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Evaluation of pesticide toxicity at their field recommended doses to honeybees, *Apis cerana* and *A. mellifera* through laboratory, semi-field and field studies

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HIGHLIGHTS

- Acute, semi-field and field toxicity of pesticides to *Apis cerana* & *A. mellifera* studied.
- Chlorpyripos, dichlorvos, malathion, profenofos, monocrotophos & deltamethrin caused 100% mortality.
- Acetamiprid and endosulfan were safer to both the bees in filter paper and topical bioassays.
- In second tier, monocrotophos, thiamethoxam, dichlorvos, profenofos and chlorpyriphos are highly toxic.
- Acetamiprid and endosulfan did not cause any repellent effect on honey bees in the field trials.

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ABSTRACT

A series of experiments were carried out to determine the acute toxicity of pesticides in the laboratory, toxicity through spray on flowering plants of mustard (Tier II evaluation) and field on both *Apis cerana* and *A. mellifera* bees. The overall mortality of honey bees through topical (direct contact) were found significantly higher than that of indirect filter paper contamination assays. Insecticides *viz*., chlorpyriphos, dichlorvos, malathion, profenofos, monocrotophos and deltamethrin when exposed directly or indirectly at their field recommended doses caused very high mortality up to 100% to both the bees at 48 HAT. The insecticides that caused less mortality through filter paper contamination viz., flubendiamide, methyl demeton, imidacloprid and thiamethoxam caused very high morality through direct exposure. Apart from all the fungicides tested, carbendazim, mancozeb, chlorothalonil and propiconazole, insecticides acetamiprid and endosulfan were found safer to both the bees either by direct or indirect exposures. Tier II evaluation by spray of pesticides at their field recommended doses on potted mustard plants showed monocrotophos as the highly toxic insecticide with 100% mortality even with 1 h of exposure followed by thiamethoxam, dichlorvos, profenofos and chlorpyriphos which are not to be recommended for use in pollinator attractive flowering plants. Acetamiprid and endosulfan did not cause any repellent effect on honey bees in the field trials endorse the usage of acetamiprid against sucking pest in flowering plants.

1. Introduction

Vegetable brassicas such as cabbage, cauliflower, broccoli, radish, mustard and several leafy greens are important crops grown worldwide in an area of approx 2.29 million ha. Asia alone accounts for more than 70% of global brassica acreage as well as production (AVRDC, 2011). In the northwestern Himalaya, cauliflower and cabbage are grown mainly for seed production during winter through spring to summer (Bhalla and Dubey, 1986). There are many reasons for the poor yield in these crops which include lack of proper pollination that is greatly accomplished by honeybee visitation. The pollinators of crucifers include honeybees, bumble bees, flies and many others (Muhammad et al., 1973; Free, 1993) and out of which honeybees are known to be the predominant (Sihag, 1986; Sushil et al., 2013). Effective increase in seed set and seed yield of many brassica crops *viz*., cabbage, cauliflower, radish, mustard, etc., with honey bee, *A. cerana* pollination were already reported (Verma and Partap, 1994; Partap and Verma, 1994) recorded.

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Cruciferous crops are attacked by many insect pests and especially aphids (*Brevicoryne brassicae, Lipaphis erysimi, Myzus persicae*) which occur in the later stage (flowering) cause significant yield loss. Pesticides used on crop plants for the control of pests have their side effects and Honey bees, being insects are highly susceptible to insecticides. The harmful effects may be due to the direct exposure of honey bees to pesticides or through indirect residual effects. Thus the insecticide formulations which minimize this hazard while still giving effective control of the pests are therefore desirable (Needham and Stevenson, 1973). An ideal pesticide should be effective against target pests but safe to pollinating insects and beneficials. Knowledge of relative safety of insecticides during flowering stage of the crop is essential to obtain the maximum benefit from the bee population (Gour and Pareek, 2004).

The study of pesticide effects on honey bees is important to control a wide variety of agricultural pests with insecticides without hurting bees that inadvertently come into contact with it while foraging (Atkins, 1992). Though there are many approaches for pesticide testing in non targets especially honeybees, a three tier assessment scheme comprising early studies in laboratory conditions followed by semi field studies and completed by field studies (Halm et al., 2006; Stanley et al., 2010) seems to be a realistic approach. The laboratory tests examine oral and contact toxicity of the pesticide under worst case exposure conditions. This increases the likelihood of detecting the adverse effects, if any (Romeis et al., 2011) on the bees. If effects are seen in tier I, the risk can further be characterized in higher tier experiments that make more realistic environmental exposure scenarios. Providing a worst case assessment under realistic conditions of exposure, a semi field cage tests can be designed to study certain hazards to honeybees (EPPO, 2010). Thus a tired risk assessment moves from tests in laboratory to evaluate adverse effects, extended to semi field to increase in realism of the test, increase in ecological complexity and reduction in generality and finally end up in field test (Romeis et al., 2011). With this view, a study was conducted to screen the commonly available pesticides used in brassica pest management at their field recommended doses to know their relative toxicity to honey bees, *Apis cerana* and *A. mellifera* through three tier evaluation i.e. laboratory (acute toxicity), semi field and field studies.

2. Materials and methods

The study was undertaken at the entomology laboratory and in mustard fields of experimental farm of V.P.K.A.S, Hawalbagh situated at 79°39′E Lat. and 29°35′N Long.; 1250 m above msl during 2009–12. Test species, *A. cerana* and *A. mellifera* were reared at the farm apiary. Toxicity of fifteen different commonly used insecticides belonging to eight classes and four classes of fungicides at their field recommended concentrations were tested to both the honeybee species through a three tier system. The field recommended dose/label dose of each insecticide, which is being used/recommended to spray at field conditions were tested and comparisons made based on the effect on honeybees at that particular dose. First tier included acute toxicity studies for all the insecticides and fungicides using topical and filter paper disc bioassays on both the honeybee species in the laboratory. Second tier included semi-field studies conducted on potted plants of mustard var. VLT 3 under caged conditions, followed by a test for repellency if any for the safest insecticides to the bees in the field.

