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Effect of Prey Density and Insecticides on Prey Consumption by *Cyrtophora citricola* (Araneae: Araneidae)

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Authors' Contribution
MKM and HMKM conceived, designed and supervised the study. HI and HGM conducted lab experiments. MI collected specimens from the field. HMT analyzed the data. MKM and HI wrote the article.

Key words
***Cyrtophora citricola*, Chlorpyrifos, Bifenthrin, Prey consumption.**

ABSTRACT

In the present study effects of prey density and sub-lethal doses of two commonly used insecticides *i.e.*, bifenthrin and chlorpyrifos, on the consumption rate of web weaving spider *Cyrtophora citricola,* was observed. Live spiders for the study were collected from citrus orchards of Sargodha and maintained individually in the laboratory. Both the control and insecticide treated spiders were offered larvae of *Drosophila melanogaster* in different densities. *C. citricola* increased prey consumption as the prey density was increased. Insecticide treated *C. citricola* consumed less prey as compared to control. Effects of chlorpyrifos on the prey consumption of *C. citricola* were more drastic than bifenthrin. It was also recorded that at low prey density, predation by *C. citricola* was delayed as compared to higher prey densities. *C. citricola* showed the type I functional response. It is concluded that both studied insecticides are not suitable for IPM program in the study area.

INTRODUCTION

Agriculture plays a significant role in the economy of Pakistan and many other countries. Insect pests inflict severe losses to our crops, fruits and vegetables that badly reduce their yield. Insecticides are applied in huge quantity to control these pests that pose a heavy burden on country's economy and also causing many health issues to humans. The commonly used, more toxic, insecticides are usually lethal to spiders, predators of insect pests (Mukhtar *et al*., 2013). The insecticides that are usually considered harmless and safe also interfere with the growth, predation potential and other behavioural aspects of spiders (Pekar, 2012).

Spiders are diverse and abundant animals on land and can play important role in controlling insect pest in agro ecosystem. They reduce prey number not only by consuming them directly but also due to wasteful killing and top down effect. Their presence in the field reduces pest attack without killing them as the insect cannot feed properly due to the fear of spiders (Maloney *et al*., 2003; Ghavami, 2008; Chatterjee *et al*., 2009; Michalko and Pekár, 2016). These are mostly generalist predators but some spiders are stenophagous that can check the population of certain insect pests (Líznarová *et al*., 2013; Pekár and Toft, 2014; Petráková *et al*., 2015). They mainly feed on insects (Marc *et al*., 1999). These all mentioned qualities make them a choice of natural predator to be used in Integrated Pest Management (IPM).

Functional response demonstrates the *per capita* eating rates of predators depending on prey density (Vucic-Pestic *et al*., 2010). It is one of the most essential behavioural features in predator prey interactions that disclose different characteristics of prey-predator interactions (Jafari and Goldasteh, 2009). Functional responses had been reviewed by different researchers since the 1920s (Holling, 1966; Royama, 1971). However, the term "functional response" was first introduced by Solomon (1949). There are three types of functional response *i.e.*, Type I (linear), Type II (Hyperbolic), and Type III (Sigmoid). These responses explain how consumption rates differ with prey density (Maloney *et al*., 2003).

Samu and Biro (1993) studied the functional response of wolf spider, *Pardosa hortensis,* under different prey densities. It indicates a Type II functional response and concluded that spiders had positive role in controlling agriculture pests in a density sensitive manner. Functional response model revealed that predation rates can be decreased by satiation when prey density is increased (Essington *et al*., 2000).

Rezac *et al*. (2010) studied the effect of five insecticides on the functional response of *Philodromus*

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cespitum. They concluded that three insecticides (Dimilin, NeemAzal and SpinTor) weakened the activity of spider while two insecticides (Mospilan and Integro) did not affect the functional response of spider, so could be used in Integrated Pest Management (IPM).

