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Research Article

Risk assessment of *Trichogramma chilonis* (Fab.) to new molecules evaluated against spotted bollworm, *Earias vittella* Ishii in cotton

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ABSTRACT: Studies were carried out in the laboratory to assess the acute toxicity of new molecules *viz.*, abamectin, emamectin benzoate, indoxacarb and spinosad to target pest, *Earias vittella* (Fab.) and selective toxicity to non-target insect, *Trichogramma chilonis* (Ishii) in terms of LD_{50}/LC_{50} and LD_{95}/LC_{95} and assessing risk hazards associated with the use of new molecules for integration in IPM. Acute toxicity of the four test molecules was found to be 0.00264, 0.00266, 0.09270 and 0.00188 µg $larva^{-1}$ and were less toxic to *T. chilonis* than the target pest when analyzed through various risk assessment methods and can be recommended for IPM. Among the four methods evaluated, hazard ratio is the best as it accounted for the field dose as criteria for determining the toxicity of the insecticides tested.

KEY WORDS: Abamectin; emamectin benzoate; indoxacarb; spinosad; *Trichogramma chilonis*; *Earias vittella*

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INTRODUCTION

In order to overcome the problems of resistance and ensuring safety to natural enemies, identification of new chemical molecules with better insecticidal properties, low mammalian toxicity, low dosage with selective action is a continuous process for integration in IPM. In search for newer insecticides, certain biologically active compounds have provided sufficient control of insect pests and one such group is the avermectins. This is the fermentation product of soil actinomycetes, *Streptomyces avermitilis* Burg. It is a broad-spectrum insecticide and highly toxic to many arthropods including selected lepidopteran pest species (Lasota and Dybas, 1991). Emamectin acts as a GABA agonist and is referred to as GABA gated chloride channel inhibitor. The benzoate salt of this compound *viz.,* emamectin benzoate has been reported effective against lepidopteran larvae (Dybas *et al.,* 1989; Stanley *et al*., 2006).

Spinosad a biologically active compound derived from an actinomycete, *Saccharopolyspora spinosa* Mertz. is effective against several lepidopteran pests. This compound has a unique mode of action and acts at the nicotinic acetyl choline receptor of the insect nervous system, with the GABA receptor sites being the secondary site of attack (Salgado, 1997; Watson, 2001). Indoxacarb is the first commercialized pyrazoline type sodium channel blocker with activity against wide range of lepidopteran, coleopteran and sucking insect pests (McCann *et al*., 2001). New insecticides, being endowed with different modes of action have lesser non-target effects and are positively safer to human beings. Since the advent of these compounds, they have been tried on various field and vegetable crops *viz.,* cotton, tomato, bhendi, cabbage and brinjal with prime focus on lepidopteran pests, and have proved to be very effective.

MATERIALS AND METHODS

Mass culturing of *Earias vittella* (Fab.)

Larvae of different instars of *E. vittella* were collected on cotton and okra from farmers' field in Masikandanpathy area of Coimbatore district, Tamilnadu state in India and were reared on okra fruits. Bioassay was done based on FAO recommended method of topical application of insecticide for the detection and measurement of resistance. Third instar larvae weighing between 0.06 and 0.08 g $larva^{-1}$ and length of 0.8 to 1.0 cm alone were used for bioassay. Dosing was done by placing one µl technical insecticide in acetone on the dorsum of each larvae using

one µl repeating dispenser (PB 600-01. Hamilton Co. Ltd) fitted with a 50 µl syringe and a rheodyne needle. The dilutions required for the insecticidal assays were prepared from technical grade insecticides of known purity diluted with analytical grade acetone and used for topical assays. Five doses/ concentrations with a minimum of 30 larvae per treatment were used for the experiment. Acetone treated larvae represented the check. After treatment, larvae were allowed individually in 12 well tissue culture trays containing okra fruits as food and they were incubated at a temperature of 25±2°C. Mortality was recorded after 24 hours. Larvae with uncoordinated movement when prodded with blunt needle were considered as dead. Necessary corrections were made for the natural mortality using Abbott's formula.

Safety test for *Trichogramma chilonis*

Trichogramma chilonis wasps were collected from the Biocontrol laboratory, TNAU, Coimbatore. An aliquot of 0.5ml concentration of the test insecticide was pipetted into glass scintillation vial of 20 ml capacity and rotated to have an uniform coating of the insecticide all over the inner surface (Lingren and Ridgeway, 1967). The vial was air dried to leave a thin dry film of the insecticide. Twenty adult wasps emerging from the parasitized egg cards were released into the vial. Mouth was covered with piece of muslin cloth fastened with rubber band and mortality was recorded after 4 hours. Endosulfan was used as standard check.