2.1. Laboratory experiments on acute toxicity of pesticides

2.1.1. Topical bioassay

Foraging worker bees of *A. cerana* and *A. mellifera*, just leaving the entrance of the hive were obtained from the apiary. The bees thus collected were calmed by chilling for two minutes in the refrigerator at 4 °C before treatment. The calmed bees were topically dosed with 1 μL drop of field recommended doses of various insecticides and fungicides prepared in analytical grade acetone as mentioned in Table 1 on their thorax using 1 μL repeating dispensor (PB 600-01; Hamilton Co. Ltd., Bonaduz, Switzerland) fitted with 50 μL syringe. Altogether, thirty bees were used per treatment with three replications having ten bees per replication. Control bees were treated only with acetone. After topical application, bees were released in plastic jars of size (9 cm × 13 cm) and provided with cotton-tissue paper cubes dipped in sugar solution. The open end of the plastic jars was closed with muslin cloth to prevent the escape of honeybees and ensure proper aeration. Observations on honeybee mortality were taken at 24 and 48 h after treatment (HAT). Moribund bees were also considered as dead. Percent mortality was worked out after correcting the

Table 1
List of insecticides and fungicides used along with recommended doses.

Insecticide	Trade name	Field dose[a] (g or mL/L)	Concentration used (ppm)	Class	Manufacturers
Endosulfan	Endocel 35 EC	2.0	700	Carbamate	Excel crop care Ltd., Gujarat
Chlorpyriphos	Tricel 20 EC	2.0	400	OP[b]	Excel crop care ltd, Gujarat
Dichlorvos	Nuvan 76 EC	1.0	760	OP	Syngenta India Ltd., Mumbai
Malathion	Tusk 50 EC	2.0	1000	OP	Shivalik Agro Chemicals, Jammu
Profenofos	Curacron 50EC	2.0	1000	OP	Syngenta India Ltd., Mumbai
Monocrotophos	Monocrown 36 SL	2.0	720	OP	Nagarjuna Agrichem Ltd., Hyderabad
Methyl demeton	Metasystox 25 EC	1.0	250	OP	United Phosphorus Limited, Gujarat
Deltamethrin	Decis 2.8 EC	1.07	30	Syn.Pyrethroid	Bayer CropScience Ltd., Gujarat
Indoxacarb	Amsac 14.5 SC	1.0	150	Oxamine	E.I. Dupont India Pvt Ltd., Gujarat
Cartap hydrochloride	Caldan 50 SP	1.0	500	Nereis toxin	Dhanuka Agritech Ltd., Haryana
Flubendiamide	Takumi 20 WG	0.35	70	Diamide	Rallis India Ltd., Mumbai
Spinosad	Tracer 45 SC	0.33	150	Spinosyn	Dow AgroSciences India Pvt Ltd., Mumbai
Imidacloprid	Tatamida 17.8 SL	0.25	50	Neonicotinoid	Saraswati Agro chemicals India Pvt Ltd., Jammu
Thiamethoxam	Actara 25 WG	0.25	50	Neonicotinoid	Syngenta India Ltd., Mumbai
Acetamiprid	Albis 20 SP	0.25	50	Neonicotinoid	Atul Ltd., Gujarat
Carbendazim	Bavistin 50 WP	0.67	334	Benzimidazole	Saraswati Agro chemicals India Pvt Ltd., Jammu
Mancozeb	Indofil M-45 75 WP	2.67	2000	EBDC compounds	Indofil chemicals company, Mumbai
Chlorothalonil	Kavach 75WP	1.50	1130	Substituted benzenes	Rallis India Ltd., Mumbai
Propiconazole	Tilt 25 EC	0.67	167	Triazoles	BioStadt India Ltd., Jammu

[a] Field realistic concentration or label dose or recommended field rates.
[b] Organophosphorus group.

treatment mortality for the control mortality, if any, using Abbot's formula.

2.1.2. Filter paper disc bioassay

Field recommended concentrations (ppm) of various insecticides and fungicides were prepared in analytical grade acetone. Specific quantity of the prepared solution (500 μL) was taken with Eppendorf's 1 mL micropipette and spread evenly over a 9 cm diameter Whatman No. 1 filter paper placed over a glass Petriplate of similar dimension. The filter paper was allowed to dry for 10 min and then shifted to another Petriplate of same dimensions. Ten *A. mellifera* bees were collected and immobilized by chilling for 2 min at 4 °C in refrigerator. The bees were then released in the glass Petriplates having treated filter paper and covered with a plastic cover of same dimension as the glass Petriplate, having holes to ensure proper aeration. The treatment was replicated thrice with 30 bees per treatment. The bees were allowed to be in contact with the filter paper for a period of half an hour. Afterwards the bees were moved to plastic jars of 9 cm × 13 cm dimensions and provided with cotton-tissue paper cubes dipped in sugar solution (sugar: water in 1:1 ratio). Similar experiments were done with *A. cerana*. The data was recorded for bee mortality at 24 and 48 HAT and percent mortality was worked out.

