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Short Communication

Toxicity of Three Commonly Used Nicotinoids and Spinosad to *Apis mellifera* L. (Hymenoptera: Apidae) Using Surface Residual Bioassays

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ABSTRACT

The current investigations were carried out to check the acute contact toxicity of commercial formulated insecticides *viz.*, imidacloprid, acetamaprid, thiamethoxam and spinosad against workers of *Apis mellifera* L. under laboratory conditions due to their large scale application against different insect pests of crops in Pakistan. Each insecticide was evaluated at four different concentrations. The mortality of honeybees was counted after 3, 6, 12 and 24 hrs post exposure. The median lethal concentration (LC_{50}) of each insecticide was determined for *Apis mellifera* L. The toxicity of insecticides increased with increase in concentration and exposure time to honeybees. The results obtained were statistically highly significant and indicate that spinosad and imidacloprid were most toxic with LC_{50} values of 13.5 and 16.6 ppm after 24 hrs post exposure to honeybees. When these results were adjusted to their commercial formulated field dose applications, they were likely to be causing potential impact to honeybees at their maximum recommended dose except acetamaprid.

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MH and MA conceived and designed the study. MAF performed the experimental work and wrote the article.

Key words
Insecticides, mortality, *Apis mellifera*, LC_{50}.

Honeybees are the economically important insects worldwide due to their pollination activities (Klein *et al.*, 2007). According to estimates, more than 80% of flowering plants are pollinated from honeybees which are important for the production of food and to maintain wild plant ecosystems (Ollerton *et al.*, 2011). Among them *Apis mellifera* L. is the most valued, and increases the yield and quality of certain crops. The application of insecticides on different flowering plants can affect various species of pollinators including honeybee foragers when they come in contact with these treated plants and when they fly through the adsorption areas of contaminated dust particles (Koch and Weisser, 1997; Prier *et al.*, 2001; Potts *et al.*, 2010). Several reports have shown the contact/relative toxicities of commonly used insecticides against honeybees (Raghunandan and Basavarajappa, 2013).

Insecticides can affect honeybee populations through direct mortality and through sub-lethal effects on behavior, such as impaired memory, learning and foraging. Impaired foraging of bees can lead to poor nutrition, and insecticides can have direct effects on the immune system of bees making them more susceptible to different diseases. In addition sub-lethal doses of insecticides may interfere with the brood development of the bees and shorten the life spans of adult worker honeybees (Desneux *et al.*, 2007; Henry *et al.*, 2012, Pettis *et al.*, 2012; Wu *et al.*, 2011). Due to indiscriminate use of insecticides, the populations of honeybees are decreasing drastically in different countries throughout the world (Raghunandan and Basavarajappa, 2013). As worldwide reduction of honeybees continues, the dependence of different agricultural plants on pollination from honeybees can critically affect food production and the natural biodiversity of plants (Fontaine *et al.*, 2006; Potts *et al.*, 2010). As a result human food security will be at risk, because pollination from insects is needed for different types of fruits, vegetables seeds, and fodder crops (Klein *et al.*, 2007).

Neonicotinoids (imidacloprid, acetamaprid and thiamethoxam) are neurotoxic in target organisms with long term persistence, act through contact as well as systemically and bind nicotinic receptors of acetylcholine esterase ultimately blocking the nerve impulses (Tomizawa and Casida, 2005). These neonicotinoids are mostly used for the control of sucking insect pests of various crops and show very strong effects on (*Apis mellifera* L.) honeybees (Bortolotti *et al.*, 2003; Desneux *et al.*, 2007; El Hassani *et al.*, 2008; Maini *et al.*, 2010). Spinosad is a bio-insecticide which is derived from the fermentation of the bacteria "*Saccharopolyspora spinosa*" and is used to control many caterpillars and sucking insect pests of vegetables and fruits by acting on the nicotinic receptors of the target organisms (Salgado,

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1997; Thompson *et al*., 1997). The current laboratory bioassays were conducted to assess the residual contact toxic effect of imidacloprid, acetamaprid, thiamethoxam and spinosad on mortality of honeybee workers because these insecticides are commonly being used on different field crops in Pakistan (Nasreen *et al*., 2005).

