Eco-toxicological effect of insecticides on the larval parasitoid, *Bracon brevicornis* Wesmael (Hymenoptera: Braconidae)

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Laboratory tests using the dry film method were conducted to assess the toxicity of different commonly used insecticides and insect growth regulators to *Bracon brevicornis.* The carbamate insecticide, carbosulfan, was highly toxic to *B. brevicornis* with a LC_{50} value of 0.0001 mg a.i./l, whereas pymetrozine and buprofezin had LC_{50} values of 0.1056 and 0.0374 mg a.i./l, respectively. Among the three neonicotinoids, acetamiprid showed the highest toxicity to *B. brevicornis* with a LC_{50} of 0.0041 mg a.i./l followed by thiamethoxam and imidacloprid. The order of toxicity based on the LC_{50} values was: carbosulfan > lambda-cyhalothrin > bifenthrin > indoxacarb > acetamiprid > thiamethoxam > imidacloprid > buprofezin > pymetrozine. Pymetrozine showed slight to moderate toxicity to *B. brevicornis,* with a risk quotient (RQ) of 1420, while the RQ value for all other insecticides were dangerously toxic to *B. brevicornis*. The mean activity of cytochrome P450 in *B. brevicornis* was 4.2817 nM/mg protein with a frequency distribution ranged from 3.001 to 5.446 nM/mg protein. The mean activity of acetyl choline esterase in *B. brevicornis* was 1.7056 nM/Min with a frequency destitution ranged from 0.7572 to 3.1951 nM/Min. The mean activity of carboxylesterase was 37.5 mOD/Bracon/min with a frequency destitution ranged from 27.8 to 47.1 mOD/Bracon/min. Use of selective insecticides to conserve *B. brevicornis* may improve the compatibility of biological control with the IPM programme.

Key words: *Bracon brevicornis*, detoxification, cytochrome P450, acetylcholine esterase, carboxylesterase.

INTRODUCTION

Braconids are an important group of larval parasitoids of several economically important lepidopteran pests in the field as well as in storage. They are the most diverse and abundant group of parasitoids (Lasalle & Gauld 1993) attacking and feeding on a very narrow range of hosts due to specialised biological and behavioural adaptations (Wharton 1993; Shaw 1994). Among the different braconid species, *Bracon brevicornis* Wesmael (Hymenoptera: Braconidae) is one of the most important larval parasitoids of *Helicoverpa armigera, Earias vitella* and *E. insulana* (Thanavendan & Jeyarani 2010). Under field condition, farmers rely on a wide range of insecticides for controlling different types of pests. Despite the advantages of being highly effective, rapid in action and adaptable to most situations, the indiscriminate use of insecticides has led to the eradication of natural enemies and has also indirectly led to pesticide-induced resistance, resurgence in target pests, and unwanted effects to human beings, non-target organisms in general and the environment (Desneux *et al*. 2007).

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In recent years, there has been a growing need for sustainable integrated pest management (IPM) programmes with a greater emphasis on biocontrol-based crop protection tactics to reduce reliance on insecticides. In order to achieve this, it is necessary to know the effect of insecticides on biocontrol agents (parasitoids and predators) before implementing any biocontrol programme in integration with the IPM programme. The use of selective insecticides with less toxicity to natural enemies than to target pests is helpful in integration of biological control and chemical applications (Hull & Beers 1985). The toxicity of various insecticides such as organophosphates, carbamates and synthetic pyrethroids to braconid parasitoids has been reported by several authors (O'Brien *et al*. 1985; Umoru *et al*. 1996; Khan *et al*. 2005; Mahdavi 2013). However, the effect of neonicotinoids, and other groups of insecticides on *B. brevicornis* and the level of detoxifying enzymes in this insect are less known. This study was aimed at determining the effect of different insecticides on adult *B. brevicornis* and the level of detoxifying enzymes in a laboratory-reared population of the test insect.

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MATERIAL AND METHODS

Rearing of parasitoid

The starting culture of the larval parasitoid *B. brevicornis* was obtained from the Biological Control Laboratory, Division of Entomology, Indian Agricultural Research Institute, New Delhi, India. The culture was maintained on larvae of *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) following the procedure described in Gautam (2008).

Insecticides

The insecticides used in the present investigation were obtained from their respective manufacturers (Table 1). In most cases, these insecticides are being used for the control of insect pests in different ecosystems. Commercial formulations of insecticides were diluted with analytical grade acetone to obtain the desired concentrations.