2.2. Semi field studies

Mustard plants (var.VLT-3) raised in pots were used for semi field studies on pesticidal toxicity to honeybees. For each treatment, sixteen pots were grouped and sprayed with the respective pesticide at their field recommended concentration during full blooming of crop (approx. 70 d from sowing) using a two litre hand sprayer. Spraying was done until the plants were completely saturated with the spray liquid. Treated plants were placed in shade for 1 h to allow the spray deposits to dry. After 1 h, the pots were shifted to the mustard field where bee colonies of both the species were introduced. Each treatment was replicated with four pots per replication. Out of sixteen pots, twelve pots in group of four each were placed inside three 1 m^2 quadrates and covered with a mosquito net. The fourth replication was left open in the field to allow bees to visit the sprayed plants. Both the honeybee species i.e. *A. cerana* and *A. mellifera* which visited at least two flowers from sprayed pots were collected in test tube and kept in separate plastic jars of 9 cm × 13 cm size, having holes in the lid. A total of five *A. cerana* and five *A. mellifera* were collected and released in first quadrate and sealed properly at sides by mosquito net to prevent escape of bees. Consecutively all quadrates were provided with bees. A total of twenty *A. cerana* and twenty *A. mellifera* were collected and released in all the four quadrates and kept in shade for 1 h to allow bees to forage. Thus a set of twenty *A. cerana* and twenty *A. mellifera* were used per pesticidal treatment. After 1 h, bees were collected back from each quadrate and kept in separate jars and provided with cotton-tissue paper cubes dipped in sugar solution. Bee mortality was recorded at 1, 24 and 48 h after treatment and percent mortality was calculated. The advantages of this method with other methods are more realistic and resemble field conditions and bees used are real foragers actively foraging in the field itself.

2.3. Field evaluation of insecticides

The insecticides found safer in the laboratory and semi field studies were further tested in the mustard fields for their field repellency, if any, to honeybees. Two fields of mustard (var. VLT-3) per treatment were sprayed with the field recommended doses of the insecticides (endosulfan and acetamiprid) and one field was sprayed with water as untreated check during peak time of flowering and foraging. Observations were taken on both the honeybee species visiting the mustard fields before and after the treatment in 8 one m^2 quadrates for 30 s in each treatment. Apart from pretreatment counts post treatment data was recorded at 0, 1, 3 and 5 d.

3. Results

3.1. Laboratory bioassay

Organophosphate insecticides including chlorpyriphos, dichlorvos, malathion, profenofos, monocrotophos and methyl demeton at their field recommended doses caused 100% mortality to both *A. cerana* and *A. mellifera* at 48 HAT in topical bioassay. Besides this, deltamethrin (a synthetic pyrethroid), spinosad (a spinosyn) and thiamethoxam (a neonicotinoid) also caused 100% mortality to both the bees at 48 HAT. Indoxacarb, cartap hydrochloride and imidacloprid were found to be slightly toxic to *A. cerana* and *A. mellifera* with mortalities ranging between 50% and 90%. Flubendiamide showed discrepancies in toxicity to both the bee species with 26.67% mortality to Indian bees and 66.67% mortality to European bees in topical bioassays at 48 HAT (Table 2). Among all the insecticides tested, only endosulfan (a carbamate) and acetamiprid (a neonicotinoid) caused least mortality of 26.67% & 16.67% to *A. cerana* while 26.67% and 36.67% to *A. mellifera* in topical bioassays. Among the fungicides tested, all the four i.e. carbendazim, mancozeb, chlorothalonil and propiconazole were found less toxic to both the bees with mortalities ranging between 5% and 30% at 48 HAT during topical bioassays.

Filter paper disc bioassays also revealed organophosphates and deltamethrin at their field recommended doses, as most toxic to both the bees with cent percent mortalities at 48 HAT with the exception of methyl demeton which caused 13.33% mortality to *A. cerana* while no mortality at all to *A. mellifera* at 48 HAT. Indoxacarb, cartap hydrochloride and flubendiamide caused mortalities in the range of 40–80% to *A. cerana* and *A. mellifera* in filter paper disc assays. Imidacloprid and thiamethoxam caused 26.67% mortality to Indian bees while 46.67% and 73.33% to European bees at 48 HAT (Table 2, Fig. 1). Spinosad caused 33.33% and 43.33% mortalities to *A. cerana* and *A. mellifera,* respectively. Filter paper disc bioassays also reported least mortality to honeybees with endosulfan and acetamiprid, where endosulfan caused 36.67% and 16.67% mortality to *A. cerana* & *A. mellifera* and acetamiprid caused 16.67% & 20.0% mortality to *A. cerana* and *A. mellifera* respectively at 48 HAT. Fungicides were also found safe to honeybees with mortalities ranging between 10% and 30% at 48 HAT. Only exception was noticed with chlorothalonil having 63.33% mortality to *A. cerana* while 53.33% to *A. mellifera* in filter paper assay at 48 HAT. Endosulfan and acetamiprid caused least mortality of 36.7% and 16.7% to *A. cerana* and 16.7% and 20.0% to *A. mellifera* in filter paper disc bioassay at 48 HAT. Besides this, indoxacarb, flubendiamide, spinosad, imidacloprid and thiamethoxam caused mortalities in the range of 30–50% to *A. cerana* in filter paper disc bioassay while all of these excluding flubendiamide were highly toxic to *A. cerana* in topical bioassay with mortalities in the range of 60% and above at 48 HAT. Among fungicides, only chlorothalonil was found slightly toxic to both the bees with mortalities in the range of 50–60% in filter paper disc bioassay at 48HAT. All the other fungicides, i.e. carbendazim, mancozeb and propiconazole caused less than 30% mortality to both *A. cerana* and *A. mellifera* and were found safer to bees in laboratory assays.

3.2. Semi field studies

The results obtained in semi field trials were also found comparable to the lab studies. Chlorpyriphos, dichlorvos, malathion,

Table 2
Acute toxicity of insecticides at their field recommended doses to honey bees in the laboratory.