Before using spiders in IPM it is important to evaluate the effect of prey density and different pesticides on prey consumption (functional response) of spiders. In Pakistan very little work has been done on the effect of insecticides on predatory performance of spiders. The present study is aimed at to evaluate the effect of prey density and pesticides (bifenthrin and chlorpyrifos) on the functional response of *Cyrtophora citricola* (Forsskål, 1775). This web weaving spider is selected as it is common in citrus orchards of Sargodha and could play a significant role in insect pest suppression. Outcome of this study will be helpful to conclude that whether the studied insecticides are safe to use in IPM of insect pest or not.

MATERIALS AND METHODS

Spider collection and their maintenance

Study was conducted from September 2011 to July 2012 at the Department of Zoology, University of Sargodha, Sargodha. For the study, live *Cyrtophora citricola* were collected from unsprayed citrus orchards of Chak No. 75 SB (25km from Sargodha). *Cyrtophora citricola* (Forsskål, 1775)*,* is a common orb-weaving spider in citrus orchard in study area. Spiders were collected by direct hand picking or by jerking the plants on a large white cloth sheet. Spiders were transferred to large plastic jars. The jars were covered with muslin cloth for aeration. In the laboratory spiders were identified using the key available in Tikader (1982).

In the laboratory spiders were kept individually in separate glass plastic jars (10cm long and 8cm wide) at room temperature. The mouths of glass plastic jars were covered with muslin cloth for aeration. To maintain humidity (65+5%), a small pieces of wet cotton was placed over each jar. Before using the spiders in the experimental trials, they were first fed with *Drosophila melanogaster* larvae to satiation level for two days, and then starved for three days to standardize their hunger level.

Insecticides

Two insecticides *i.e.*, bifenthrin (Talstar) and chlorpyrifos (Lorsban) used in the study were purchased from local market. Sub-lethal concentrations of bifenthrin (1ml/50 l water) and chlorpyrifos (5ml/20 l water) were used. Sub-lethal dose were determined by conducting the bioassay tests against various concentrations of selected insecticides. These concentrations did not cause mortality of spiders but may affect their behaviour.

Determination of effect of prey density on the consumption by C. citricola

To test the effect of prey density on the prey consumption rate of *C. citricola,* five plastic jars were taken and numbered 1 to 5. A single spider was added in each jar. Spider of jar 1 was offered one larva of *D. melanogaster*. However three, five, 10 and 15 larvae were added in the jar 2, 3, 4 and 5, respectively. The number of prey consumed by the spiders in each jar was recorded after every four hour till 24 h. plastic jars were covered with muslin cloth for proper aeration. *D. melanogaster* were reared in the laboratory. Larvae of uniform age were offered to the spiders as prey.

Determination of effect of insecticide on the consumption by C. citricola

To evaluate the effect of insecticides (bifenthrin and chlorpyrifos) on feeding performance of *C. citricola,* filter papers (140mm) were dipped into the sub-lethal dose of bifenthrin (1ml/50 liter water) or chlorpyrifos (5ml/20 liter) and allowed to dry at room temperature. The filter papers were placed in Petri plates (150mm x 15mm) and spiders were exposed to dried filter paper for one hour. After that each spider was placed in separate plastic jars (10cm long and 8cm wide) and jars which were numbered 1-5. Rest of the experiment was same as described in the above experiment. Experiments were repeated three times.

Statistical analysis

First normality of the data was assessed and then Mann–Whitney U test was used to compare the consumption rate of control and bifenthrin or chlorpyrifos treated spiders. We also compared the consumption rate of bifenthrin and chlorpyrifos treated *C. citricola* using same test. Statistical software MINITAB 14 was used for analyzing the data.

RESULTS

The results of the study showed that prey consumption rate of *C. citricola* were increased with the increase of prey density. When one larva was offered, it was consumed after 4 h, whereas when three larvae were offered, spiders consumed two of them within 8 h. When five larvae were offered, spiders consumed four of them within 8 h and 5th larva was not consumed by the spider even after 24

h. When 10 and 15 larvae were offered, spiders consumed seven and 11 of them, respectively within 16 h. After this time remaining larvae were not consumed by the spiders (Fig.1).

