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Assessment of safety of flubendiamide 20 WG against non-target organisms

B Lincy Kirubhadharsini, K Gunasekaran and SV Krishnamoorthy

Abstract
Laboratory studies were conducted to assess the safety of flubendiamide 20 WG on non-target organisms *viz.*, Egg parasitoid, *Trichogramma chilonis*, Predator, *Chrysoperla zastrowii silemi* and four different species of honey bees. Flubendiamide 20 WG at higher doses (70 and 60 g a.i. ha^{-1}) was better than stand alone products *viz.*, quinalphos 25 EC, novaluron 10 EC and emamectin benzoate 5 SG in terms of toxicity to non-target organisms. The hatchability and parasatisation of *T. chilonis* was not affected by flubendiamide 20 WG at 70 and 60 g a.i. ha^{-1} as they recorded more than 65 per cent of hatchability and parasitisation. Flubendiamide 20 WG 70 and 60 g a.i. ha^{-1} was found safer to predator *C. zastrowi sillemi* as the hatchability per cent was above 50 per cent and grub mortality was below 50 per cent. The contact toxicity of flubendiamide 20 WG to honey bees *viz., Apis cerana indica* F., *A. florea* F., *A. dorsata* F. and *Trigona iridipennis* at 70, 60, 50 and 40 g a.i. ha^{-1} recorded the lowest mortality (<40%) when compared to standard checks *viz.*, quinalphos 25 EC, novaluron 10 EC and emamectin benzoate 5 SG at 24 HAT.

Keywords: Flubendiamide, *Trichogramma* sp, *Chrysoperla* and Honey bees

Introduction
Recent days chemical pesticides are drastically used by farmers as first choice for the management of pest. They give immediate knockdown effect on insects and save the crops from damage which in-turn reduce the economic loss. Though these insecticides are effective against targeted pests, most of them are found to be toxic to natural enemies. Conservation of these beneficial organisms is very much important for any agro ecosystem. Hence, various groups of newer molecules like diamides, neonicotinoides, phenyl pyrazoles, pyridine and tetramic acid derivatives are developed to overcome the problem and are recommended to be used in the Integrated Pest Management (IPM) programme. These insecticides are reported to be relatively safe against natural enemies. IPM emphasize the combination of chemical and other biological control methods to be incorporated together to maintain the pest at their economic threshold level (Roubos *et al.*, 2014) [16]. Thus, using little amount of chemical pesticides which does not affect the beneficial organisms in the environment is very important (Gonzalez-Zamora *et al.*, 2013) [6]. Hence, the chemicals which are relatively safe to natural enemies has to be identified for the use in IPM programme (Carmo *et al.*, 2009) [2]. The greatest asset of these chemicals are, they show minimal effect on the beneficial insects.
Flubendiamide is one such novel insecticide developed by Nihon Nohyaku and Bayer Crop Science and it belongs to phthalic acid diamide group. IRAC (Insecticide Resistance Action Committee) grouped flubendiamide as the first member of the new group 28 (ryanodine receptor modulator) under mode of action classification scheme (Nauen, 2006) [13]. This insecticide is widely tested for the management of various lepidopterous pests which infests the major crops and found to be effective by interfering with the ryanodine receptors (Tohnishi *et al.*, 2005) [21]. The broad spectrum activity of flubendiamide and their effectiveness towards the major group of lepidopteran pests make it a promising insecticide among the farmers. So, it is necessary to understand the eco-toxicological profile of insecticide flubendiamide to be appropriately used in the IPM programme. Hence, the study was taken to assess the safety of flubendiamide 20 WG against the non-targeted organisms at laboratory level.

Materials and Methods
Laboratory experiments were conducted to investigate the safety of flubendiamide 20 WG against the non-target organisms *viz.*, Egg parasitoid, *T. chilonis*, Predator, *C. zastrowii silemi* and Honey bees like *A. cerana indica*, *A. florea*, *A. dorsata* and *T. irridipennis*.

The experiment was laid out in Completely Randomized Design (CRD) with eleven treatments replicated thrice.