Insecticides	Percent mortality							
	A. cerana				*A. mellifera*			
	Filter paper disc		Topical assay		Filter paper disc		Topical assay	
	24 HAT	48 HAT	24 HAT	48 HAT	24 HAT	HAT	24 HAT	48 HAT
Endosulfan	10.0^{c}	36.67^{ef}	16.67^{b}	26.67^{bc}	13.33^{de}	16.67^{b}	13.33^{d}	26.67^{bc}
Chlorpyriphos	100^{h}	100^{j}	100^{e}	100^{f}	100^{h}	100^{h}	100^{g}	100^{f}
Dichlorvos	100^{h}	100^{j}	100^{e}	100^{f}	100^{h}	100^{h}	100^{g}	100^{f}
Malathion	100^{h}	100^{j}	100^{e}	100^{f}	76.67^{g}	86.67^{g}	100^{g}	100^{f}
Profenofos	100^{h}	100^{j}	100^{e}	100^{f}	100^{h}	100^{h}	100^{g}	100^{f}
Monocrotophos	100^{h}	100^{j}	100^{e}	100^{f}	100^{h}	100^{h}	100^{g}	100^{f}
Deltamethrin	100^{h}	100^{j}	100^{e}	100^{f}	100^{h}	100^{h}	100^{g}	100^{f}
Indoxacarb	0.0^{a}	46.67^{g}	63.33^{d}	86.67^{e}	13.33^{de}	56.67^{de}	3.33^{ab}	73.33^{e}
Cartap hydrochloride	40.00^{g}	80.00^{i}	36.67^{c}	83.33^{e}	13.33^{de}	60.00^{e}	13.33^{d}	56.67^{d}
Flubendiamide	20.00^{de}	43.33^{fg}	16.67^{b}	26.67^{bc}	36.67^{f}	43.33^{c}	30.00^{e}	66.67^{de}
Spinosad	26.67^{ef}	33.33^{de}	100^{e}	100^{f}	33.33^{f}	43.33^{c}	100^{g}	100^{f}
Methyl demeton	0.0^{a}	13.33^{a}	100^{e}	100^{f}	0.0^{a}	0.0^{a}	16.67^{d}	100^{f}
Imidacloprid	10.00^{c}	26.67^{cd}	60.00^{d}	66.67^{d}	0.0^{a}	46.67^{cd}	50.00^{f}	66.67^{de}
Acetamiprid	10.00^{c}	16.67^{ab}	0.0^{a}	16.67^{a}	0.0^{a}	20.00^{b}	10.00^{cd}	36.67^{c}
Thiamethoxam	23.33^{ef}	26.67^{cd}	100^{e}	100^{f}	43.33^{f}	73.33^{f}	100^{g}	100^{f}
Carbendazim	6.67^{b}	13.33^{a}	16.67^{b}	30.00^{bc}	6.67^{bc}	16.67^{b}	0.0^{a}	6.67^{a}
Mancozeb	13.33^{cd}	20.00^{bc}	16.67^{b}	26.67^{c}	3.33^{ab}	3.33^{a}	6.67^{bc}	6.67^{a}
Chlorthalonil	30.00^{f}	63.33^{h}	0.0^{a}	33.33^{c}	20.00^{e}	53.33^{cde}	16.67^{d}	16.67^{b}
Propiconazole	10.00^{c}	33.33^{de}	0.0^{a}	20.00^{ab}	10.00^{cd}	16.67^{b}	0.0^{a}	6.67^{a}
CD (0.05)	5.43	4.89	4.02	5.74	7.02	6.85	6.73	7.94
CV (%)	7.74	5.60	4.42	5.45	10.73	8.26	8.36	7.89

In a column, means followed by a common superscript letter are not significantly different at $p = 0.05$ by LSD.

profenofos, monocrotophos, deltamethrin, spinosad, methyl demeton and thiamethoxam caused highest mortalities in the range of 75% and above to both the honeybee species at 48 HAT. Indoxacarb and imidacloprid were found more toxic to *A. cerana* with 70–80% mortality compared to *A. mellifera* with a mortality of 60%. Cartap hydrochloride caused 40% mortality to *A. cerana* and 65% mortality to *A. mellifera* at 48 HAT while Flubendiamide caused 35% mortality to *A. mellifera* and 60% mortality to *A. cerana* under semi field conditions (Table 3). Endosulfan and acetamiprid were found least toxic to both the honeybees with less than 25% mortality at 48 HAT. Besides these, all the four fungicides viz., carbendazim, mancozeb, chlorothalonil and propiconazole caused less than 25% mortality to *A. cerana* and *A. mellifera* in semi field studies.

3.3. Field studies

The insecticides reported least toxic from first and second tier evaluation were carried forward and used for field sprays at their field recommended doses. Fields sprayed with endosulfan and acetamiprid revealed a total number of 2.25, 3.25 and 2.13, 3.00 *A. cerana* and *A. mellifera* per m^2 per 30 s just after the spray whereas this figure was 2.13 and 2.88 in untreated plot. One day after treatment, the number of *A. cerana* and *A. mellifera* was 2.38 and 3.38 in endosulfan sprayed field and 2.13 and 3.25 in acetamiprid sprayed field while in untreated plots it was 2.25 and 3.38 *A. cerana* and *A. mellifera* bees per 30 s (Table 4). Similarly on third day after treatment the number of honeybees in plots sprayed with endosulfan and acetamiprid were not less than the unsprayed plots. Likewise on fifth day after treatment also no significant reduction in bee visits were noticed in any of the insecticide sprayed fields or in the bee species when compared to untreated control.

4. Discussion

This study indicates that insecticides present significantly different risks to honey bees and thus can be used to decide selective and non-selective insecticides to bees and select the safest one for use in fields. Bioassays revealed organophosphates as the most toxic group causing maximum mortality to *A. cerana* and *A. mellifera*. Exposure to organophosphates and pyrethroid insecticides has been associated with bee poisonings in many crops (Kevan, 1975; Johansen, 1977; Kearns et al., 1998). Based on the recommendations of IOBC–WPRS working group (Hassan, 1989; Stanley et al., 2009) endosulfan and acetamiprid which caused 36.7% and 16.7% mortality to *A. cerana* and 16.7% and 20.0% to *A. mellifera* were found least toxic to bees in first tier evaluation. Among all insecticides tested, organophosphates including chlorpyriphos, dichlorvos, malathion, profenofos, monocrotophos and methyl demeton were highly toxic to both the bees with more than 80% mortality. Besides, deltamethrin (a synthetic pyrethroid), indoxacarb (oxamine), cartap hydrochloride (nereis toxin), flubendiamide (diamide), spinosad (spinosyn), and some neonicotinoids as imidacloprid and thiamethoxam were found to be slightly to moderately toxic to bees with mortalities in the range of 40–90%. These results corroborate with Kapil and Lamba (1974) who concluded methyl demeton, endrin and dieldrin as moderately toxic whereas, malathion, parathion, phosphamidan, lindane, phorate and mevinphos as toxic insecticides to *A. cerana* based on LC_{50} values. Deshmukh (1991) also reported malathion as highly toxic to honey bees in field experiments resulting in minimum visits. Such compounds could potentially intoxicate pollinators through direct contact, exposure to residues, or spray contamination of nectar and pollen (Burgett and Fisher, 1980; Johansen et al., 1983).