Materials and methods

Insecticides

Commercial formulations of imidacloprid, acetamaprid, thiamethoxam and spinosad (Table I) were purchased from their respective manufacturing companies to check their residual contact toxicity to *Apis mellifera* L. under laboratory conditions. Individual stock solutions for each of four insecticides (1000, 500, 250 and 125ppm) were prepared in acetone. These concentrations (ppm) were made according to active ingredient of the commercial formulated insecticides.

Table I.- List of insecticides with formulations and chemical groups used in bioassays.

S.No.	Insecticide	Formulation	Group
1	Imidacloprid	Confidor@200SL	Nicotinoid
2	Acetamaprid	Mospilan@20 SP	Nicotinoid
3	Thiamethoxam	Actara@25 WG	Nicotinoid
4	Spinosad	Tracer@240 SC	Bio Insecticide

Test bees

Adult honeybee workers of *Apis mellifera* L. were collected from the bees' hives from University of Agriculture, Faisalabad- Pakistan. The hives at the time the bees were collected were free of diseases. No hive treatments to control diseases were conducted before collection of bees. Hives were exposed to smoke twice for 30–60 sec prior to collection. Honeybees were collected into a plastic container and were brought to laboratory. These bees were fed upon 50% sucrose solution in the laboratory. The bees were immobilized by keeping them in a deep refrigerator for about 5 minutes. The bee workers were allowed to recover from cold treatment before exposure. The bees were maintained at 28±2°C and 65±5% R.H under constant darkness in the laboratory for about 20 minutes prior to experiments.

Insecticides exposure procedure

Stock solutions of different insecticides according to their active ingredients in ppm (1000, 500, 250 and 125) were prepared in acetone which was put in flask and a calculated amount of insecticide were added into this solvent through micropipette. The surface residual method in glass jars (Radwan and Taha, 2012) with some modifications was used for testing contact toxicity of insecticides to honeybees (*A. mellifera)*. Ten ml from each stock solution was taken in an injection syringe and was applied into each jar for contact toxicity. Uniform and complete spread of the solution over the inner surface of the jars was ensured. 20 bees were released into each jar after it was completely air dried. Besides these concentrations, there was a control treatment with acetone only. Muslin cloths were cut into small pieces which were easily adjustable on mouth of jars. These jars were placed on smooth and clean surface at 28±2 ^{0}C in the dark immediately after treatment application.

Data analysis

Mortality of honeybees was assessed after 3, 6, 12, and 24 hrs post exposure. Total number of dead bees was counted as mentioned in results below. Statistical analysis was performed using probit procedure (Finney, 1971) to determine the median lethal concentration (LC50), 95% Confidence interval and Chi- square goodness- of- fit test for each insecticide tested. Each LC50 determination was based on the four different concentrations of commercial formulated insecticides. The percent mortality was calculated and corrected using Abbott's formula (Abbot, 1925) as follows:

$$\text{Corrected mortality \%} = \frac{\text{Observed mortality - Control mortality}}{\text{100 - Control mortality}} \times 100$$

Results and discussion

Recently dramatic losses in honeybee populations have been recorded worldwide due to colony collapse disorder (Oldroyd, 2007) and bee species have been reported to vary in susceptibility to insecticides (Mayer *et al*., 1998; Devillers *et al.*, 2003). The LC_{50} is used to determine the appropriate field doses of pesticides used near beekeeping areas. The current bioassays were performed according to the requirements and criteria set by EPPO (1992), with the honeybees' mortality rate less than 10% in control treatments.