Egg parasitoid, *T. chilonis*: Mass culturing of the host insect rice moth, *Corcyra cephalonica* was carried out in the laboratory following the procedure from Singh and Jalali (1994) [18]. Both the host insect (rice moth) and parasitoid *T. chilonis* was cultured using the eggs collected from the mass culturing of *C. cephalonica* described by Prabhu (1991) [14]. To evaluate the safety of flubendiamide 20 WG against *T. chilonis*, the bioassay method given by Jalali and Singh (1997) [9] was adopted. Different doses of flubendiamide 20 WG and other standard check insecticides were sprayed using hand atomizer on 1 cm^2 bits of parasitized egg cards. The untreated control egg cards were sprayed with distilled water. After treatment, the egg cards were shade dried and transferred to test tubes (15 x 2.5 cm) for adult emergence. After 24 and 48 hours of treatment, observations on the number of parasitoids emerged were made and per cent adult emergence was worked out using the formula,

$$\text{Adult emergence (\%)} = \frac{\text{Number of wasps emerged}}{\text{Total number of eggs in 1 cm}^2} \times 100$$

Fresh sterilized *C. cephalonica* eggs treated with respective insecticides were exposed to parasitoids at 6:1 ratio and the per cent parasitization was worked out.

$$\text{Parasitization (\%)} = \frac{\text{Number of parasitized eggs}}{\text{Total number of } \textit{C. cephalonica} \text{ eggs}} \times 100$$

Predators Green lacewing, *Chrysoperla zastrowi sillemi*: The mass rearing techniques of *C. zastrowi sillemi* using the eggs of *C. cephalonica* as feed described by Swamiappan (1996) [19] were adopted for the current study. The assays were carried out with the required number of eggs and grubs which were collected from the mass culturing. Eggs of *C. zastrowi sillemi* were laid on the brown paper with long stalks. These brown paper strips with eggs were treated with different concentrations of insecticides using a hand atomizer. The egg strips were shade dried for ten minutes and transferred to small plastic cups covered with muslin cloth. Treatments were replicated thrice with 30 eggs per treatment. Hatchability (%) were calculated by counting the total number of grubs emerged from each treatment using the following formula.

$$\text{Hatchability (\%)} = \frac{\text{No. of grubs hatched}}{\text{Total number of eggs}} \times 100$$

Grub mortality test: The bioassay method described by McCutchen and Plapp (1988) [12] was adopted with modifications according to Chelladurai (1999) [3]. Insecticidal solutions were prepared using acetone for the experiment. Required amount (0.5 ml) of insecticidal solutions were equally spread inside the glass scintillation vials of 20 ml capacity. The vials were rotated horizontally on a table using palm to make it dry. Second instar grubs of *C. zastrowi sillemi* obtained from the mass rearing were released into the treated vials @ 30 per treatment and exposed for one hour. The vials were covered with muslin cloth and secured using a rubber band. After one hour, the grubs were removed from the vials and transferred to fresh test tubes where the grubs were given with 1cc of *Corcyra* eggs as feed. After 24 hours of treatment the mortality of the grubs were recorded.

Honey bees: Four different species of honey bees were assessed for the effect of flubendiamide using contact toxicity method. Bioassays were conducted on honey bees in a plastic containers with adequate perforations on the upper lid which provided proper aeration. The insecticidal solutions were prepared using distilled water and they are sprayed using hand atomizer on a filter paper discs (9 cm diameter) held using forceps. Insecticide treated filter paper discs were shade dried and placed inside the plastic containers. Later the honey bees were transferred to the containers at the rate of 10 numbers each. They were exposed to insecticide for one hour and transferred back to polythene bags. Cotton swab dipped in 40 per cent sucrose solution were provided as feed. After 24 hours of treatment the mortality of bees were recorded and calculated using the formula,

$$\text{Mortality (\%)} = \frac{\text{No. of bees dead}}{\text{Total number of bees}} \times 100$$

Statistical analysis: The mortality per cent in laboratory studies were corrected using Abbot's
(Abbot, 1925) [1] formula,

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

The corrected per cent mortalities were subjected to Finney's method of probit analysis using Microsoft excel to calculate the median lethal dose/ concentration/ time and confirmed using EPA Probit Analysis Version 1.5. For safety studies, the corrected per cent mortalities were transformed to arcsine percentage and subjected to statistical analysis adopting completely randomized design. The mean values of treatments were then separated by Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984) [5].