Bailey et al. (2005) reported intermediate toxicities of spinosad and imidacloprid to honey bees, *A. mellifera* in laboratory experiments by direct contact of bees with the spray liquid. Hasan et al. (1986) also reported monocrotophos and synthetic pyrethroids as toxic to the bees. Our results are also in line with Thomos and Phadke who in 1994 found chloropyriphos as most toxic to *A. cerana* foragers. Neonicotinoids showed higher mortality to *A. mellifera* compared to *A. cerana* in lab tests which is in accordance with earlier reports, in which several neonicotinoids showed very strong toxicity to *A. mellifera*, causing other effects also which are seldom easily identifiable, such as behavioral disturbances, orientation difficulties and impairment of social activities (Guez et al., 2001; Bortolotti et al., 2003; Medrzycki et al., 2003; Decourtye

Table 3
Toxicity of insecticides to honey bees tested through potted plants of mustard – IInd tier evaluation.

Insecticides	Percent mortality					
	A. cerana			*A. mellifera*		
	1 HAT	24 HAT	48 HAT	1 HAT	24 HAT	48 HAT
Endosulfan	10.0^{bc}	10.0^{a}	30.0^{c}	0.0^{a}	0.0^{a}	20.0^{bc}
Chlorpyriphos	50.0^{ef}	100.0^{e}	100.0^{h}	20.0^{cd}	100.0^{e}	100.0^{f}
Dichlorvos	60.0^{f}	100.0^{e}	100.0^{h}	100.0^{f}	100.0^{e}	100.0^{f}
Malathion	45.0^{ef}	100.0^{e}	100.0^{h}	15.0^{c}	100.0^{e}	100.0^{f}
Profenofos	35.0^{de}	100.0^{e}	100.0^{h}	20.0^{cd}	100.0^{e}	100.0^{f}
Monocrotophos	100.0^{h}	100.0^{e}	100.0^{h}	100.0^{f}	100.0^{e}	100.0^{f}
Deltamethrin	20.0^{cd}	65.0^{d}	85.0^{g}	5.0^{ab}	100.0^{e}	100.0^{f}
Indoxacarb	5.0^{ab}	25.0^{c}	75.0^{f}	0.0^{a}	5.0^{b}	60.0^{e}
Cartap hydrochloride	0.0^{a}	25.0^{c}	40.0^{d}	15.0^{c}	60.0^{d}	65.0^{e}
Flubendiamide	0.0^{a}	20.0^{bc}	60.0^{e}	0.0^{a}	10.0^{c}	35.0^{d}
Spinosad	5.0^{ab}	100.0^{e}	100.0^{h}	15.0^{c}	100.0^{e}	100.0^{f}
Methyl demeton	50.0^{ef}	100.0^{e}	100.0^{h}	55.0^{e}	100.0^{e}	100.0^{f}
Imidacloprid	15.0^{c}	65.0^{d}	80.0^{fg}	35.0^{d}	60.0^{d}	60.0^{e}
Acetamiprid	20.0^{cd}	20.0^{bc}	30.0^{c}	0.0^{a}	10.0^{a}	20.0^{bc}
Thiamethoxam	85.0^{g}	100.0^{e}	100.0^{h}	95.0^{f}	100.0^{e}	100.0^{f}
Carbendazim	0.0^{a}	10.0^{a}	25.0^{bc}	0.0^{a}	0.0^{a}	10.0^{ab}
Mancozeb	0.0^{a}	15.0^{ab}	15.0^{a}	0.0^{a}	0.0^{a}	15.0^{a}
Chlorthalonil	10.0^{bc}	20.0^{bc}	25.0^{bc}	0.0^{a}	15.0^{c}	25.0^{c}
Propiconazole	10.0^{bc}	20.0^{bc}	20.0^{ab}	10.0^{bc}	15.0^{c}	15.0^{ab}
CD (0.05)	10.08	5.12	5.53	10.45	7.38	5.36
CV (%)	17.40	4.41	4.26	19.24	6.81	4.24

In a column, means followed by a common superscript letter are not significantly different at $p = 0.05$ by LSD.

Table 4
Field evaluation of repellency of insecticides on honey bees at peak time of flowering and foraging.

Period	No. of bees per m^2/30 s									
	Pre treatment		0 DAT		1 DAT		3 DAT		5 DAT	
	A. cerana	*A. mellifera*	*A. cerana*	*A. mellifera*	*A. cerana*	*A. mellifera*	*A. cerana*	*A. mellifera*	*A. cerana*	*A. mellifera*
Endosulfan	2.13 (1–4)	3.13 (2–4)	2.25 (1–5)	3.25(2–4)	2.38 (1–4)	3.38 (1–5)	2.00 (1–3)	3.00 (1–4)	2.25 (1–4)	3.25 (2–5)
Acetamiprid	2.00 (0–4)	3.13 (1–5)	2.13 (1–5)	3.00 (1–5)	2.13 (0–5)	3.25 (2–5)	2.63 (0–4)	3.75 (1–5)	2.25 (0–5)	3.38 (1–6)
Untreated	2.00 (1–5)	3.00 (1–5)	2.13 (1–4)	2.88 (1–4)	2.25 (0–4)	3.38 (2–4)	2.38 (0–5)	3.38 (2–5)	2.25 (0–4)	3.13 (1–5)

Mean of 8 observations.
Figures in the parentheses are range.
DAT – days after treatment.

