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Effects of Pesticides on Agrobiont Spiders in Laboratory and Field

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Abstract.- Present study was designed to evaluate the toxicity of Confidor and Buctril-M on two dominant spiders of wheat ecosystem of Punjab, Pakistan *i.e., Lycosa terrestris* Butt, Anwar & Tahir, 2006 and *Oxyopes javanus* Thorell, 1887. Residual toxicity of pesticides on these spiders in the laboratory and in the fields was also studied. Buctril-M was found less toxic compared to Confidor for both species. Mortality of both spider species declined with the increase of post sprayed time for both pesticides. Altogether we captured 2260 individuals of *L. terrestris* (707 from treated field and 1553 from control field) -. Similarly, in total of 637 individuals of *Oxyopes javanus* were collected (212 from treated and 425 from control) from the studied plots. It is concluded that toxicity of Confidor is higher than Buctril-M. Moverover, Confodor remains active for longer time in the field compared to Buctril-M.

Keywords: Confidor, Buctril-M, toxicity, spiders.

INTRODUCTION

In agro-ecosystems communities of natural enemies are usually affected by pesticide application, either due to direct contact or drifting of spray. Many studies have reported the adverse effects of pesticides on non target organisms including natural predators that feed on herbivorous insect pests (Paul and Thygarajan, 1992). The extensive use of pesticides may reduce the efficiency of natural predators in the agro-ecosystem. Pesticides may also cause death of boil-control agents or may change their longevity, reproduction, development, physiology and mobility (Moura *et al.*, 2006; Tahir *et al.*, 2012).

Spiders are most dominant and important bio-control agents that suppress the populations of insect pests of different crops (Holland *et al.*, 2004; Schmidt *et al.*, 2004; Pearce and Zalucki, 2006; Chauhan *et al.*, 2009; Tahir and Butt, 2009). They feed on variety of small sized and soft bodied preys without inflicting damage to field crops (Holland *et al.*, 2004). Spiders are important due to their abundance and high predatory potential (Nyffeler and Benz, 1987; Wise, 1993). Pesticides affect the populations of spiders in all types of crops.

In the fields efficacy of pesticides depends on several factors including types of the solvent, soil type, moisture, organic matter and temperature of time of day of spraying. Furthermore, the hunting style, prey preference and behaviour of spiders also influence their response to pesticide application (Marc *et al.*, 1999). Although pesticides are not ecologically desirable yet they are still most effective means of combating insect pests. Spiders are less resistant to insecticides than some insect predators (Toft and Jensen, 1998). Field application of pesticides often led to reduce diversity of spiders for several weeks (Dinter and Pöehling, 1995). Some agrobiont spiders, however, recolonize the habitat quickly.

Although wheat aphids seldom cross the economic threshold level (Nawaz, 2000) but the recommendation for insecticide application against the cereal aphids by the agrochemical sector in Pakistan has adversely affected biodiversity and abundance of natural predator's fauna. Aphids are the major insect pests of wheat crop. Confidor 20SL, the first neonicotinoid insecticide, is particularly effective against sucking insect pests such as aphids, whiteflies, several beetles and flies with its systemic and broad-spectrum activities. Although use of Confidor against sucking insects is considered safe for natural predators of pests including spiders, predatory beetles and bugs (Hough- Goldstein and Whalen 1993; James, 1997; Kunkel *et al*., 1999; James and Vogele, 2001; Elzen,

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2001), but some studies reveal that confidor is highly toxic to spiders (Mizell and Sconyers, 1992; Stark *et al*., 1995; Delbeke *et al*., 1997; Sclar *et al*., 1998; James and Coyle, 2001). The extensive use of confidor in wheat crop may reduce the diversity, abundance and efficiency of spiders. Buctril-M is used to control broad leaved weeds of wheat crop. It is reported that fungicides and weedicides are less effective against the spiders (Yardim and Edwards, 1998).