Insecticide bioassay

The dry-film residue method was followed to assess the toxicity of different insecticides to *B. brevicornis* as per the standard procedure given by Hassan *et al*. (1998) and Desneux *et al*. (2004) with slight modifications. Preliminary range-finding tests were conducted to fix the test concentrations that caused 10 to 90 % mortality to the parasitoid. Different concentrations of each insecticide were prepared using acetone and water in the ratio 80:20 and used for bioassays. Glass scintillation vials of 15 ml capacity with an internal surface area of 50 cm^2 were used. The vials were soaked in soapy water overnight, whereafter they were cleaned thoroughly and rinsed with acetone and air-dried for at least 4 h before use. Six different concentrations of each insecticide were selected for this study. The inner surfaces of the vials were treated with 0.5 ml of insecticides, which was enough to cover the internal surface area of the glass vial and they were rotated manually until no more droplets were seen on the glass vial and were left for 1 h at room temperature, *i.e*. 30 ± 2 °C to ensure complete evaporation of the excess solution before introducing parasitoids. For the untreated control, 0.5 ml of acetone:water in the ratio 80:20 was used.

Twenty newly emerged adult wasps were released into the vial and they were provided with honey streaks as a source of food. Thereafter the vials were covered with insecticide-treated muslin cloth and was secured with a rubber band. The entire experiment was carried out under controlled conditions, *i.e*. 27 ± 2 °C and 65 ± 5 % RH. After 4 h of exposure, the wasps were placed in a clean test tube and mortality was recorded at 24 and 48 h after treatment (HAT). Each treatment was replicated five times with a total of 100 wasps per treatment. Necessary corrections were made with respect to natural mortality in the control using Abbott's formula (Abbott 1925) and then the data were subjected to probit analysis as per Finney (1971) and the log concentration probit mortality curve was obtained. Probit analysis was carried out using SAS version 9.2 (SAS 2011).

Risk assessment method

The risk quotient method was used to assess the safety of pesticides to predators and parasitoids.

Table 1. Insecticides and their respective manufacturers.

Group	Insecticide	Trade name of the formulation	Manufacturer
Neonicotinoids	Imidacloprid	Confidor 17.80 %, SL	Bayer Crop Science Limited, Mumbai
	Acetamiprid	Baadshah 20 % SP	Hindustan Pulverising Mills, Jammu
	Thiamethoxam	Actara 25 % WG	Syngenta India Limited, Mumbai
Synthetic pyrethroids	Bifenthrin	Talstar 10 % EC	FMC India Private Limited, Kanchipuram
	Lambda-cyhalothrin	Karate 5 % EC	Syngenta India Limited, Mumbai
Carbamate	Carbosulfan	Marshal 20 % EC	FMC India Private Limited, Kanchipuram
Oxadiazine	Indoxacarb	Avaunt 15.8 % EC	Dupont India Private Limited, Gurgaon
Pyrindinazomethane	Pymetrozine	Endeavor 50 % WG	Syngenta India Limited, Mumbai
Chitin synthesis inhibitor (CSI)	Buprofezin	Applaud 25 % SC	Rallis India Limited, Mumbai

The risk quotients for the insecticides were calculated from the LC_{50} values based on the equation given by Preetha *et al.* (2009).

$$\text{Risk quotient} = \frac{\text{Recommended field rate (g a.i./ha)}}{\text{LC}_{50}\text{ of beneficiial insect (mg a.i./ha)}}$$

Risk quotient	*Category*
<50	Harmless
50–2500	Slightly to moderately toxic
>2500	Dangerous

Carboxylesterase (COE) activity of laboratory-reared B. brevicornis

Total COE activity was estimated by kinetic microplate assay using 1-naphthylacetate as substrate (Stumpf & Nauen 2002) at 450 nm. The adult parasitoids survived after the insecticides were collected and they were used for halogenate preparation. The homogenates of individual insects were prepared using 100 μl ice-cold sodium phosphate buffer (0.1 M, pH 7.5), containing 0.1 % [w/v] Triton™ X-100). The homogenate was centrifuged at 10 000 *g* and the supernatant was used as the enzyme source. COE activity was measured by adding 25 μl supernatant in 200 μl substrate solution containing Fast Blue RR salt in sodium phosphate buffer (0.2 M, pH 6.0) and 1 mM 1-naphthylacetate. Wells with only buffer served as control for the non-enzymatic reaction. The COE activity was then measured continuously at 27 °C in a SPECTRAmax® *plus*^{384} microplate spectrophotometer (Molecular Devices Corporation, California, U.S.A.) for 10 min. Using SOFTmax software, linear regression data were determined. Enzyme activity was measured from the reaction rate as a change in absorbance at 450 nm over time and the initial velocity of the enzymatic reaction determined from the linear portion of the curve and expressed in mOD/min.