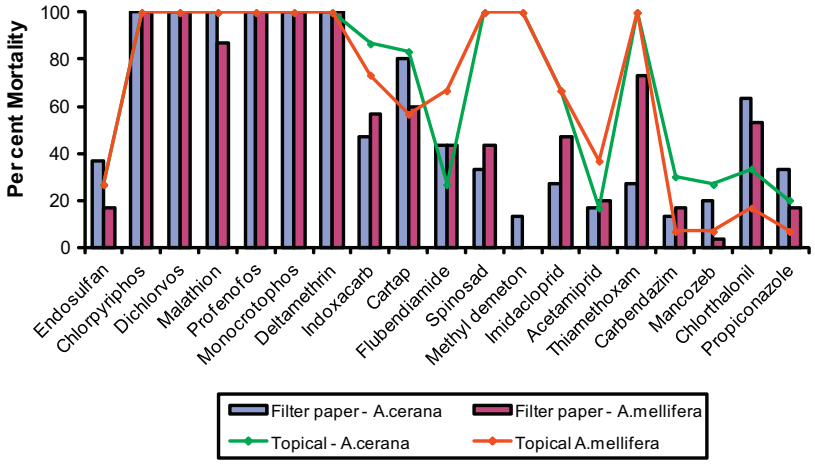

Fig. 1. Acute toxicity of insecticides and fungicides to honeybee, *A. cerana* and *A. mellifera*.

et al., 2004a; Decourtye et al., 2004b; Desneux et al., 2007; El Hassani et al., 2008; Maini et al., 2010). Suchail et al. (2000) reported high acute contact and per os toxicity to honey bee for imidacloprid. Direct exposure of imidacloprid is inherently toxic to bees (Stark et al., 1995; Mayer and Lunden, 1997; Schmuck et al., 2001). In the present study it was found that neonicotinoids like imidacloprid and thiamethoxam posed higher toxicities to both the honeybees, although acetamiprid, which is also a neonicotinoid, was found least toxic. Higher toxicity of imidacloprid and thiamethoxam may be due to the presence of nitro group in the neonicotinoid while lower toxicity of acetamiprid to bees may be due to cyano substitution as reported by Iwasa et al.

(2004). He further stated that nitro group in neonicotinoids produces high topical toxicity to honey bees in the laboratory as in imidacloprid, thiamethoxam, clothianidin, etc., and neonicotinoids like acetamiprid and thiacloprid with the cyano substitution have significantly lower bee toxicity. Laurino et al. (2011) also confirmed higher mortality of honey bees to thiamethoxam even at a concentration of 20–200 times lower than the field recommended concentration in laboratory through ingestion and indirect contact with Spanish chestnut leaves while acetamiprid was found safer with no honeybee mortality even at 72 HAT in ingestion and indirect contact tests.

Among the fifteen insecticides tested, only two insecticides viz., Endosulfan (a carbamate), a contact insecticide and acetamiprid (a neonicotinoid), a systemic insecticide proved harmless to both the bees at their field recommended doses in the laboratory as well as semi field studies. It was observed that even after spraying the insecticides, the number of honeybee foragers of both species was almost same in pre and post treatments in comparison to untreated plots on all days respectively. The normal bee population in both sprayed and unsprayed plots revealed that these insecticides did not repel bees from foraging. This is in line with the results of Needham and Stevenson (1973) which showed that endosulfan at the field application rate is much safer to honeybees than azinphos-methyl or malathion as proved by higher mortality in *A. mellifera* hives kept adjacent to malathion treated field shortly after spraying compared to no mortality noticed in bee hives next to endosulfan sprayed field. Deshmukh (1991), Misra and Verma (1982), Kapil and Lamba (1974), Reddy (1997), Singh et al. (1997) also found endosulfan as less toxic pesticide against honeybees while Johansen (1977) reported minimal hazards of endosulfan in honeybees when applied during late evening or night time under field conditions. Hasan et al. (1986) and Thomas and Phadke (1994) also reported endosulfan as least toxic to *A. cerana.* Acetamiprid has been reported safe to honeybees, *A. mellifera* through direct contact, ingestion and indirect contact studies (Takahashi et al., 1992; Suchail et al., 2000; Laurino et al., 2011) and bumble bees (Takahashi et al., 1992).

All the four fungicides tested at their field recommended doses were found safe to both the honeybees. Our results are in conformity with Kubik et al. (1999) who stated that fungicides have relatively low toxicity for bees and thus spraying of crops during bloom is allowed and for most plant protection products the prevention time i.e. the time elapsed from spraying, is about 1 h. Besides he also stated that however fungicides may not affect bees but residues can be found in pollen grains and nectar collected by bees from treated plants. Mussen et al. (2004) also reported that most of the fungicides are not toxic to honeybees in the quantities ingested or contacted during foraging but in some cases they have shown to deter feeding and hypothermia in adult bees. However, chlorothalonil, a contact and slightly volatile fungicide, was found to be a marker for entombing behavior in honey bee colonies associated with poor health (vanEngelsdorp et al., 2009).

In the present study, two insecticides endosulfan and acetamiprid proved safer to honey bees in lab and semi field experiments. Further these pesticides were not found to deter bees from visiting the sprayed plants as revealed by field experiments. Of this, endosulfan has lately been banned for use in agricultural crops in many countries. Acetamiprid, a systemic insecticide found safer to honeybees in the study is reported to be effective against sucking pests as aphids in various crops. Cruciferous crops are also attacked by aphids (Rouf and Kabir, 1997) which is distributed in many countries of the world (Setokuchi, 1983). Small population of aphids can be eliminated frequently by parasites and predators. (Liu and Sparks, 2011) but when aphid populations exceed acceptable levels and rapid control is needed, insecticides become a necessary part of management (Liu and Sparks, 2011). At this time acetamiprid can be recommended for use during pest infestation in crop bloom without affecting honey bee populations. Our studies have mostly focused on lethal effects of insecticides on honey bee foragers but the use of synthetic insecticides may also cause alteration in the social behavior of honeybees as an increase in agitation, aggressiveness and pollen contamination (Johansen, 1984). Therefore further studies can be undertaken on sublethal effects of these insecticides on honey bee populations. Not only foragers visiting the crops are exposed to pesticides but hive bees and larvae fed on pollen and nectar stored in the combs are also exposed. Therefore effects of pesticide exposure on different life stages of honeybees can also be studied.

