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The predatory efficacy of three *Pardosa* spider species against American bollworm (*Helicoverpa armigera*)

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Abstract

The predatory efficacy was observed in the Arachnology laboratory; Department of Zoology, Wildlife and Fisheries, GC University Faisalabad. After mating ten female spiders were selected for research in separate cages. On hatching newly born spiders-lings were climbed on the back of the female spider for 4 days, and then they were separated from the mother back, and a group of seventy separated spider-lings was randomly selected. To prevent cannibalism each spider-ling was introduced into a separate cage. The selected *Pardosa* species was used as a bio-control agent of the American bollworm (*Helicoverpa armigera*). The feeding potential of the *Pardosa* species was determined under controlled conditions against various stages of *H. armigera* insect pests was evaluated. The analysis of the variance regarding the feeding showed that three selected *Pardosa* spider species (7^{th}& 8^{th}Instar) had significant difference ($P < 0.05$) for the predation on 2^{nd} instar of *H. armigera*. The three *Pardosa* spider species (2^{nd} to 8^{th} Instar) had a significant difference for the predation on 1^{st} instar of *H. armigera*.

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Introduction

Most of the agricultural crops are damaged by various insect pests (khushk *et al.*, 1988; Haque, 1991). *Helicoverpa armigera* is highly polyphagous pest (Fit, 1989) and one of the leading pests of horticultural and agricultural crops in Pakistan (Karim, 2000). It has been reported from various countries like USA, Mexico, Manitoba, Africa, Canada, Asia Europe, America, Argentina Peru and Brazil having 182 hosts with maximum population on okra, groundnuts, squash, sorghum, tomato, chili, peas, cabbage, cauliflower, sunflower, maize, chickpea and cotton etc. (Ravi *et al.*, 2005; Gowda, 2005) with a wide range of geographical distribution (Cunningham *et al.*, 1999).

Helicoverpa armigera has been controlled by different insecticides andhas developed resistance against the most of synthetic insecticides due to the continuous use (Forrester, 1994; Ahmad *et al.*, 1997). Further research showed that resistance to endosulfan, profenofos, cypermethrin and chloropyrifos reached its maximum level till 1997 (Ahmad *et al.,* 1998).

The aim of the study was to increase the interest towards the Integrated Pest Management (IPM) to prevent the crops loss of socio-economic importance. In this way, to reduce dependence on chemical pest control, and make sure by elevating the long term sustainability of agro-ecosystem. Several strategies are being used in this approach to reduce or eliminate pest problems. So Integrated Pest Management program need to be enhanced.

Materials and methods

Collection of spiders was performed from the cotton and tomato fields of Ayub Agricultural Research Institute Faisalabad, Postgraduate Agriculture Research Station (PARS), and surroundings of the Faisalabad District, Pakistan. Research work was conducted at Arachnology Laboratory in the Department of Zoology, Wildlife and Fisheries at GC University, Faisalabad.

Rearing of Pardosa species

The identified male and female *Pardosa* spiders were kept in separate mating chamber pair-wise and after mating the male was removed. After incubation, the spider-lings were replaced to the rearing cages (10 cm x 7 cm x 5 cm), a clear perplex freezer dishes (sandwich boxes) 2 cm diameter ventilation hole was covered by mesh No. 4.0 milk strainer cloth (brass screen). Moisture was constantly given to the inner side of the cage by cotton swab.

To avoid from cannibalistic behavior, spiders were placed in separate cages and reared on man-made diet with some modification after conditions optimization. Different instars of *H. armigera* were given after regular period (2 hours). Laboratory conditions were adjusted at 27±2 °C, 70±5 RH and 12:12 L: D photoperiod.

After mating ten female spiders with cocoon were chosen for investigation. On hatching, newly borne spider-lings were attached on the back of the female spider for 2-5 days. After detachment of spider-lings from the back of the mother, a group of 70 spider-lings were separated from every cage for examination and each spider-ling of the group was shifted to a separate cage to prevent cannibalism. The spider-lings were nourished on each instar of *H. armegera* under same laboratory conditions. Daily observations were noted on each cage to know the complete picture of development from selected *Pardosa* species.