The application of selective pesticides may prove an important tactic in pest management programs. Therefore, it is imperative to get information at least on the susceptibility/resistance of agrobiont spider species to Confidor (imidacloprid) and Buctril–M (Bromoxynil+MCPA) in wheat ecosystem. In the present study, we assessed the toxicity (topical application method) of Confidor and Buctril-M aganist *Lycosa terrestris* and *Oxyopes javanus*, which are dominant spiders of wheat ecosystems in Punjab, Pakistan. We also studied the residual toxicity of both pesticides on these spiders in the laboratory as well as in the fields.

MATERIALS AND METHODS

Chemicals

In the present study we evaluated the toxicity of Buctril-M (herbicide which is used to control broad leaved weeds in wheat crop) and Confidor (imidacloprid) (a systemic insecticide which used to control variety of soft bodied sucking insect pests of different crops. Field application rates of Buctril-M and Confidor are 0.5 liter per acre (1.250 liter per hectare) and 0.1 liter per acre (0.25 liter per hectare), respectively.

Test organisms

For the study two agrobiont spiders species *i.e., Lycosa terrestris* and *Oxyopes javanus* were selected (Tahir and Butt, 2008). *L. terrestris* is a lycosid spider which spent most of the time on ground. This spider species can climb up on plant to chase its prey (Tahir and Butt, 2009). However, *O. javanus* is foliage living and very active hunting spider.

Collection of test organisms

Both species of spiders were collected using handy vacuum (Simens VK 20C) from un-treated fields of wheat crop at Adaptive Research Farm, Sheikhupura during February through April 2011. Collected specimens were placed separately into the collection bottles (10 cm long and 5.5 cm wide) at room temperature 30±5°C, relative humidity of 50±10% and 12:12 light dark photoperiod. Each bottle was filled with half an inch thick layer of moist sand to maintain humidity. Mouth of each bottle was covered with thin cotton cloth. Spiders were fed with different larvae of insects until used in the different trials. Only the adult males and females were used in tests.

Topical exposure

In all bioassays, four concentrations of Confidor and Buctril-M were topically applied i.e., field rate, half field rate, one third and one fourth of field rate. Concentrations less than field rate represented the effect of spray drift on the spiders. Field rate was based on 100 liter of water spray per acre (250 liter per hectare). Dilutions of pesticides were prepared in water. Prior to pesticide application, spiders were anesthetized by carbon dioxide from a cylinder supply. A twenty seconds exposure was sufficient to immobilize a batch of ten spiders for approximately two minutes. By using syringe, one droplet of 0.5 µl of a pesticides concentration was applied topically onto the dorsum of each spider. Control group was treated only with distilled water. After the application of pesticides, spiders were placed singly in glass bottles at room temperature. Food was not offered to the spiders during the test. Each experiment was repeated thrice on different days. Spiders were examined at different time intervals till 24 hours after the exposure. The mortality was defined as no movement observed after stimulation.

Residual toxicity

To evaluate the toxic effects of pesticide residues in the laboratory, wide mouth pots (10cm diameter) were used. All pots were filled with soil collected from the wheat fields. Each pot was sprayed either with field rate of Buctril-M or Confidoror using knapsack hand sprayer. To

simulate natural degradation under natural conditions, the pots were kept outside the laboratory in open space. We sheltered the experimental pots from direct sunlight and rain. Control containers were sprayed with water only. Spiders were released in these pots after 4h or 1, 2, 5, 10 and 20 days of spray. At each experiment, 20 specimens of spiders were used and experiment was repeated twice. During the experiments no feed was provided to the spiders. Mortality of spiders due to pesticide residues was assessed after 24 hours.