Cytochrome P450 activity of B. brevicornis

The cytochrome P450 activity was estimated and expressed in terms of general oxidase, which is an indirect measure of cytochrome P450 by heme peroxidation using 3,3′,5,5′-tetramethylbenzidine dihydronchloride as a substrate (Brogdon *et al.* 1997). For oxidase assays, individual insects drawn from the colonies raised in the laboratory were homogenised in 50 μl ice-cold potassium phosphate buffer (0.1 M, pH 7.2), and then spun in a centrifuge at 10 000 *g* for 10 min. The resultant supernatant was used as the enzyme source. The reaction mixture consisted of 80 μl of 0.625 M potassium phosphate buffer (pH 7.2), 20 μl of enzyme source, 200 μl TMBZ (3,3′,5,5′-tetramethylbenzidine) solution, 25 μl of hydrogen peroxide (3.0 %) making up a final volume of 325 μl. The substrate solution was made by dissolving 2 mg of TMBZ in 2.5 ml of methanol and 7.5 ml of 0.25 M sodium acetate buffer (pH 5.0). Absorbance was read at 620 nm against blanks (wells containing all reaction components except enzyme source) in a SPECTRAmax® *plus*^{384} microplate spectrophotometer for 5 min, and by utilising SOFTmax software, linear regressions were obtained.

Acetylcholine esterase (AChE) activity of B. brevicornis

Activity of AChE in individual *B. brevicornis* adults was measured according to the method given by Ellman *et al.* (1961), using acetylthiocholine (ATChI) as substrate analogue, and was determined colorimetrically by the absorbance of 2-nitro-5-thiobenzoate at 405 nm, after the reaction of dithionitrobenzoate (DTNB) with the liberated thiocholine. The homogenates of individual females were prepared using 100 μl ice-cold sodium phosphate buffers (0.1 M, pH 7.5), containing 0.1 % (w/v) Triton™ X-100. The homogenate was centrifuged at 10 000 *g* and the resulting supernatant used as enzyme source. The amount of enzyme source used was 25 μl of supernatant to 75 μl sodium phosphate buffer (0.1 M, pH 7.5). The buffered substrate solutions of ATChI and DTNB were added (100 μl each) to the wells of a microtitre plate containing 25 ml enzyme solution in 75 ml buffer, giving a final concentration of 0.5 mM each in a final volume of 300 ml. AChE activity was measured continuously at 25 °C in a SPECTRAmax® *plus*^{384} absorbance microplate reader (Molecular Devices) for 10 min, and using SOFTmax software linear regression data were determined. Changes in absorbance per minute were converted into nmol DTNB conjugated/min using the molar extinction coefficient (εmolar) of the resulting 5-thio-2-nitrobenzoate: $\varepsilon_{412\,nm} = 1.36 \times 10^4$/mM/cm. All measurements were done in five replicates. The wells containing all reactions components, except enzyme source were used as blank.

Protein estimation

The standard protocol given by Bradford (1976) was followed for the estimation of total protein content in *B. brevicornis*.

RESULTS

Contact toxicity of insecticides to *B. brevicornis*

Differential variation in toxicity was found within the group and between the group of insecticides. Among the various insecticides used in the study, the contact toxicity of carbosulfan (carbamate) was the highest with a LC_{50} value of 0.0001 mg a.i./l, whereas the new generation insecticides such as pymetrozine and buprofezin showed the lowest toxicity with LC_{50} values of 0.1056 and 0.0374 mg a.i./l, respectively. Overall, carbamates, synthetic pyrethroids and neonicotinoids exhibited the highest intrinsic acute toxicities, whereas pymetrozine and buprofezin showed the lowest.