Results and Discussion

Effect of flubendiamide 20 WG on *T. chilonis*: *Trichogramma* parasitized *Corcyra* eggs sprayed with different doses of flubendiamide showed a maximum adult emergence per cent upto 85.23. The emergence was 80.77 and 84.20 % for flubendiamide 20 WG at 60 and 50 g a.i. ha^{-1}, respectively. Flubendiamide 20 WG at 70 g a.i.ha^{-1} resulted in 77.91 % emergence and was on par with flubendiamide 480 SC at 48 g a.i. ha^{-1} (81.66 %). The standard check quinalphos 25 EC 250 g a.i.ha^{-1} recorded the least emergence per cent of 53.15 and the untreated check recorded the highest emergence of 96.58 % (Table 1). Flubendiamide 20 WG was also found to have less effect on the parasitisation of *T. chilonis.* The highest per cent parasitisation was recorded in untreated check (96.01%). Among the different doses, the parasitisation of 87.76 % was recorded in flubendiamide 20 WG at 40 g a.i. ha^{-1} and 79.74 % from flubendiamide 20 WG at 70 g
a.i. ha^{-1}. The lowest per cent parasitisation of 52.69 was recorded in quinalphos 25 EC at 250 g a.i. ha^{-1} treated eggs (Table 1). Flubendiamide was reported as safe to the natural enemies (Latif *et al.*, 2009) [11]. Different life stages of *T. chilonis* were tested for the harmful effects of certain chemical insecticides and the results revealed that

flubendiamide was the most selective and safest insecticides which does not affect their development, survival and egg laying capacity of the insect (Shahid *et al.*, 2011) [17].

Effect of flubendiamide 20 WG on *C. zastrowi sillemi*: The results on the ovicidal action of flubendiamide 20 WG on *C. zastrowi sillemi* indicated that flubendiamide 20 WG did not affect the hatchability and it was in the range of 83.33 to 70.00 % (Table 2). The standard check quinalphos recorded only 43.33 % which was lesser than the higher dose of flubendiamide 20 WG at 70 g a.i. ha^{-1}. The results on the influence of flubendiamide 20 WG to *C. zastrowi sillemi* grubs at 24 HAT in the present investigation revealed that all the doses caused less mortality (16.67 – 36.67 %) when compared to the standard check quinalphos 25 EC which recorded highest mortality of 73.33 % (Table 2) and this result is in conformity with the results of Reddy and Divakar (1998) [15] who indicated that quinalphos 25 EC was more toxic to

C. carnea. Similar results were reported by Tohnishi *et al.* (2005) and Dilbar *et al*. (2012) [4] that, the flubendiamide was inactive against *Chrysoperla* larvae. Hirooka *et al*. (2007) [8] also suggested that flubendiamide was safe to green lace wing and would be compatible with applications in IPM programs.