Field assays

Field study was conducted from December, 2010 through April, 2011in Herdev village of district Sheikhupura. Twelve wheat plots, one acre each, were randomly selected for the field assays. Wheat variety 'Inqilab 91' was sown through drill during the last week of November, 2011. Recommended doses of fertilizers (NPK at the rate of 128, 114 and 62 kg per hectare), as approved by the agriculture department, were used in all the fields. From selected field we sprayed six plots randomly on January 9, 2011 with herbicide Buctril-M at the field rate (500 ml per acre), recommended by manufacturing company using knapsack hand sprayer. Then on March 9, 2011 herbicide treated plots were sprayed with confidor at the field rate of 100 ml per acre to control wheat aphids. Control plots (remaining six) were not treated with any pesticide. However, other inputs such as seed rate, variety, fertilizers and irrigations etc. were kept constant at all sampling plots. All the wheat plots were harvested in the last week of April, 2011.

To study the effects of pesticides on density of spiders we used pit fall traps and visual search method. Each pitfall traps consisted of 250 ml glass jar (6 cm in diameter and 13 cm in depth) and contained 150 ml of 70% ethyl alcohol. Few drops of 5% liquid detergent were added to each pitfall trap to lower the surface tension. Sixteen traps were operated in each plot in four parallel rows. The distance between two rows and in traps within a row was 15 meter. The traps were emptied after 72 h (trapping session) and reinstalled after twelve days. During visual searching from each plot, 20 tillers of wheat were selected randomly and searched for *L. terrestris* and *O. javanus* thoroughly. Foliage sampling was done at the next day of pitfall traps installation. Sampling of spiders from foliage of plants was commenced on January 30, 2011 and continued till the harvesting of crop. Collected spiders were brought to the laboratory and identified to species level.

Statistical analyses

The percent spider mortality was calculated for both pesticides. Results obtained from dose response series or time response series after 24 hours were subjected to Probit Analysis (Lichfield and Wilcoxon, 1949) to determine LD_{50} and $LT_{50.}$). The normality of the field data (log N+1 transformed) was checked using Kolmogorov–Smirnov test. Field data were subjected to general linear model ANOVA to estimate the difference in the abundance between treated and control fields, different plots of the control and treatment and in trapping sessions. Variation in the mortality of both agrobiont species was assessed using t-test. All statistical analyses were done by using Minitab package 14.

RESULTS

Topical exposure

The susceptibility of spiders against Confidor increased both with amount of insecticide applied and time period in experiments (Fig. 1). In *L. terrestris*, 75 percent mortality was recorded at field rate concentration after 24 hours. However, in *O. javanus* we observed 65 paercent mortality for the same concentration and dose. The calculated LD50 values for *L. terrestris* and *O. javanus* were 0.47 (0.43-0.50) ml per liter and 0.62 (0.57- 0.67) ml per liter, respectively (Table I). Statistically susceptibility of two species against Confidor did not differ ($t=2.61$, $P=0.08$). Buctril-M was found less toxic for the studied species (Fig. 1). The calculated LD50 value for *L. terrestris* was 15.63 (11.53-18.63) ml per liter, and for *O. javanus* 20.02 (14.31- 25.71) ml per litre (Table I). The susceptibility of the studied species did not differ significantly from each other ($t = 1.78$; $P>0.05$).