The LC_{50} values of the synthetic pyrethroids lambdacyhalothrin and bifenthrin to *B. brevicornis* was 0.0002 and 0.0005 mg a.i./l, respectively. Among the three neonicotinoids tested, acetamiprid showed the highest toxicity to *B. brevicornis* with a LC_{50} of 0.0041 mg a.i./l followed by thiamethoxam and imidacloprid. Based on the LC_{50} values, acetamiprid was twice more toxic than imidacloprid and thiamethoxam. The LC_{50} of indoxacarb to *B. brevicornis* was 0.0006 mg a.i./l and found to be high toxic as compared to pymetrozine. The order of toxicity based on LC_{50} values were: carbosulfan > lambdacyhalothrin > bifenthrin > indoxacarb > acetamiprid > thiamethoxam > imidacloprid > buprofezin > pymetrozine (Table 2).

Risk assessment

All the tested insecticides were classified into three different groups based on their risk quotient (RQ) value (Table 3). Pymetrozine was found to be slightly to moderately toxic to *B. brevicornis,* with a risk quotient of 1420, whereas the RQ value for all other insecticides on *B. brevicornis* were dangerously toxic (>2631).

Detoxifying enzyme level in adult *B. brevicornis*

The estimated activity of cytochrome P450,

Table 2. Effect of different insecticides on *Bracon brevicornis* adults.

Insecticide	Heterogeneity (d.f. = 4)	LC_{50} (mg a.i./l)	Regression equation	Fiducial limits
Imidacloprid	9.5970	0.0095	$Y = 6.8651 + 0.9214x$	0.0040–0.0178
Acetamiprid	5.4896	0.0041	$Y = 7.0299 + 0.8521x$	0.0030–0.0056
Thiamethoxam	3.7438	0.0092	$Y = 6.5239 + 0.8881x$	0.0144–0.0257
Bifenthrin	4.1651	0.0005	$Y = 7.8816 + 0.8697x$	0.0003–0.0007
Carbosulfan	8.0149	0.0001	$Y = 10.201 + 1.3355x$	0.0001–0.0002
Lambdacyhalothrin	3.9954	0.0002	$Y = 8.1815 + 0.84199x$	0.0001–0.0002
Indoxycarb	2.6770	0.0006	$Y = 6.8409 + 0.5736x$	0.0004–0.0010
Pymetrozine	1.8757	0.1056	$Y = 5.5254 + 0.53823x$	0.0610–0.1828
Buprofezin	8.3192	0.0374	$Y = 6.3846 + 0.97031x$	0.0284–0.0494

Table 3. Effect of different insecticides on *Bracon brevicornis* adults based on RQ values.

Insecticide	Recommended dose (g a.i./ha)	LC_{50} (mg a.i./l)	Risk quotient	Category
Imidacloprid	25	0.0095	2631	Dangerous
Acetamiprid	80	0.0041	19512	Dangerous
Thiamethoxam	25	0.0092	2717	Dangerous
Bifenthrin	50	0.0005	100000	Dangerous
Carbosulfan	250	0.0001	2500000	Dangerous
Lambdacyhalothrin	30	0.0002	150000	Dangerous
Indoxycarb	75	0.0006	125000	Dangerous
Pymetrozine	150	0.1056	1420	Slightly to moderately toxic
Buprofezin	200	0.0374	5347	Dangerous

Table 4. Enzyme activities of *Bracon brevicornis*.

Mean activity of COE (mOD/min)	± S.E.	Mean activity of AChE (nM//min)	± S.E.	Mean activity of cytochrome P450 (nM/mg protein)	± S.E.
37.5(27.8–47.1)*	1.1	15.5 (12.5–18.5)§	0.3	22.0 (16.0–28.0)#	0.8

* n = 17; § n = 20; # n = 9. CI = confidence interval. S.E. = standard error.

AChE and CAE are shown in Table 4 and Figs 1–3. The level of cytochrome P450, one of the major detoxifying enzymes, was estimated in the laboratory at 4.2817 nM. The frequency distribution of cytochrome P450 (general oxidase) activity in *B. brevicornis* ranged from 3.001 to 5.446 nM/mg of protein. The frequency distribution of AchE activity in *B. brevicornis* towards 5,5′-dithio-bis2-nitrobenzoic acid was 1.7056 ± 0.1584 nM/min/mg protein. The mean activity of acetyl choline esterase in *B. brevicornis* was 1.7056 nM/min with a frequency distribution ranged from 0.7572 to 3.1951 nM/min. The mean activity of COE was 37.5 mOD/Bracon/min with a frequency distribution ranging from 27.8 to 47.1 mOD/Bracon/min.