The results of present studies are presented in Table II and showed that these insecticides were found to be very toxic against *Apis mellifera* L. adult workers at different exposure time periods and concentrations used. The results obtained were significantly different; $P<0.001$ with highly toxic effects even after minimum exposure time period of 3 hrs. However, maximum toxicity was recorded after 24 hours when bees were exposed to different concentrations of insecticides. The results revealed that the mortality of test bees increased with increase in concentrations of insecticides and exposure periods. The LC_{50} value of imidacloprid recorded was 16.6 ppm, for acetamaprid it was 38.7, thiamethoxam showed 24.6 ppm and LC_{50} of spinosad was 13.5 ppm

Table II.- Toxicity of different insecticides against *Apis mellifera* L. by surface residual method at different time intervals.

Insecticides	Time (Hrs)	LC_{50}	Slope±SE	X^2	F. C. I (95%)	P-Value
Imidacloprid	3	798.9	0.00150±0.00026	0.221	642.5-1088.9	<0.001
	6	369.7	0.00194±0.00029	0.194	272.9-458.5	<0.001
	12	253.2	0.00285±0.00060	0.014	137.9-341.2	<0.001
	24	16.6	0.00353±0.00062	0.162	-196.3-112.0	<0.001
Acetamaprid	3	828.3	0.00150±0.00026	0.933	743.3-1124.4	<0.001
	6	441.4	0.00192±0.00028	0.318	312.6-690.3	<0.001
	12	210.9	0.00255±0.00040	0.212	144.5-394.4	<0.001
	24	38.7	0.00349±0.00079	0.196	-112.4-156.6	<0.001
Thiamethoxam	3	711.8	0.00114±0.00025	0.452	563.7-993.2	<0.001
	6	316.5	0.00159±0.00027	0.251	183.7-421.7	<0.001
	12	100.6	0.00192±0.00032	0.15	12..9-196.8	<0.001
	24	24.6	0.00224±0.00062	0.074	-61.9-116.7	<0.001
Spinosad	3	768.2	0.00150±0.00026	1.090	644.7-959.7	<0.001
	6	441.4	0.00192±0.00028	0.318	350.0-534.5	<0.001
	12	175.7	0.00212±0.00035	0.218	44.66-264.4	<0.001
	24	13.5	0.00273±0.00046	0.184	-202.9-107.5	<0.001

Note: LC_{50} are expressed as ppm of solution, F.C.I, Fiducially Confidence Interval and P<0.001, results are highly significant.