Preliminary range finding tests were conducted to attain series of doses which gave mortality range from 10 to 100 per cent. Bioassay was conducted based on the dosages obtained from the preliminary range finding test to construct log dose probit mortality line.

Statistical analysis

The data obtained in the study were subjected to Finneys method of probit analysis and confirmed in EPA probit analysis program used for calculating LC/EC values version 1.5.

Risk assessment factors – Selectivity ratio

Selectivity ratio was calculated as

$$\text{Selectivity ratio} = \frac{LD_{50}/LC_{50} \text{ of } X}{LD_{50} \text{ /or } LC_{50} \text{ of } Y}$$

where X = the beneficial species (µg/g)

Y = the pest species (µg/insect)

Probit substitution

This method was used to determine relative toxicity of beneficial species at particular level of pest mortality. This method involved substituting the log LD_{90} (µg/insect) and its 95 per cent fiducial limits for the pest species, *E. vittella,* into modified probit equations (Finney, 1971) for each insecticide and beneficial species. The equation used is as follows,

$$Y = 5 + m(x - [\log LC_{50} \text{ of beneficial species}])$$

Where, Y = probit value,

m = the slope of the probit line for the beneficial species,

x = log of the fiducial limits for the LD_{90} of the pest species (*E. vittella*).

Solving for Y gives a probit value which is then converted to percentage of mortality using a conversion table (Finney, 1971).

Hazard ratio

The hazard ratio was calculated as given below on the lines of Felton *et al*. (1986). Hazard ratio = recommended field rate (g a.i.ha^{-1}) LC_{50} (µg/g).

Insecticides with hazard ratio

Less than 50	harmless
50 to 2,500	slightly to moderately toxic
More than 2,500	dangerous

Sequential testing scheme

Hazards of insecticides to nontargets were assessed using Sequential testing scheme on the lines of Johansen and Mayer (1990).

LC_{50} (µg/g) = > 100 µg/g = nontoxic product

LC_{50} (µg/g) = 11-100 µg/g = slightly toxic product

LC_{50} (µg/g) = 2 to 10.9 µg/g = moderately toxic product

LC_{50} (µg/g) = < 2 µg/g = highly toxic product.

RESULTS AND DISCUSSION

The acute toxicity of new molecules *viz.,* abamectin, emamectin benzoate, indoxacarb and spinosad to *E. vittella* by topical method was 0.00264, 0.00266, 0.09270 and 0.00188 µg larva^{-1} whereas the LD_{95} being 0.01373, 0.02210, 1.14192 and 0.01416 µg larva^{-1} (Table 1). In the present study, spinosad was found to be toxic to

E. vittella than the other new molecules. Spinosad has both contact and stomach toxicity, appears to be unique with primary site of attack being the nicotinic acetyl choline receptor and a secondary site of attack being GABA receptors (Salgado, 1997; Watson, 2001). This mechanism of action suggests that resistance due to changes in the target sites of many other insecticides would not result in cross resistance to spinosad (Toshio and Scott, 2003). The order of toxicity of new molecules in terms of LD_{50} (µg $larva^{-1}$) to *E. vittella* was spinosad (0.00188) > abamectin (0.00264) > emamectin benzoate (0.00266) > indoxacarb (0.09270), respectively (Table 1). However, Gupta *et al*. (2005) reported that LC_{50} of abamectin, emamectin benzoate, spinosad and indoxacarb were 0.0001, 0.0004, 0.0004 and 0.0237 per cent for five day old larvae of *E. vittella.* Differences observed may be due to method of application *viz*., topical bioassay followed in present study. Also, it could be due to the differences in the age of the larvae considered for the study.

The acute toxicity of new molecules *viz.,* abamectin, emamectin benzoate, indoxacarb, spinosad and endosulfan to *T. chilonis* by dryfilm method was 0.02812, 0.05912, 9.9586, 1.4609 and 3.6476 ppm where as the LC_{95} being 0.44681, 3.05928, 124.5896, 8.4416 and 16.4967 ppm (Table 2). Indoxacarb was found to be less toxic than the standard check endosulfan. The order of insecticides safer to *T. chilonis* in terms of LC_{50} (ppm) was indoxacarb (9.9586) > endosulfan (3.6476) > spinosad (1.4609) > emamectin benzoate (0.05912) > abamectin (0.02812) (Table 2). Our findings concur with Ruberson (2003) who reported that indoxacarb and spinosad had no negative effects on the development of *Trichogramma pretiosum.* Indoxacarb appears to be entirely compatible with *T. pretiosum*. However, spray droplets caused harm to Trichogramma wasps and other parasitoids (Suh *et al.,* 2000; Tilman and Mullrooney, 2000). Nevertheless, once the spray droplets dry, they are generally safer for beneficial insects. Nian *et al*. (1997) found that *T. chilonis* was safer when treated with the most toxic B1 component of abamectin at 40 mg l^{-1} concentration. Emamectin was far safer to Trichogramma (Aston *et al.,* 2001; Shobanadevi, 2003; Udikeri *et al.,* 2004) and coccinellids (Udikeri *et al.,* 2004). But, the present study revealed that both emamectin benzoate and abamectin were toxic to *T. chilonis* when compared to other new molecules used in the study.