References

Atkins, E.L., 1992. Injury to honey bee by poisoning. In: Graham, J.E. (Ed.), The Hive and the Honey Bee. Dadant and Sons, Hamilton, pp. 1153–1208.

AVRDC, 2011. Asian vegetable research and development centre. In: Srinivasan, R., Shelton, A.M., Collins, H.L. (Eds.), Proc. The Sixth International Workshop on Management of the Diamondback Moth and Other Crucifer Insect Pests 21–25 march, 2011 at Kasetsart University Thailand.

Bailey, J., Scott-Dupree, C., Harris, R., Tolman, J., Harris, B., 2005. Contact and oral toxicity to honey bees (*Apis mellifera*) of agents registered for use for sweet corn insect control in Ontario, Canada. Apidol 36, 623–633.

Bhalla, O.P., Dubey, J.K., 1986. Bionomics of the Diamondback Moth in the Northwestern Himalayan. In: Talekar, N.S., Griggs, T.D. (Eds.), Diamondback Moth and Other Crucifer Pests. AVRDC, Tainan, Taiwan, pp. 55–62.

Bortolotti, L., Montanari, R., Marcelino, J., Medrzycki, P., Maini, S., Porrini, C., 2003. Effects of sub-lethal imidacloprid doses on the homing rate and foraging activity of honey bees. Bull. Insectol. 56, 63–67.

Burgett, M., Fisher, G.C., 1980. Recovery of Penncap-M from foraging honey bees and pollen storage cells. Environ. Entomol. 9, 430–431.

Decourtye, A., Armengaud, C., Renou, M., Devillers, J., Cluzeau, S., Gauthier, M., Pham-Delegue, M.H., 2004a. Imidacloprid impairs memory and brain metabolism in the honeybee (*Apis mellifera* L.). Pestic. Biochem. Physiol. 78, 83–92.

Decourtye, A., Devillers, J., Cluzeau, S., Charreton, M., Pham-Delegue, M.H., 2004b. Effects of imidacloprid and deltamethrin on associative learning in honeybees under semi-field and laboratory conditions. Ecotoxicol. Environ. Saf. 57, 410–419.

Deshmukh, P.B., 1991. Toxicity of some insecticides to Indian honeybee *Apis cerana* F. Pestol 15, 31–33.

Desneux, N., Decourtye, A., Delpuech, J.M., 2007. The sublethal effects of pesticides on beneficial Arthropods. Ann. Rev. Entomol. 52, 81–106.

EL Hassani, A.K., Dacher, M., Gary, V., Lambin, M., Gauthier, M., Armengaud, C., 2008. Effects of sublethal doses of acetamiprid and thiametoxam on the behavior of the honeybee (*Apis mellifera*). Arch. Environ. Contam. Toxicol. 54, 653–661.

EPPO, 2010. Side-effects on honeybees. In: Efficacy Evaluation of Plant Protection Products. Bull. OEPP/EPPO Bull. 40, 313–319.

Free, J.B., 1993. Insect Pollination of Crops, 2nd ed. Academic Press, London.

Gour, I.S., Pareek, B.L., 2004. Effect of insecticides on the incidence of honey bee, *Apis cerena indica* Fab.on mustard bloom under semi-arid region of Rajasthan. Madras Agric. J. 91, 430–432.

Guez, D., Suchail, S., Gauthier, M., Maleszka, R., Belzunces, L.P., 2001. Contrasting effects of imidacloprid on habituation in 7 and 8 day old honeybees (*Apis mellifera*). Neurobiol. Learn. Mem. 76, 183–191.

Halm, M., Rortais, R., Arnold, G., Taseai, J.N., Rault, S., 2006. New risk assessment approach for systemic insecticides: the case of honey bees and imidacloprid (Gaucho). Environ. Sci. Technol. 40, 2448–2454.

Hassan, S.A., 1989. Testing methodology and concepts of IOBC–WPRS working group p 1–18. In: Jepson, P.C. (Ed.), Pesticides and non-target invertebrates, intercept, Wimborm, Dorset, 240p.

Hasan, S.B., Deo, P.G., Majunder, S.K., 1986. Relative toxicity of insecticides to household and beneficial insects. Indian Bee J. 48, 42–44.

Iwasa, T., Motoyama, N., Ambrose, J.T., Michael, R., 2004. Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera*. Crop Protec. 23, 371–378.

Johansen, C.A., 1977. Pesticides and pollinators. Ann. Rev. Entomol. 22, 177–192.

Johansen, C.A., 1984. Behavior of pollinators following insecticide exposure. Am. Bee J. 124, 225–227.

Johansen, C.A., Mayer, D.F., Eves, J.D., Kious, C.W., 1983. Pesticides and bees. Environ. Entomol. 12, 1513–1518.

Kapil, R.P., Lamba, D.P.S., 1974. Toxicity of some important insecticides to *Apis cerana* Fab. Indian J. Ent. 36, 6–10.

Kearns, C.A., Inouye, D.W., Waser, N.M., 1998. Endangered mutualisms: the conservation of plant pollinator interactions. Annu. Rev. Ecol. Syst. 29, 83–112.

Kevan, P.G., 1975. Pollination and environmental conservation. Environ. Conserv. 2, 293–298.