It was observed that with the application of sub-lethal dose of bifenthrin, the prey consumption by *C. citricola* was delayed and the delay was more pronounced in smaller prey densities (Fig. 2A; Table I). However, the results of Mann–Whitney U test showed that although bifenthrin treated *C. citricola* consumed less preycompared to control but statistically the difference was non-significant (*P> 0.05*) (Table II).

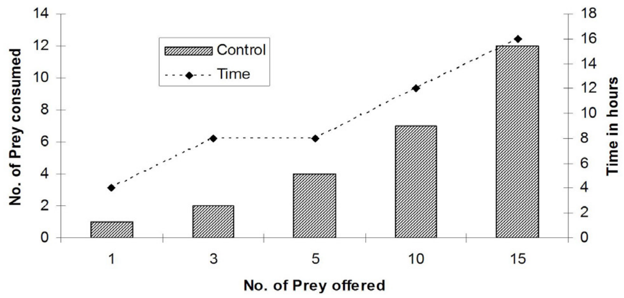

Fig. 1. Effect of prey density on the consumption rate of *C. citricola.*

Table I.- Number of prey consumed by *Cyrtophora citricola* treated with bifenthrin, chlorpyrifos and water.

Time (h)	Treatment	No of prey consumed at prey density				
		1	3	5	10	15
4 h	Control	0.00	0.00	0.33	1.00	2.33
	Bifenthrin	0.00	0.00	0.00	0.00	0.00
8 h	Control	1.00	1.33	1.00	1.00	1,667
	Bifenthrin	0.00	0.00	0.00	0.33	1.66
12 h	Control	-	1.66	1.00	2.00	1.00
	Bifenthrin	0.33	0.00	1.33	2.33	1.66
16 h	Control	-	-	1.00	1.33	1.33
	Bifenthrin	0.66	1.00	0.33	1.00	1.00
20 h	Control	-	-	0.667	1.00	1.33
	Bifenthrin	-	1.00	2.00	1.00	1.33
24 h	Control	-	-	-	0.667	1.00
	Bifenthrin	-	0.00	0.33	0.66	0.33
Total number of prey consumed	Control	1.00	3.00	4.66	7.33	8.00
	Chlorpyrifos	1.00	2.00	2.66	3.66	3.66
4 h	Control	0.00	0.00	0.33	1.00	2.33
	Chlorpyrifos	0.00	0.00	0.00	0.00	0.00
8 h	Control	1.00	1.33	1.00	1.00	1,667
	Bifenthrin	0.00	0.00	0.00	0.33	1.66
	Chlorpyrifos	0.00	0.00	0.00	0.00	0.00
12 h	Control	-	1.66	1.00	2.00	1.00
	Chlorpyrifos	0.00	0.00	0.00	0.00	1.00
16 h	Control	-	-	1.00	1.33	1.33
	Bifenthrin	0.66	1.00	0.33	1.00	1.00
	Chlorpyrifos	0.33	0.33	0.33	1.00	1.00
20 h	Control	-	-	0.667	1.00	1.33
	Chlorpyrifos	0.66	1.00	1.00	1.66	1.66
24 h	Control	-	-	-	0.667	1.00
	Chlorpyrifos	-	0.66	1.00	0.33	0.33
Total number of prey consumed	Control	1.00	3.00	4.66	7.33	8.00
	Chlorpyrifos	1.00	2.00	2.66	3.66	3.66

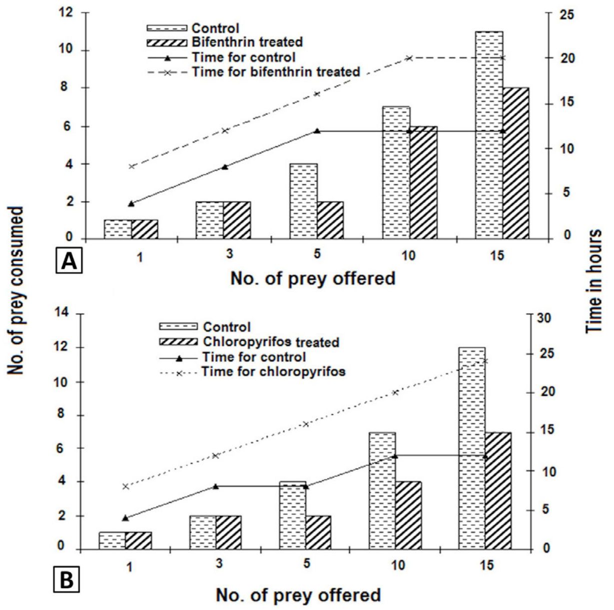

Fig. 2. Effect of bifenthrin (A) and chlorpyrifos (B) on the consumption rate of *C. citricola.*