The selectivity ratio of less than one indicates the selectivity favouring pest and a value of more than one represents selectivity favouring non-target. The ratio was

Table 1. Acute toxicity of new molecules to *Earias vittella* by topical application

New molecules	Regression Equation	Chi square χ^2	LD_{50} µg/larvae	Fiducial limits		LD_{95} µg/ larvae	Fiducial limits	
				LL	UL		LL	UL
Abamectin	Y = –2.871 + 2.299x	3.941	0.00264	0.00207	0.00338	0.01373	0.00846	0.02227
Emamectin benzoate	Y = –1.132 + 1.789x	4.464	0.00266	0.00196	0.00361	0.02210	0.0113	0.0439
Indoxacarb	Y= –2.492 + 1.508x	1.383	0.09270	0.06203	0. 13853	1. 14192	0.53264	2.44813
Spinosad	Y = –1.018 + 1.838x	0.110	0.00188	0.00142	0.00249	0. 01416	0.00724	0.03005

Table 2. Acute toxicity of new molecules to *Trichogramma chilonis*

Insecticide	Regression Equation	Chi square χ^2	LC_{50} ppm	Fiducial limits		LD_{95} ppm	Fiducial limits	
				LL	UL		LL	UL
Abamectin	Y = 3.015 + 1.369x	0.876	0.02812	0.01572	0.05027	0.44681	0.13852	1.44116
Emamectin benzoate	Y = 3.299 + 0.959x	2.247	0.05912	0.03003	0.11411	3.05928	0.53213	17.58819
Indoxacarb	Y = -0.993 + 1.578x	1.578	9.9586	6.1722	16.0678	124.5896	49.00679	316.74045
Spinosad	Y = -1.831 + 2.158x	0.7019	1.4609	1.0549	2.0231	8.4416	3.7131	19.2029
Endosulfan	Y = -3.940 + 2.509x	1.558	3.6476	2.8336	4.6956	16.4967	8.5602	31.7913

more than one for *E. vittella vs T. chilonis* was based on LD_{50}/LC_{50} as 10.65 for abamectin, 22.23 for emamectin benzoate, 107.43 to indoxacarb and 777.07 for spinosad, all favouring non-target beneficials (Table 3). Selectivity ratio based on toxicity to *E. vittella* indicated that all the new molecules are highly toxic to *E. vittella* than the beneficials insects. Spinosad was more selective than

Table 3. Selectivity ratio of new molecules to *Trichogramma chilonis*

New Molecules	LC_{50} of Non-target insects/ LC_{50} (or) LD_{50} of Target pest	LD_{50} /LC_{50}	LD_{95} /LC_{95}
Abamectin	*T. chilonis* / *E. vittella*	10.65	32.542
Emamectin benzoate	*T. chilonis* / *E. vittella*	22.23	138.42
Indoxacarb	*T. chilonis* / *E. vittella*	107.43	109.11
Spinosad	*T. chilonis* / *E. vittella*	777.07	578.19

the other new molecules as revealed by increased selectivity ratio. Our studies revealed that a range of physiological selectivity exists among the new molecules *viz.,* abamectin, emamectin benzoate, indoxacarb and spinosad, with spinosad being more selective than others as indicated by higher selectivity ratio. The increasing order of selectivity ratio for *T. chilonis* was spinosad > indoxacarb > emamectin benzoate > abamectin. If selectivity ratio method is taken as criteria of evaluation it can be concluded that new molecules used in the study can be recommended for use in IPM programmes with spinosad as superior one.

Based on the probit substitution method, the dose of abamectin which caused 90 per cent mortality in *E. vittella* will cause 26.0 per cent mortality to *T. chilonis*. Emamectin is more or less similar to abamectin in terms of saftey to beneficials. For indoxacarb the predicted mortality of *T. chilonis* was 3.1 per cent at the dose which can cause 90 per cent mortality to *E. vittella. Trichogramma chilonis* were not affected by the LD_{90} dose of spinosad to *E. vittella* (Table 4). All the chemicals tested in probit substitution were found to be safer, with spinosad and indoxacarb being the superior one and can be included in IPM.