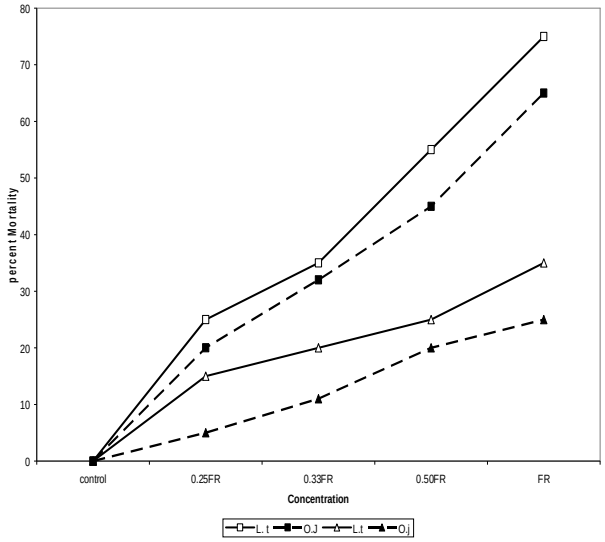

Fig. 1. Response of spiders to different concentrations of pesticides exposed. ■ represent mortality of *L. terretris* and □ *O. javanus* to cofidor. Mortality due to Buctril- M was represented by ▲in *L. terretris* and Δ in *O. javanus.* (FR = Field application rate)

Table I.- LD_{50} of Confidor and Butril-M for the agrobiont spider species,

Insecticide/ Species	LD50 ± S.E. (ml per L)	Slope	Chi square	Probability
Confidor				
L. terrestris	0.47±0.04	1.626	0.819	0.664
O. javanus	0.62±0.05	1.473	0.779	0.601
Buctril-M				
L. terrestris	15.63±3.60	0.861	1.68	0.43
O. javanus	20.02±5.96	1.07	3.86	0.19

Residual toxicity

Mortality of both spider species declined with the increase of post sprayed time for both pesticides (Figs. 2, 3). Residual toxicity of Confidor differ significantly between two species (t=15.65, P<0.001). In *L. terrestris*, after 2.65 days of confidor spray, mortality rate become less than 50 percent. While *O. javanus* appeared more resistant to the confidor and its survival rate increase more than 50 percent just after 2.17 days (Fig. 2). The residual toxicity of Buctril-M was not different for both spider species (t= 1.75, P=0.154, Fig. 3). LT_{50} values of both spider species against Confidor and Buctril-M are given in the Table II.

Table II.- LT_{50} of confidor and butril-M for spider species.

Insecticide/ Species	LD50 ± S.E. (ml per L)	Slope	Chi square	Probability
Confidor				
L. terrestris	2.68±0.27	-1.009	2.61	0.623
O. javanus	2.17±0.22	- 0.995	1.45	0.834
Buctril-M				
L. terrestris	-0.62±0.38	- 0.551	1.46	0.832
O. javanus	-1.83±0.67	- 0.452	1.68	0.793

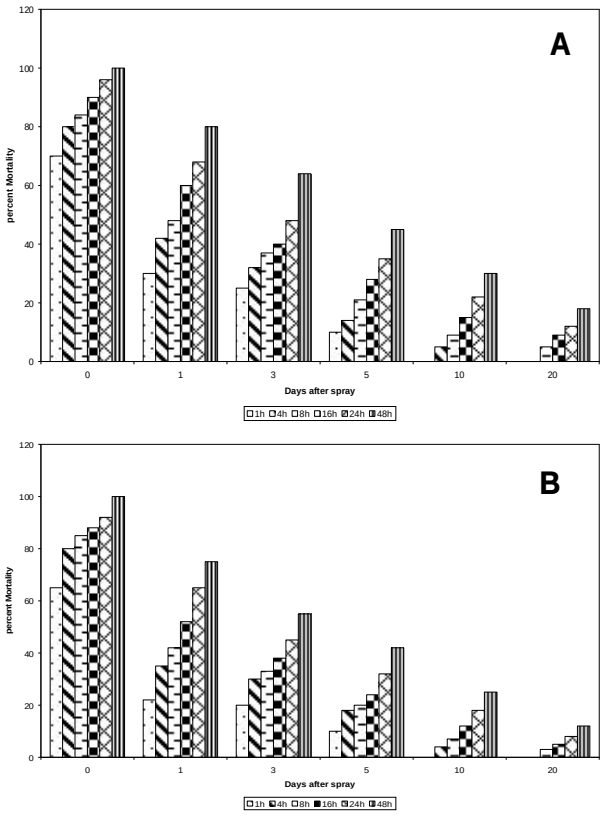

Fig. 2. Laboratory Response of *L. terrestris* (A) and *O. javanus* (B) against confidor residues of different age.