Effect of flubendiamide 20 WG on Honey bees: Worker honey bees of *A. cerana indica* were used for the experiment. At 24 hours after treatment (HAT) higher doses of flubendiamide 20 WG at 70 and 60 g a.i. ha^{-1} recorded 30.00 and 26.67% mortality followed by its lower doses at 50 (23.33%) and 40 g a.i. ha^{-1} (16.67%). The lower dose of flubendiamide 20 WG at 40 g a.i. ha^{-1} was on par with untreated check (6.67%) and standard check quinalphos 25 EC at 250 g a.i. ha^{-1} recorded the highest mortality of 73.33% (Table 3). Contact toxicity of flubendiamide 20 WG to little bees revealed that the higher dose of 70 g a.i. ha^{-1} recorded 40.00 % mortality at 24 HAT which was on par with flubendiamide 20 WG at 60 g a.i. ha^{-1} (33.33%). The standard checks *viz.*, quinalphos 25 EC at 250 g a.i. ha^{-1} was found to be highly toxic causing mortality of 63.33 % respectively (Table 3). For rock bees flubendiamide 20 WG at lower dosage of 40 and 50 g a.i.ha^{-1} were found less toxic and recorded the least mortality rate of 13.33 and 20.00 % respectively (Table 3). The standard check, quinalphos 25 EC at 250 g a.i. ha^{-1} was highly toxic and recorded mortality of 66.67 % and untreated check recorded the least mortality of 6.67 at 24 HAT. Influence of flubendiamide 20 WG to stingless bees revealed that it was safer. Flubendiamide 20 WG at 70 g a.i. ha^{-1} recorded 36.67 % mortality at 24 HAT. Flubendiamide at lower doses of 40, 50 and 60 recorded 13.79, 17.24 and 24.14 % mortality and the standard check Quinalphos 25 EC at 250 g a.i. ha^{-1} proved to be highly toxic recording a mortality rate of 73.33 % (Table 3). The findings are in tune with Thilagam (2006) [20] who reported that, among the bees tested, little bees were the most affected due to the application of flubendiamide than Indian and Italian bees. The mortality increased as the time of exposure increased. Gradish *et al.*, (2012) [7] revealed that flubendiamide was non-toxic to both larvae and adults of *Megachile rotundata* and is predicted to pose little hazard in the field. As the bee activity will be more during the morning hours, spraying can be recommended during evening hours. Hence, the natural enemies in general will have less direct contact with the pesticides which will reduce the toxicity level for the beneficial ones.

Conclusion
The present investigation concludes that flubendiamide 20 WG when sprayed at recommended dosage was found to be relatively safe compared to the other standard check insecticides tested against the non-target organisms *viz.*, *T. chilonis*, *C. zastrowi sillemi*, Indian bees, Little bees, Rock bees and Stingless bees. Thus, current study reveals that flubendiamide 20 WG can be incorporated as a part of IPM programme in major crops at field level without disturbing the natural enemies in the environment and thus helps in maintaining the ecological balance.

Table 1: Toxicity of flubendiamide 20 WG to parasitoid, *Trichogramma chilonis* Ishii (Mean of three observations)

S.No	Treatments	Dose (g a.i. ha^{-1})	Adult emergence (%)	Parasitisation (%)
1.	Flubendiamide 20 WG	40	85.23^{b}	87.76^{b}
2.	Flubendiamide 20 WG	50	84.20^{bc}	82.82^{c}
3.	Flubendiamide 20 WG	60	80.77^{cd}	82.49^{cd}
4.	Flubendiamide 20 WG	70	77.91^{d}	79.74^{de}
5.	Acephate 75 SP	562.5	73.78^{e}	76.87^{e}
6.	Acephate 75 SP	750	67.01^{f}	69.18^{g}
7.	Quinalphos 25 EC	250	53.15^{h}	52.69^{i}
8.	Emamectin benzoate 5 SG	11	70.57^{ef}	72.46^{f}
9.	Novaluron 10 EC	75	60.51^{g}	65.38^{h}
10.	Flubendiamide 480SC	48	81.66^{bcd}	80.46^{cd}
11.	Untreated check	-	96.58^{a}	96.01^{a}

In a column means followed by a common letter are not significantly different at P = 0.05 by LSD.
Figures in parentheses are arc sine transformed values.

Table 2: Toxicity of flubendiamide 20 WG to eggs and grubs of green lacewing bug, *Chrysoperla zastrowi sillemi* Esben-Petersen. (Mean of three observations)

S. No	Treatments	Dose (g a.i. ha^{-1})	Per cent egg hatchability	Grub mortality at 24 HAT	
				Per cent mortality	Corrected mortality (%)
1.	Flubendiamide 20 WG	40	83.33 (66.14)^{b}	16.67 (23.86)^{b}	10.71
2.	Flubendiamide 20 WG	50	76.67 (61.22)^{bc}	23.33 (28.78)^{c}	17.86
3.	Flubendiamide 20 WG	60	73.33 (59.00)^{bcd}	30.00 (33.21)^{d}	25.00
4.	Flubendiamide 20 WG	70	70.00 (57.00)^{cd}	36.67 (37.22)^{e}	32.14