The hazard ratio of less than 50 is considered harmless and more than 2500 indicates that the chemical is dangerous, respectively to the nontargets. Indoxacarb and spinosad fall under the harmless category recording a hazard ratio of 7.531 and 41.070, respectively to *T. chilonis* whereas emamectin benzoate and abamectin were slightly

Table 4. Predicted mortality of *Trichogramma chilonis* at the dose resulting in 90 per cent mortality of *E. vittella* (Probit substitution method)

Insecticides	LC_{50} of *T. chilonis* (ppm)	LD_{90} *E. vittella* (ug/larvae)	Probit value (Y)*	Predicted mortality of *T. chilonis*
Abamectin	0.02812	0.00954 (0.0064 – 0.0192)	4.35 (4.12–4.58)	26.0 (19.1–34.0)
Emamectin benzoate	0.05912	0.01386 (0.0080 – 0.0239)	4.38 (4.16–4.63)	27.0 (20.1–35.9)
Indoxacarb	9.9586	0.6558 (0.36162 – 1.18933)	3.13 (2.72–3.54)	3.1 (1.2 - 7.3)
Spinosad	1.4609	0.00936 (0.0052 – 0.0167)	0.27 (–0.27 –0.82)	0.0

* $Y = 5 + m(x - [\log LD_{50}$ of beneficial species$])$

to moderately toxic and the values are 186.062 and 515.647, respectively (Table 5).

Based on the sequential testing scheme for *T. chilonis* indoxacarb and spinosad were considered to be moderately toxic products with a LC_{50} values of 9.9586 and 1.4609 µg/ g, whereas abamectin and emamectin benzoate were toxic with LC_{50} values of less than 0.1 µg/ g (Table 6).

All the chemicals tested were less toxic to natural enemies than the target pest when analyzed through various risk assessment methods *viz.*, selectivity ratio, probit substitution, hazard ratio and sequential testing scheme, so it can be recommended for Integrated Pest

Table 5. Hazard ratio of new molecules for *Trichogramma chilonis*

New Molecules	Recommended field rate g a.i. ha^{-1}	Hazard ratio* for *T. chilonis*	Category
Abamectin	14.5	515.647	Slightly to moderately toxic
Emamectin benzoate	11	186.062	Slightly to moderately toxic
Indoxacarb	75	7.531	Harmless
Spinosad	60	41.070	Harmless

* Hazard ratio is recommended field rate (g [a.i.] / LD_{50} or LC_{50} (µg/g)
A ratio greater than 2,500 indicates hazardous product

Table 6. Sequential testing scheme for new molecules against *Trichogramma chilonis*

Insecticides	*T. chilonis*	
	LC_{50} (µg /g)	Category
Abamectin	0.02812	Toxic
Emamectin benzoate	0.05912	Toxic
Indoxacarb	9.9586	Moderately toxic
Spinosad	1.4609	Moderately toxic

Management Programmes. Sequential testing scheme alone revealed that abamectin and emamectin benzoate were toxic to non target insects (Table 7). However, sequential testing scheme does not incorporate field dose of pesticide application and rough estimate of field exposure. Neem products can be added to these insecticides, to avoid contact with the insecticides immediately after application; the chemicals (emamectin benzoate) with halflife dislodgeable residues of 10 hours (Amechi *et al*., 1997) will dissipate to a low level of toxicity to non targets. The natural enemy release, if any, can be done after reduced toxicity, which will result in low risk to non targets.

The new molecules used in this study except indoxacarb, are synthetic analogues of natural pesticide from microorganisms. They have a long residual toxicity even at lower doses and superior performance against insect pests with unique modes of action. Hence, may have higher environmental fitness with rapid degradation and can be used to control the pests that exhibit resistance to pyrethroids and organo phosphates. Thus, they offer a good scope for utilization in IPM programmes and in situations that warrant IRM.

Table 7. Comparison of risk assessment methods for recommendation in IPM in cotton ecosystem (based on toxicity to *Earias vittella*)

Risk assessment methods	Recommendation in IPM programme			
	Abamectin	Emamectin benzoate	Indoxacarb	Spinosad
Selectivity ratio	Yes	Yes	Yes	Yes
Probit substitution	Yes	Yes	Yes	Yes
Hazard ratio	Yes	Yes	Yes	Yes
Sequential testing scheme	No	No	Yes	Yes

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