(73.12) ^b	(35.42) ^{bc}	(37.48) ^c	(23.21) ^{cd}	(11.91) ^e	(56.13) ^b	(17.48) ^g
<i>Azadirachtin</i>	97.24	93.78	77.44	59.79	89.93	33.13	74.67	30.85	8.51	14.09	27.24
0.15EC @ 0.4 %	(81.62) ^a	(76.46) ^b	(62.00) ^a	(50.94) ^c	(72.02) ^b	(35.43) ^{bc}	(60.12) ^b	(34.04) ^b	(17.45) ^{cd}	(22.43) ^g	(31.77) ^c
Control (water)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	(4.05) ^h	(4.05) ^g	(4.05) ^g	(4.05) ^h	(4.05) ^h	(4.05) ^g	(4.05) ^j	(4.05) ^g	(4.05) ^f	(4.05) ⁱ	(4.05) ^j
SEM	1.72	1.50	0.88	1.12	1.10	1.49	1.20	0.92	1.84	0.75	0.74
CD (p=0.05)	5.08	4.42	2.60	3.31	3.25	4.38	3.46	2.65	5.30	2.16	2.12

Paranthesis data angular values. Data superscripted with same alphabet statistically similar to each other. Based on corrected mortality (%) (E). Insecticides were grouped according to protocols (Classes: 1 = Harmless (E < 30%), 2 = Slightly Harmful (30 < E < 79%), 3 = Moderately Harmful (80 < E < 99%), 4 = Harmful (E > 99%))

Observation was also reported here for subsequent parasitism and adult emergence after its contact exposure with insecticides. Harmless toxicity for parasitism occurred in cyantraniliprole, azadirachtin, novaluron + indoxacarb, flubendiamide and chlorantraniliprole having respective mortality ranged from 7.49 to 23.02%. It was slightly harmful in broflanilide, emamectin benzoate, chlorfenapyr and spinosad with respective mortality varied from 46.50 to 70.74%. Whereas, teflubenzuron proved it as moderately harmful with 91.11% mortality. Harmless residual toxicity resulting 3.27 to 27.24% mortality for adult emergence was recorded in most treatments except slightly harmful broflanilide and flubendiamide with 37.61 to 42.91% mortality, respectively. Considering pooled toxicity (Table 2), the lowest lethality observed in chlorantraniliprole and flubendiamide followed by emamectin benzoate, cyantraniliprole, novaluron + indoxacarb, broflanilide, teflubenzuron. The highest lethality recorded in spinosad, chlorfenapyr and Azadirachtin. Some variation was noted in regard to persistent toxicity among different insecticides. Chlorantraniliprole proved to be the lowest persistency trailed by cyantraniliprole, flubendiamide and broflanilide. The persistent effect was highest in Spinosad followed by azadirachtin, emamectin benzoate, chlorfenapyr, teflubenzuron and novaluron + indoxacarb. So, it is confirmed that chlorantraniliprole and flubendiamide had most harmless direct and persistent average toxic effect on adults and pre imaginal different developmental stages of *T. japonicum*. It was also more or less harmless with cyantraniliprole, novaluron + indoxacarb and broflanilide. The same effect was slightly harmful with teflubenzuron, chlorfenapyr and azadirachtin. Harmless to slightly harmful effect was observed in emamectin benzoate, whereas spinosad depicted slightly harmful to moderately harmful average toxicity.

Treated method wise discussion apropos acute and persistent effect of novel insecticides on developmental stages of *T. japonicum* is evaded to make it somewhat practically easy for understanding and ultimately to draw a tangible conclusion from this study. So, an innovative new concept to obtain cumulative toxicity (instant and residual) of these insecticides on different developmental stages of *T. japonicum* has been introduced in this manuscript. Based on this, the major discussion has been highlighted here. Here, it revealed mostly harmless to slightly harmful effect for all the treatments except spinosad. Our results largely concur with findings from other studies as discussed below with