Field assay

During the study, 2260 individuals of *L. terrestris* (707 from treated field and 1553 from control field) were collected from all the 12 plots. However, the abundance of *L. terrestris* did not

differ in all studies plots (P>0.05). In contrast to plot type, the density of *L. terrestris* was negatively associated with the insecticide spray ($F_{1,104}$ = 220.41, P<0.001). The abundance of *L. terrestris* (Fig. 4) also varies with the trapping session ($F_{9,104}$ = 42.18, P <0.001). Paired t-test showed that in each trapping session the number of wolf spider varies in treated and control plots just after the application of insecticides confidor (t=2.44, P=0.037; Fig. 4A).

A total of 637 individuals of *Oxyopes javanus* were collected (212 from treated and 425 from control) from the studied plots. Results showed significant difference in the number of *O. javanus* in treated and control plots ($F_{1,82}$ = 193.26, P = 0.000) and in different trapping sessions ($F_{7, 82}$= 81.91, P<0.001). Comparison of each trapping session of treated and control plots also showed significant difference in the density of *O. javanus* after the application of confidor (t=2.85, P=0.025; Fig. 4B). However, all types of studied plots were similar to each other.

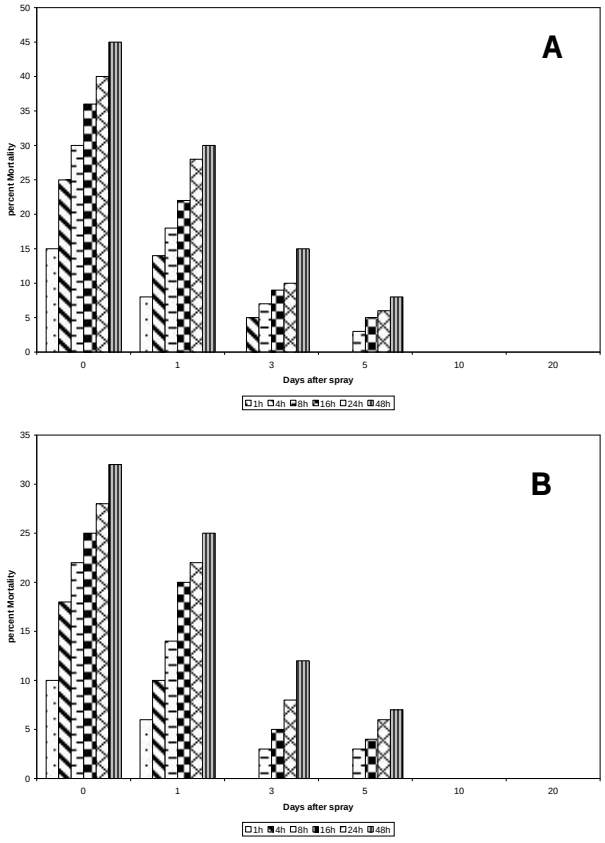

Fig. 3. Laboratory Response of *L. terrestris* (A) and *O. javanus* (B) against Buctril-M residues of different age.