DISCUSSION

Use of selective insecticides to conserve *B. brevicornis* may improve the compatibility of biological control with the IPM programme. Various methods have been used to study the effect of pesticides on natural enemies (Desneux *et al*. 2006). The parasitoids and predators are exposed to pesticides by any of the three methods such as (i) direct contact (ii) indirect contact with residues on plant surfaces and (iii) by ingestion of contaminated hosts. The safety of a particular pesticide may be assessed by exposing the natural enemies to a range of concentrations (Preetha *et al*. 2010). The toxicity of the pesticides on natural enemies was determined by knowing the effect on survival rate of parasitoids at a given time.

In the present study, carbosulfan was found to be more toxic to *B. brevicornis* as documented by Baker *et al*. 1995. Similarly, the other insecticides such as lambdacyhalothrin, bifenthrin and indoxacarb were also found to be more toxic to the *Bracon* sp. (Tillman & Mulrooney 2000). The ill effects of carbamate and synthetic pyrethroids to hymenopteran parasitoids were well documented by several authors (Rafiee *et al*. 2008; Mahdavi

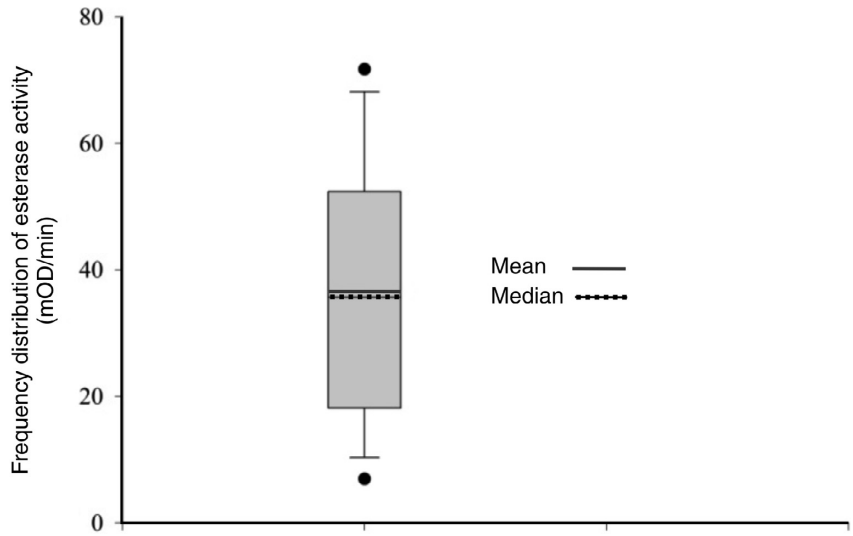

Fig. 1. Frequency distribution of non specifc COE activity in *Bracon* towards α-naphthyl acetate. Mean = 36.514 ± 7.42.

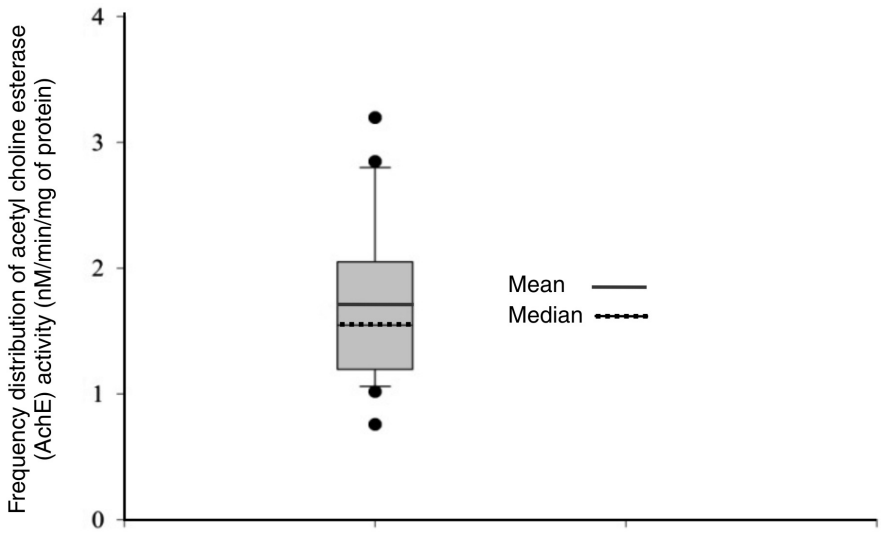

Fig. 2. Frequency distribution of acetyl choline esterase (AChE) activity *Bracon* towards 5,5'-dithio-bis (2 nitrobenzoic acid). Mean ± S.E. = 1.7056 ± 0.1584.