Collection of the pests

Eggs and larvae of *H. armigera* were collected from different tomato fields in spring 2012 from Ayub Agriculture Research Institute Faisalabad and brought in the Arachnology laboratory at Zoology, Wildlife and Fisheries department, GC University Faisalabad.

Rearing of Helicoverpa armigera (Hubner)

In plastic vials, larvae were collected from different fields and each vial was covered up with a finely pores mesh to allow the exchange of air. Collected larvae were shifted to the rearing trays (30 cmx15cmx4.5

cm) housed in the Arachnology laboratory, Zoology, Wildlife and Fisheries department, GC University, Faisalabad. Each rearing tray was separated into 24 squared chambers. The lid of the tray had 24 holes (diameter: 2cm) covered up with brass screen for air. The larvae were put in these separate chambers (one larva in each). The chambers were cleaned daily to avoid contamination of the feces excreted by the larvae. The larvae were provided with artificial diets (Table 1) (Gupta *et al.* 2004).

The pupae were replaced to the pupal chamber (18 cmx18 cmx20 cm) having floor covered sand. Pupae were sorted sex wise and kept in separate pupal chambers. Moths gathered from these chambers were kept in glass chimneys having muslin cloth on both openings. A Petri-dish was placed in the bottom of the chimney. Ten to fifteen pairs of moths were kept in single chimney, and given 10% sugar solution as feed present in plastic vial having cotton pad. Sugar solutions swab the cotton pads by capillary action and moths sucked the solution from cotton. Female moths laid eggs and gathered daily from muslin cloth present on the openings of the chimney and were transferred in plastic bags. These bags were marked with date and placed for incubation and adjusted at 27 ± 2 °C, 70 ± 5 % RH and a 12: 12 (L: D) photoperiod.

Newly borne larvae (1st instars), were placed in large sized Petri-dish in which pieces of artificial diet were placed. This was put in a box covered with black cloth. Up to third instars larvae remained in the Petri-dish and then transferred into rearing trays given with artificial diet. Pupae were allowed to develop the larvae which were put in pupa chamber and the whole life cycle was repeated. The purpose of rearing of *H. armigera* was to easily availability of each instar in order to determine predatory efficacy of different instar of *Pardosa* species against various instars of selected prey.

Predator- Prey Interactions

To evaluate the feeding capabilities of predatory spiders on bollworm (*H.armigera*) was tested. Firstly, the live pests were placed in the Petri-dishes (5 cm diametre) on fresh leaves of cotton. Afterward, the predatory spiders of *Pardosa* spp. were released on the separate pests using camel hair brush. As such, five replications (Petri-dishes) were put for knowing the feeding efficacy of predator species which were collected from same cotton plant considering same age of predators. These predators were made hungry for 24hours and then the pests were given for 24 hours. Ten (10) larvae of the American bollworm were given to the each predator. *H. armigera* fed by each predatory spider after 24 hours and predator efficacy was calculated.

To evaluate how much predation on different stages of pest (*H. armigera)* calculated. ANOVA were used to compare the mean predation on different stages of *H. armigera* by different stages of selected *Pardosa* spider spp.

Results

In the present study the predatory efficacy of three selected *Pardosa* species (*Pardosa birmanica, P.leucopalpis* and *P.oakleyi*) belonging to family Lycosidae against different life stages of *Helicoverpaarmigera*was studied under standard laboratory conditions to understand their prey and predator relationship.

Table 1. Ingredients of artificial feed of American bollworm, *Helicoverpa armigera* (GUPTA *et al.* 2004).

Ingredients	Quantity
Chickpea flour	94.0 g
Distilled water	825 ml
Agar	15.0 g
Dried yeast powder	24.5 g
Casein	15.0 g
Ascorbic acid	6.0 g
Methyl-p-hydroxybenzoate	2.0 g
Sorbic acid	1.2 g
Streptomycin sulfate	0.2 g
Cholesterol	0.6 g
Formaldehyde (40%)	1.0 ml
Multivitamin capsules	2 capsule

The Predatory efficacy of Pardosa species

This study was formulated to test the predation of *Pardosa* spider on the American bollworm (*Helicoverpa armigera*) in the laboratory conditions (27± 2 °C and 70±5 RH).