5.	Acephate 75 SP	562.5	63.33 (52.78)^{de}	46.67 (43.08)^{g}	42.86
6.	Acephate 75 SP	750	56.67 (48.85)^{e}	56.67 (48.85)^{h}	53.57
7.	Quinalphos 25 EC	250	43.33 (41.07)^{f}	73.33 (59.00)^{i}	71.43
8.	Emamectin benzoate 5 SG	11	63.33 (52.77)^{de}	43.33 (41.15)^{fg}	39.29
9.	Novaluron 10 EC	75	53.33 (46.92)^{ef}	53.33 (46.92)^{h}	50.00
10.	Flubendiamide 480SC	48	73.33 (59.00)^{bcd}	40.00 (39.23)^{ef}	35.71
11.	Untreated check	-	96.67 (83.85)^{a}	6.67 (12.29)^{a}	0.00

HAT- Hours after treatment
In a column means followed by a common letter are not significantly different at P = 0.05 by LSD
Figures in parentheses are arcsine transformed value

Table 3: Toxicity of flubendiamide 20 WG to Honey bees

Treatment	Doses (g a.i/ha)	Mortality of honey bees at 24 HAT							
		Apis cerana indica		*Apis florea* F.		*Apis dorsata* F.		*Trigona iridipennis* (Smith)	
		Percent Mortality	Corrected mortality (%)	Per cent mortality	Corrected mortality (%)	Per cent mortality	Corrected mortality (%)	Per cent mortality	Corrected mortality (%)
Flubendiamide 20 WG	40	16.67 (23.86)^{ab}	10.71	20.00 (26.57)^{b}	14.29	13.33 (21.14)^{ab}	7.14	23.33 (28.78)^{b}	17.86
Flubendiamide 20 WG	50	23.33 (28.78)^{bc}	17.86	26.67 (31.00)^{bc}	21.43	20.00 (26.57)^{bc}	14.29	30.00 (33.21)^{bc}	25.00
Flubendiamide 20 WG	60	26.67 (30.79)^{bcd}	21.43	33.33 (35.22)^{cd}	28.57	26.67 (31.00)^{cd}	21.43	33.33 (35.22)^{bcd}	28.57
Flubendiamide 20 WG	70	30.00 (33.00)^{bcde}	25.00	40.00 (39.23)^{de}	35.71	33.33 (35.22)^{de}	28.57	36.67 (37.22)^{cde}	32.14
Acephate 75 SP	562.5	40.00 (39.15)^{def}	35.71	46.67 (43.08)^{ef}	42.86	40.00 (39.15)^{ef}	35.71	40.00 (39.15)^{cdef}	35.71
Acephate 75 SP	750	53.33 (46.92)^{f}	50.00	56.67 (48.85)^{gh}	53.57	53.33 (46.92)^{gh}	50.00	46.67 (43.08)^{ef}	42.86
Quinalphos 25 EC	250	73.33 (59.00)^{g}	71.43	63.33 (52.78)^{h}	60.71	66.67 (54.78)^{i}	64.29	73.33 (59.00)^{g}	71.43
Emamectin benzoate 5 SG	11	43.33 (41.15)^{ef}	39.29	50.00 (45.00)^{fg}	46.43	46.67 (43.07)^{fg}	42.86	43.33 (41.07)^{def}	39.29
Novaluron 10 EC	75	53.33 (46.92)^{f}	50.00	56.67 (48.85)^{gh}	53.57	56.67 (48.84)^{h}	53.57	50.00 (45.00)^{f}	46.43
Flubendiamide 480SC	48	36.67 (37.23)^{cde}	32.14	36.67 (37.23)^{d}	32.14	33.33 (35.21)^{de}	28.57	36.67 (37.23)^{cde}	32.14
Untreated check	-	6.67 (12.29)^{a}	0.00	6.67 (12.29)^{a}	0.00	6.67 (12.29)^{a}	0.00	6.67 (12.29)^{a}	0.00

In a column means followed by a common letter are not significantly different at P = 0.05 by LSD
Figures in parentheses are arcsine transformed value

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