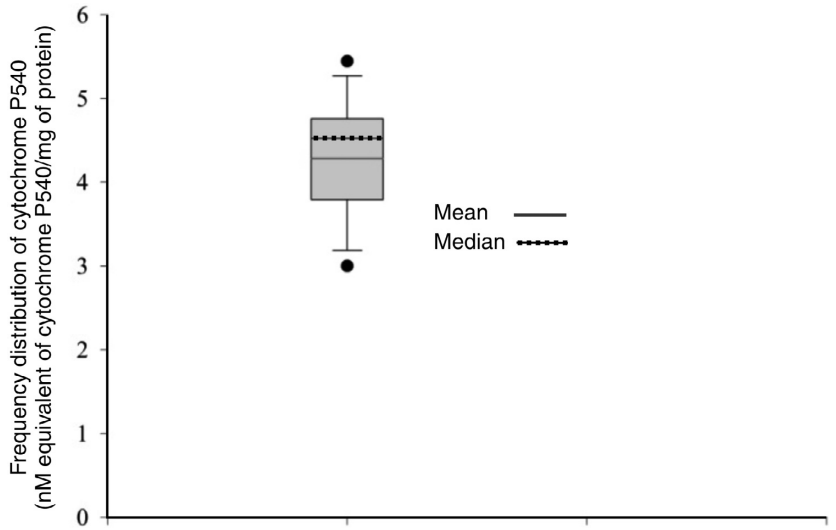

Fig. 3. Frequency distribution cytochrome P450 (general oxidase) activity in *Bracon* towards 3,3′,5,5′-tetramethylbenzodine (TMBZ). Mean ± S.E. = 4.2817 ± 0.2568.

2013). Imidacloprid showed less toxicity compared to other neonicotinoids. Results from this study indicate that acetamiprid was more toxic to *B. brevicornis* than the other two neonicotinoids as evidenced against *Bemisia tabaci* by Horowitz *et al*. (1998). Therefore, their use in IPM programmes needs to be carefully evaluated. Indoxacarb is active against lepidopteran larvae but is more toxic to *B. brevicornis*. Acute toxicity of indoxacarb to the adults of different *Cotesia* sp. revealed the high toxic nature of indoxacarb to the parasitoids and it caused 100 % mortality at 48 h after treatment (Haseeb *et al*. 2001; Halappa *et al.* 2012). Buprofezin, an insect growth regulator, acts as chitin synthesis inhibitor which was found to be less toxic compared to other synthetic insecticides. Pymetrozine did not affect the rate of parasitism and behaviour of *Anagrus nilaparvatae*, a parasitoid of *Nilaparvata lugens* and did not differ significantly compared to the untreated control (Liu *et al*. 2012).

The biochemical basis of insecticide resistance is one of the major mechanisms in insects, which is essentially required to overcome the toxicity of the insecticides. This is possible because enzymatic systems such as cytochrome P450-dependent microsomal monooxygenases (P450s), esterases and glutathione-S-transferases (GSTs) may use synthetic compounds from different chemical groups as substrates. Enhanced carboxyl esterase activity was observed in the resistant populations of *Cotesia vestalis*. Variations in populations and degree of resistance accounted for the detoxifying enzyme activity (Srinivasa Murthy 2014). The efficacy of the insecticides against insect pests with relative safety to the natural enemy is considered to be of greatest importance. Substitution of the currently used conventional insecticides with newer insecticides should go a long way towards minimising disruption of beneficial insects and in improving the effectiveness of parasitoids.

CONCLUSION

Use of selective insecticides to conserve *B. brevicornis* may improve the compatibility of biological control in IPM programmes. The insecticides belonging to carbamate (carbosulfan) and synthetic pyrethroid and neonicotinoids had highest intrinsic acute toxicities to *Bracon* spp., whereas pymetrozine and buprofezin were found to be the safest. The present study can be validated further under field conditions for developing area-wide pest management strategies using biological control agents.