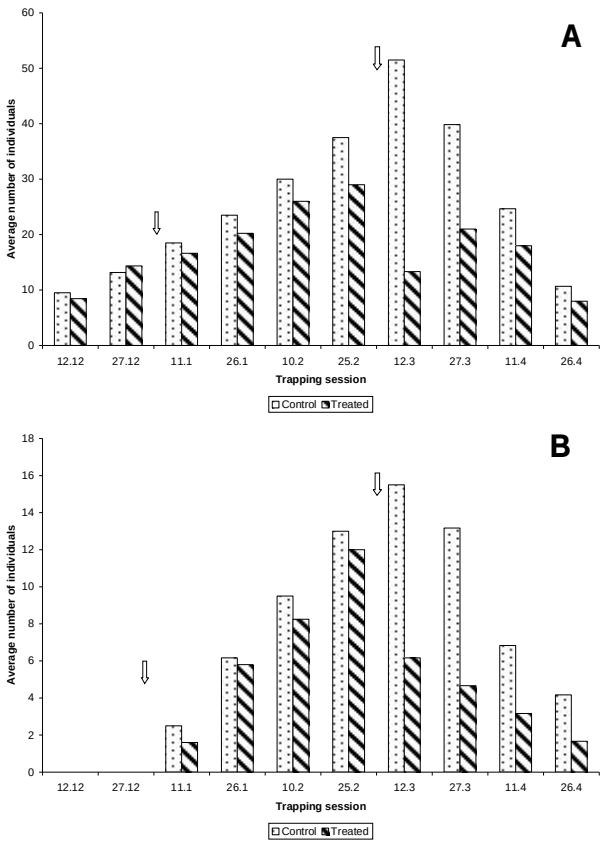

Fig. 4. Effect of pesticide on the abundance of *L. terrestris* (A) and *O. javanus* (B) after the treatment of crop field. In A and B first arrow represents spray of Buctril-M and 2nd arrow of Confidor.

DISCUSSION

Spiders are highly sensitive to different insecticides in field and laboratory conditions (Amalin *et al.,* 2000; Shaw *et al*., 2006; Marshall, 2006). In our study susceptibility of agrobiont spiders species (*Lycosa terrestris and Oxyopes javanus*) against two pesticides (Confidor and Buctril-M) was investigated in the laboratory and field. The LD_{50} values of topical exposure showed that confidor is highly toxic at field rate in the laboratory. Approximately half concentration of field rate was enough to kill 50% population in the

laboratory. Tietjen and Cady (2007) reported that toxicity to spiders ranged from less mortality for herbicides to medium mortality for pyrethroid and organophosphate and to high mortality for cyclo-compounds.

Spiders are known to be relatively resistant to starvation (Wise, 1993), therefore individuals in the present experiment were fed to satiated level. However, there may be still variation in the degree of satiation between experimental animals. Pederson *et al.* (2002) found that starved *Pardosa amentata*, or individual fed on low quality prey, were more susceptible to the effect of dimethoate.

The results of present study showed that residual toxicity of Confidor was higher than Buctril-M. The post spray exposure showed that reduction in mortality was very steep in Buctril-M than Confidor. The difference in mortality may be due to difference in the interaction of pesticides with the substrate and environment in the field.In the short term, absorption to soil and volatilization from soil will determine the fate and bio-degradability of pesticides (Arnold and Briggs, 1990).

Toxicity of confidor was higher than Buctril-M in topical and residual toxicity. The toxicity of weedicide is less for both species. However, imidacloprid was proved toxic for both species. The susceptibility of both species varies. The susceptibility of *L. terrestris* was more compared to the *O. javanus*. The difference in the susceptibility may be due to difference in the insecticide detoxifying enzymes. Furthermore, *L. terrestris* is exposed more to the insecticides compared to *O. javanus* as it not only reside on the ground but also spent time on foliage to search prey.

In field experiment, the application of herbicide did not affect the population of both species. However, the population of both species declined immediately after the application of confidor. However, recolonization took place just after 15 days. After that the population of both species again declined due to maturity of wheat crop in April.

LT_{50} of Confidor was more as compared to Buctril-M. So confidor exists long time in the soil. That is why residual toxicity of Confidor was higher than Buctril-M. However, these different performances of pesticides cannot be transferred to the field situation, where many other complex environmental factors are also working simultaneously. However, the current work has urged the need to evaluate the impact of other pesticides, and the effect of different mode of exposures (topical, residual and ingestion) in order to gain more realistic information of what may occur in pesticide treated crop. Furthermore, field based assessments are required to provide the most reliable results to be extrapolated into real environmental situation.

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