Kubik, M., Nowacki, J., Pidek, A., Warakomska, Z., Michalczuk, L., Goszczyñski, W., 1999. Pesticide residues in bee products collected from cherry trees protected

during blooming period with contact and systemic fungicides. Apidol 30, 521–532.
Laurino, D., Porporato, M., Patetta, A., Manino, A., 2011. Toxicity of neonicotinoid insecticides to honeybees: laboratory tests. Bull. Insectol. 64, 107–113.
Liu, T.X., Sparks, J.R., 2011. Aphids on Cruciferous Crops: Identification and Management. Texas Agrilife Extension. 01 Feb. 2011 assessed through <https://agrilifebookstore.org/publications_details.cfm?whichpublication=1477>. (23.07.12).
Maini, S., Medrzycki, P., Porrini, C., 2010. The puzzle of honey bee losses: a brief review. Bull. Insectol. 63, 153–160.
Mayer, D.F., Lunden, J.D., 1997. Effects of imidacloprid insecticide on three bee pollinators. Hortic. Sci. 29, 93–97.
Medrzycki, P., Montanari, R., Bortolotti, L., Sabatini, A.G., Maini, S., Porrini, C., 2003. Effect of imidacloprid administered in sub-lethal doses on honey bee (*Apis mellifera* L.). Bull. Insectol. 56, 59–62.
Misra, R.C., Verma, A.K., 1982. Relative toxicity of some insecticides to *Apis cerana* F. Indian Bee J. 44, 69–71.
Muhammad, S., Gondal, A., Manzoor-ul-Haq, 1973. Studies on the role of *Apis indica* F. in the pollination of cauliflower (*Brassica oleracea* Var. *botrytis Linn.*) and radish (*Raphanus sativus* Linn). S.U. Res. J. 7, 87–93.
Mussen, E., Lopez, J., Peng, C., 2004. Effects of selected fungicides on growth and development of larval honey bees, *Apis mellifera* L. (Hymenoptera: Apidae). Physiol. Ecol. 33, 1151–1154.
Needham, P.H., Stevenson, J.H., 1973. The toxicity to foraging honeybees, *Apis mellifera,* of endosulfan, malathion and azinphos – methyl applied to flowering oil seed rape, *Brassica napus*. Ann. Appl. Biol. 75, 235–240.
Partap, U., Verma, L.R., 1994. Pollination of radish by *Apis cerena*. J. Apic. Res. 33, 237–241.
Reddy, M.L.K., 1997. Relative toxicity of some insecticides to honeybees (*Apis cerana indica* Fab.). J. Res. ANGRAU 25, 26–28.
Romeis, J., Hellmich, R.L., et al., 2011. Recommendations for the design of laboratory studies on non-target arthropods for risk assessment of genetically engineered plants. Transgenic Res. 20, 1–22.
Rouf, F.M.A., Kabir, K.H., 1997. Economic efficacy of some insecticides for the control of mustard aphid, *L. erysimi* Kalt. Bangladesh J. Entomol. 7, 1–7.
Schmuck, R., Schoning, R., Stork, A., Schramel, O., 2001. Risk posed to honeybees (*Apis mellifera* L., Hymenoptera) by an imidacloprid seed dressing of sunflowers. Pest Manag. Sci. 57, 225–238.
Setokuchi, O., 1983. Seasonal prevalence of *Myzus persicae* Sulzer. and *L. etysimi* Kalt. (Homoptera: Aphididae) Kagoshima prefecture. Japan. J. Appl. Entomol. Zool. 21, 219–233.
Sihag, R.C., 1986. Insect pollination increases seed production in cruciferous and umbelliferous crops. J. Apic. Res./Bee World 31 (2), 83–95.
Singh, P.B., Singh, D.S., Naim, M., Srivastava, V.S., 1997. Contact toxicity of some insecticides to oriental honeybee, *Apis cerana indica* foragers. Ann. Plant Prot. Sci. 5, 207–209.
Stanley, J., Preetha, G., Chandrasekaran, S., Kuttalam, S., 2009. Honeybees of the cardamom ecosystem and the selective toxicity of diafenthiuron to four different bee species in the laboratory. J. Apic. Res./Bee World 48 (2), 91–98.
Stanley, J., Chandrasekaran, C., Preetha, G., Kuttalam, S., 2010. Toxicity of diafenthiuron to honey bees in laboratory, semi-field and field conditions. Pest Manag. Sci. 66, 505–510.
Stark, J.D., Jepson, P.C., Mayer, D.F., 1995. Limitations to use of topical toxicity data for predictions of pesticide side effects in the field. J. Econ. Entomol. 88, 1081–1088.
Suchail, S., Guez, D., Belzunces, L.P., 2000. Characteristics of imidacloprid toxicity in two *Apis mellifera* subspecies. Environ. Toxicol. Chem. 19, 1901–1905.
Sushil, S.N., Stanley, J., Hedau, N.K., Bhatt, J.C., 2013. Enhancing seed production of three brassica vegetables by honeybee pollination in North-western Himalayas of India. Univ. J. Agric. Res. 1 (3), 49–53.
Takahashi, H., Mitsui, J., Takakusa, N., Mastuda, M., Yoneda, H., Suzuki, J., Ishimitsu, K., Kishimoto, T., 1992. NI-25, a new type of systemic and broad spectrum insecticide. Brighton Crop Protection Conference, Brighton, UK. Pests Diseases 1, 27–36.
Thomas, J., Phadke, K.G., 1994. Relative toxicity of oxydemeton methyl, chlorpyriphos and guinalphos to honeybee *Apis cerana indica*. Indian J. Agric. Sci. 64, 207–209.
vanEngelsdorp, D., Evans, J.D., Donovall, L., Mullin, C., Frazier, M., Frazier, J., Tarpy, D.R., Hayes, J., Pettis, J.S., 2009. Entombed pollen: A new condition in honey bee colonies associated with increased risk of colony mortality. J. Invertebr. Pathol. 101, 147–149.
Verma, L.R., Partap, U., 1994. Foraging behaviour of *Apis cerana* on cauliflower and cabbage and its impact on seed production. J. Apic. Res. 33, 231–236.