some variations in direct and persistent susceptibility of different stages of *Trichogramma spp.* Adults and different developing stages of *Trichogramma* within host eggs appear to be well protected from some insecticides. Teflubenzuron was classified as harmless or slightly harmful to *T. pretiosum* immature stages (Bueno et al., 2008). Evidence is available to consider flubendiamide as considerably safe, neem oil as mildly toxic and spinosad as highly toxic for *T. chilonis* (Sattar et al., 2011). Sub-acute susceptibility of adult *T. chilonis* by chlorfenapyr and spinosad was determined previously by Wang et al., 2012. Safety report with emamectin benzoate against *T. Ostrinia* (Wang et al., 2012a) and *T. nubilale* (Wang et al., 2012b) has a direct support with present findings to *T. japonicum*. Spinosad was confirmed as moderately harmful and novaluron as harmless in respect to parasitization and adult emergence of *T. chilonis* from the treated host eggs. Spinosad was also found greatly lethal to egg, larval and pupal stages of *T. chilonis*. Whereas, novaluron was found to be safe (Narendra et al., 2013). Chlorantraniliprole and emamectin benzoate were rated as 'harmless' as per IOBC safety classification whereas spinosad and flubendiamide were found 'slightly harmful' to adults *T. japonicum* (Uma et al., 2014).

Deshmukh et al. (2018) revealed chlorantraniliprole, flubendiamide and azadirachtin as harmless for parasitisation when irradiated host eggs were exposed to *T. japonicum*. Emamectin benzoate caused 32.50% parasitisation and 56.58% adult emergence of *T. chilonis* from parasitized host eggs (Singh et al., 2018). There is report about harmless effect of chlorantraniliprole on the parasitoid *T. achaeae* and killing of all female offspring by spinosad (Fontes et al., 2018). Flubendiamide, chlorantraniliprole, indoxacarb and novaluron had harmless effect on adult emergence of *T. chilonis* from treating parasitized host eggs (Duraimurugan and Lakshminarayana, 2018). Chlorantraniliprole and chlorantraniliprole showed harmless effect against pupa of *Trichogramma* species (Wahengbam et al., 2018). Neemazol @ 5 mL/ L caused 29.42% acute mortality for adult emergence of *T. japonicum* in field condition. Comparative high mortality (97.24%) by azadirachtin @ 6 mL/L obtained in current experiment might be due to higher dose in laboratory condition. Here in both cases, slightly harmful persistency for parasitization was seen (Sharma and Aggarwal, 2019). Azadirachtin, flubendiamide and chlorantraniliprole were found safe towards parasitisation of *T. japonicum* on previously treated host egg (Pawar et al., 2020). Harmless response for adult emergence of *T. japonicum* from treated pupa

was obtained in flubendiamide, chlorantraniliprole and emamectin benzoate (Patel, 2021). He also confirmed chlorfenapyr as moderately harmful for the same.

Azadirachtin and chlorfenapyr caused sublethal effects on *T. pretiosum*, reducing parasitism in F1 and F2 generations (Alano et al., 2021). Slightly harmful contact toxicity of flubendiamide and chlorantraniliprole was observed against adult *T. japonicum* (Mohapatra and Shinde, 2021). Transgenerational effects on parasitisation and adult emergence of *T. pretiosum* occurred in chlorfenapyr treated parasitized host eggs. Teflubenzuron was safe insecticide (Costa et al., 2022). Neem seed extract treated 1 day old parasitized host eggs resulted 19% adult emergence of *T. chilonis* (Asrar et al., 2022). They also noticed 96% mortality of adults *T. chilonis* after 24 hr of exposure through dipped surface residue bioassay. Nidagundi et al. (2022) revealed spinosad as moderately toxic and chlorantraniliprole, flubendiamide and emamectin benzoate as less toxic to adults *T. chilonis* following dry film residue method. The treatments flubendiamide, chlorantraniliprole and indoxacarb are relatively safe, whereas emamectin benzoate showed a high level of toxicity against *T. chilonis* (Manzoor et al., 2023). The present results on broflanilide reveal that it can be generally regarded as having comparatively overall low toxicity to *T. japonicum*.

The results of the present study suggest that new generation insecticides like chlorantraniliprole and flubendiamide are safe towards different biological parameters of *T. japonicum*. Broflanilide, cyantraniliprole, novaluron + indoxacarb and emamectin benzoate are harmless to slightly harmful. On the other hand, teflubenzuron, chlorfenapyr and azadirachtin behaved as slightly harmful and only spinosad depicted as slight to moderately harmful for the developmental stages of *T. japonicum*. Thus conclusions can be drawn for compatible use of safer chemicals with lepidopteran egg parasitoid *Trichogramma* spp.

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AUTHOR CONTRIBUTION STATEMENT

MIZ conducted the study analyzed the data and drafted the report. LCP designed the study, supervised the work and reviewed the manuscript.

COFLICT OF INTEREST

No conflict of interest.

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