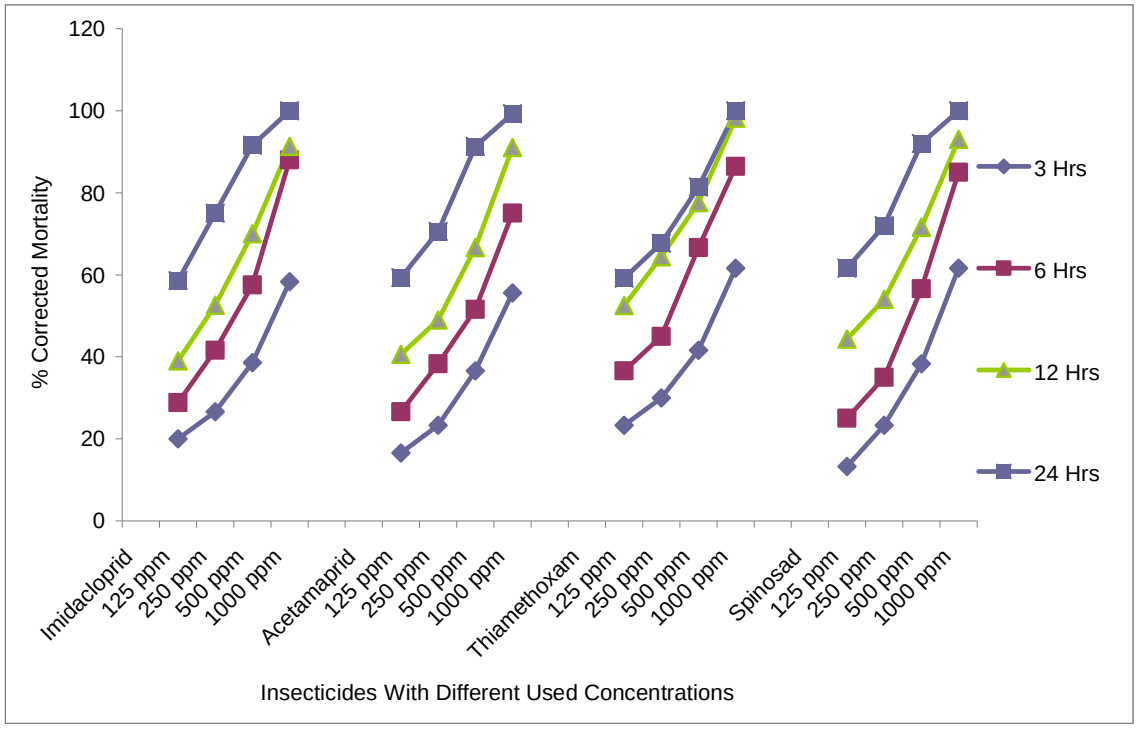

Fig.1. Comparison of contact toxicity of imidacloprid, acetamaprid, thiamethoxam and spinosad against *Apis mellifera* L. at different concentrations (125 ppm, 250, 500 and 1000 ppm). Each specific time interval showing mortality of test bees with different concentrations of insecticide used.
Note: Tukey HSD at 0.05% of significance was performed for statistical test.

after maximum exposure period of 24 h. However, there was major variation in LC_{50} and mortality of test bees after 3, 6 and 12 h, respectively. The comparison of % corrected mortality of test bees at different concentrations and exposure time periods is given in Figure 1.

The toxicity of nicotinoids and spinosad to

honeybees has been reported previously from many studies which are in full agreement to our current findings and showed highly toxic effects of these insecticides to honeybees. For example, the LD_{50} values for imidacloprid were in the range of 0.0067-0.0239 ppm after 24 hours (Stark *et al.*, 1995; Suchail *et al*., 2000) in the previous studies. The sub-lethal effects of imidacloprid such as trembling, tumbling and lack of co-ordination in honeybees were recorded as well by Suchail *et al.* (2001) after 24 h of its exposure. The results of Iwasa *et al.* (2004) are also in full support to our present findings and showed that imidacloprid caused significant toxicity to honeybees by contact exposure with LD_{50} of 0.008 ppm under laboratory conditions. Similarly, Senn *et al*. (1998) reported the highly toxic effect of thiamethoxam to honeybees with a LD_{50} value of 0.024 ppm after maximum time interval of exposure. However, these previous findings showed much lower values of LD_{50} as compared to our results due to different application procedures of these insecticides to honey bees as they directly injected the different concentrations of insecticides on thorax of honey bees. The findings of Bailey *et al*. (2005) also showed significant residual contact toxic effects of imidacloprid and thiametxomam to honeybees under laboratory conditions which are also in accordance with current studies. The results of previous studies (Halsall and Gray, 1998; Miles, 2003; Morandin *et al.*, 2005; Scott-Dupree *et al*., 2009) have reported highly significant effects of spinosad to honeybees by contact routes of exposure. However, the dry residues of spinosad has been considered safe (Mayer *et al*., 2001) to bees. The laboratory assessments of contact toxicity of insecticides are only one measure to check the potential impact of insecticides, and mortality of honeybees can differ partially or completely under field conditions due to different abiotic factors (Scott-Dupree *et al*., 2009). However, these current investigations showed that these insecticides were harmful and caused 98-100% mortality of test bees with maximum concentration used (1000 ppm) after 24 h exposure time when adjusted to their commercial formulated field dose applications.

Conclusion

The present investigations of contact toxicity of insecticides to honeybees (*Apis mellifera* L.) showed that all insecticides tested, proved to be highly toxic when bees were exposed to different concentrations of insecticides with surface residual method under laboratory conditions and point the urgent need of their limited use during blooming periods of flowers. So the current findings suggest that there is need to conduct consistent reviews of different insecticides which are bring used on different field crops to ensure sustainable development and management of beekeeping for better pollination.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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