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REFERENCES

ABBOTT, M.S. 1925. A method of computing effectiveness of an insecticide. *Journal of Economic Entomology* **18**: 265–267.

BAKER, J.E., WEAVER, D.K., THRONE, J.E. & ZETTLER, J.L. 1995. Resistance to protectant insecticides in two field strains of the stored-products insect parasitoid, *Bracon hebetor* (Hymenoptera: Braconidae). *Journal of Economic Entomology* **88**: 512–519.

BRADFORD, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**: 248–254.

BROGDON, W.G., McALLISTER, J.C. & VULULE, J. 1997. Heme peroxidase activity measured in single mosquitoes identifies individuals expressing an elevated oxidase for insecticide resistance. *Journal of the American Mosquito Control Association* **13**: 233–237.

DESNEUX, N., DECOURTYE, A. & DELPUECH, J.M. 2007. The sublethal effects of pesticides on beneficial arthropods. *Annual Review of Entomology* **52**: 81–106.

DESNEUX, N., DENOYELLE, R. & KAISER, L. 2006. A multistep bioassay to assess the effect of deltamethrin on the parasitic wasp *Aphidius ervi*. *Chemosphere* **65**: 1697–1706.

DESNEUX, N., RAFALIMANANA, H. & KAISER, L. 2004. Dose-response relationship in lethal and behavioural effects of different insecticides on the parasitic wasp *Aphidius ervi*. *Chemosphere* **54**: 619–627.

ELLMAN, G.L., LOURTNEY, D.K., ANDRES, V. & GMELIN, G. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology* **7**: 88–95.

FINNEY, D.J. 1971. *Probit Analysis*. 3rd edition. Cambridge University Press, Cambridge, U.K.

GAUTAM, R.D. 2008. *Biological Pest Suppression*. Westville Publishing House, New Delhi, India.

HALAPPA, B., AWAKNAVAR, J.S. & ARCHANA, D. 2012. Toxicity of insecticides to larval endoparasitoid, *Cotesia plutellae* (Kurdj.) of diamondback moth, *Plutella xylostella* (L.) under laboratory condition. *Pesticide Research Journal* **24**: 231–234.

HASEEB, M.Y., KOBORI, H., AMANO & NEMOTO, H. 2001. Population dynamics of *Plutella xylostella* (Lepidoptera: Plutellidae) and its parasitoid *Cotesia plutellae* (Hymenoptera: Braconidae) on two varieties of cabbage in an urban environment. *Applied Entomology and Zoology* **36**: 353–360.

HASSAN, S.A., HAFES, B., DEGRANDE, P.E., & HERAI, K. 1998. The side effects of pesticides on the egg parasitoid, *Trichogramma caecoeciae* Marchal (Hym. Trichogrammatidae), acute dose response and persistence tests. *Journal of Applied Entomology* **122**: 569–573.

HOROWITZ, A.R., MENDELSON, Z., WEINTRAUB, P.G & ISHAAYA, I. 1998. Comparative toxicity of foliar and systemic applications of acetamiprid and imidacloprid against the cotton whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Bulletin of Entomological Research* **88**: 437–442.

HULL, L.A. & BEERS, E.H. 1985. Ecological sensitivity modifying chemical control practices to preserve natural enemies. In: Hay, M.A. & Herzog, D.L. (Eds) *Biological Control in Agricultural IPM Systems*. 103–121. Academic Press, New York and Orlando, FL, U.S.A.

KHAN, R.R., ASHFAQ, M. & RANA, S.A. 2005. Some studies on the toxicity of conventional and new chemistry insecticides against *Bracon hebetor* (Say) (Hymenoptera: Braconidae) under laboratory conditions. *Pakistan Entomologist* **27**: 19–21.

LASALLE, J. & GAULD, I.D. 1993. *Hymenoptera and Biodiversity*. CAB, Wallingford, Oxon, U.K.

LIU, F., ZHANG, X., GUI, Q.Q. & XU, Q.J. 2012. Sublethal

effects of four insecticides on *Anagrus nilaparvatae* (Hymenoptera: Mymaridae), an important egg parasitoid of the rice planthopper *Nilaparvata lugens* (Homoptera: Delphacidae) *Crop Protection* **37**: 13–19.