Similarly, the application of sub-lethal dose of chlorpyrifos also delayed the prey consumption by *C. citricola* as observed in bifenthrin treated *C. citricola* (Fig. 2B; Table I). Significant difference in consumption rate of control and chloropyrifos treated *C. citricola* ($P< 0.05$) was found (Table II). Prey capture by the spiders was much more delayed with exposure to chloropyrifos as compared to bifenthrin and only one prey could be consumed after 12 h even when 15 preys were offered whereas at lower prey densities, prey consumption was delayed till 16 h (Table I). Significant difference was also observed when consumption rate of bifenthrin and chloropyrifos treated *C. citricola* was compared ($P< 0.05$) and the functional response was significantly reduced in chloropyrifos as compared to bifenthrin (Table II).

Table II.- Comparison of mean no. of prey consumed by *Cyrtophora citricola* (a) between Control and bifenthrin (b) control and chlorpyrifos (c) between bifenthrin and chlorpyrifos.

Treatments	Mean prey consumed (±S.E) prey density		
	5 (n=3)	10 (n=3)	15 (n=3)
Control	4.66±0.33	7.33±0.33	8.00±0.57
Bifenthrin	3.33±0.33*	5.33±0.33*	5.66±0.33*
Control	4.66±0.33	7.33±0.33	8.00±0.57
Chlorpyrifos	2.66±0.33*	3.66±0.33*	3.667±0.33*
T-value	2.00	2.50	6.369
P-value	0.184	0.130	< 0.001

DISCUSSION

Our study revealed that *Cyrtophora citricola* respond positively to the increasing prey density, increase rate of prey consumption at higher prey density. At higher density of prey, spider spent less time to capture prey. This was not suspiring as less searching time is required for capturing prey at higher prey densities (Reis *et al*., 2003; Rocha and Redaelli, 2004). Spiders consume more food in the laboratory when prey is offered ad libitum than they take in the field (Nyffeler and Benz, 1988; O'Neil, 1990; Ives *et al*., 1993).

C. citricola is orb-web spiders that make a web to capture (Lubin, 1980). When prey was offered to *C. citricola* it did not consume it for few hours. The delay time in pesticides treated *C. citricola* was higher to control. The delay in prey consumption time in both control and insecticides (bifenthrin and chlorpyrifos) treated groups might be due to the reason that the offered prey may not be preferred prey of the *C. citricola.* Furthermore, more delay in insecticides treated group is due to neurotoxic effects of insecticides which might have affected the web building, feeding and chemical signaling of spiders (Benamú *et al*., 2013).

Our results, that spiders show type II fictional response is in accordance with the findings of Maloney *et al*. (2003). Some researchers have also reported type III functional response in spiders (Provencher and Coderre, 1987; Breene *et al*., 1990). In the field spiders may exhibit various types of functional responses depending upon type, size, nutrients and the density of prey. Feeding behavior of predator may also differ in the laboratory and field.

Insecticides directly affect locomotion, predation, web-building, reproduction, development and physiology of spiders (Amalin *et al*., 2000; James and Price, 2002; Tietjen, 2006; Deng *et al*., 2008). Similar results that insecticides directly affect the feeding of spiders have been reported by Stark *et al*. (1995) and Rezac *et al*. (2010). Our result that Chlorpyrifos is more toxic to spiders is also supported by the studies of Fountain *et al*. (2007) and Venkateswara *et al*. (2005).

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Statement of conflict of interest

Authors have declared no conflict of interest.

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