MAHDAVI, V. 2013. Residual toxicity of some pesticides on the larval ectoparasitoid, *Habrobracon hebetor* Say (Hymenoptera: Braconidae). *Journal of Plant Protection Research* **53**(1): 27–31.

O'BRIEN, P.J., ELZEN, G.W. & VINSON, S.B. 1985. Toxicity of azinophos methyl and chlordimeform to parasitoid *Bracon mellitor* (Hymenoptera: Braconidae): Lethal and sub lethal effects. *Environmental Entomology* **14**: 891–894.

PENILLA, R.P., RODRÍGUEZ, A.D., HEMINGWAY, J., TERJO, A., LÓPEZ, A.D. & RODRÍGUEZ, M.H. 2007. Cytochrome P450-based resistance mechanism and pyrethroid resistance in the field *Anopheles albimanus* resistance management trial. *Pesticide Biochemistry Physiology* **89**: 111–117.

PREETHA, G., STANLEY, J., SURESH, S. & SAMIYAPPAN, R. 2010. Risk assessment of insecticides used in rice on mirid bug, *Cyrtorhinus lividipennis* Reuter. *Chemosphere* **80**: 498–503.

PREETHA, G., STANLEY, J., SURESH, S. & KUTTALUM, S. 2009. Toxicity of selected insecticides to *Trichogramma chilonis*: assessing their safety in the rice ecosystem. *Phytoparasitica* **37**: 209–215.

RAFIEE, D.H., HEJAZI, M.J., NOURI, G.G. & SABER, M. 2008. Toxicity of some biorational and conventional insecticides to cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae) and its ectoparasitoid, *Habrobracon hebetor* (Hymenoptera: Braconidae). *Journal of Entomological Society of Iran* **28**: 27–37.

SAS® (Statistical Analysis System). 2011. Ver. 9.3 System options, 2nd edition. SAS Institute Inc., Cary, NC, U.S.A.

SHAW, M.R. 1994. Parasitoid host ranges, In: Hawkins, B.A. & Sheehan, W. (Eds) *Parasitoid Community Ecology*. 111–144. Oxford University Press, Oxford, U.K.

SHAW, M.R. & HUDDLESTON, T. 1991. Classification and biology of braconid wasps (Hymenoptera: Braconidae). In: Dolling, W.R. & Askew, R.R. (Eds) *Handbooks for the Identification of British Insects*, *Vol. 7, Part 11*. 1–126. Royal Entomological Society of London, London, U.K.

SRINIVASA MURTHY, K., RAMYA, S.L., VENKATESAN, T., JALALI, S.K. & VERGHESE, A. 2014. Biochemical basis of insecticide resistance and determination of esterase enzyme patterns in field collected populations of *Cotesia vestalis* (Haliday) (Hymenoptera: Braconidae) from India. *Annals of Biological Research* **5**: 7–15.

STUMPF, N. & NAUEN, R. 2002. Biochemical markers linked to abamaectin resistance in *Tetranychus urticae* (Acari: Tetranychidae). *Pesticide Biochemistry and Physiology* **72**: 111–121.

THANAVENDAN, G. & JEYARANI, S. 2010. Effect of different temperature regimes on the biology of *Bracon brevicornis* Wesmael (Braconidae: Hymenoptera) on different host larvae. *Journal of Biopesticides* **3**: 441–444.

TILLMAN, P.G. & MULROONEY, J.E. 2000. Effect of selected insecticides on the natural enemies *Coleomegilla maculata* and *Hippodamia convergens* (Coleoptera: Coccinellidae), *Geocoris punctipes* (Hemiptera: Lygaeidae), and *Bracon mellitor*, *Cardiochiles nigriceps*, and *Cotesia marginiventris* (Hymenoptera: Braconidae) in cotton. *Journal of Economic Entomology* **93**: 1638–1643.

UMORU, P.A., POWELL, W. & CLARK, S.J. 1996. Effect of pirimicarb on the foraging behaviour of *Diaeretiella rapae* (Hymenoptera: Braconidae) on host-free and infested oilseed rape plants. *Bulletin of Entomological Research* **86**: 193–201.

WHARTON, R.A. 1993. Bionomics of the Braconidae. *Annual Review of Entomology* **38**: 121–143.

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