Predation by three Pardosa spider species (7th& 8th instars) on 2nd instars of H. armigera

Three spider species were significantly different for the predation on 2nd instar *of H. armigera*. Two instars (7th& 8th) of *Pardosa* species had significant predation on 2nd instars of *H. armigera*. The interaction between species and instars had non significant difference on 2nd instars of *H. armigera* (Table 2).

The 7th instar of *P. birmanica* had 3.41±0.509 per day consumption rate on 2nd instars of *H. armigera* and 8th instars had per day consumption rate 4.6±0.509.The 8th instars of *P. birmanica* had higher per day consumption rate as compared to 7thinstars of this species. The *P. birmanica* species had a higher predation as compared to other two *Pardosa* species (viz., *P. leucopalpis & P. oakleyi*).The 7th instar of *P. leucopalpis* had 2.4±0.509 per day consumption rate on 2nd instars of *H. armigera* and 8th had a 3.8±.583 per day. The 7th instar of *P. oakleyi* showed (2.2±0.374) per day consumption rate and 8th instar had 2.8±0.374 per day consumption rate. The 7th& 8th instar of *P. birmanica* had higher per day consumption rate which was followed by *P. leucopalpis* and *P. leucopalpis* was followed by *P. oakleyi* (Fig 1).

Predation by both sexes of three Pardosa spider species feeding on 1st& 2nd instars H. Armigera.

The spider species showed non significant difference for the predation of 2nd instars of *H. armigera*. Male and female showed non significant difference for the predation of 2nd instars of *H. armigera*. The interaction between species and sex was non significant for the predation of 2nd instars of *H. armigera* (Table 2).

Table 2. ANOVA for the predation by three spider species (7th and 8th instar) feeding on 2nd instar of *Helicoverpaarmigera*.

S.O.V	d.f	S.S.	M.S.	F.Value	Pr(>F)
Spider species	2	1.198	0.5991	3.970	0.0324*
Instars	1	1.016	1.0162	6.734	0.0159*
Species: Instars	2	0.073	0.0365	0.242	0.7872
Residual	24	3.622	0.1509		

NS = Non significant at P>0.05; * =significant at P<0.05; **=highly significant at P<0.01.

Three adult female *Pardosa* spider species viz., *P. birmanica*, *P. leucopalpis, P. oakleyi* showed 8.4±0.447/day, 7.4±0.678/day, 6.4±0.927/day consumption rate respectively on 1st instar, respectively. The female *P. birmanica* had higher per day predation rate as compared to other two species on 1st instars of *H. armigera*. These three spider species viz., *P. birmanica*, *P. leucopalpis P. oakleyi* showed 5.8±0.663/day, 6.6±0.678/day, 5.6±0.812/day consumption rate on 2nd instars of *H. armigera* respectively. The *P. leucopalpis* showed higher per day consumption rate as compared with other two spider species. The *P. leucopalpis* had a higher per day consumption rate on 2nd instar of *H. armigera* which was followed by *P. birmanica* and *P. birmanica* followed by *P. oakleyi* (Fig 2).

The adult male viz., *P.birmanica*, *P. leucopalpis P.oakleyi* showed 7.8±0.357/ day, 7±0.707/day, 6.2±0.583 consumption rate respectively on 1st instars of *H. armigera*. The *P. birmanica*had a higher per day consumption rate on 1st instars of *H. armigera* which was followed by *P. leucopalpis* and *P. leucopalpis* followed by *P. oakleyi*. These three spider species viz., *P. birmanica*, *P. leucopalpis P. oakleyi* showed 6±0.509/day, 5.6±0.707/day, 5.20±0.583/day consumption rate on 2nd instars of *H. armigera* respectively. The male *P. birmanica*,

had higher consumption rate on 2nd instars of *H. armigera* which was followed by *P. birmanica* and *P. birmanica* followed by *P. oakleyi* (Fig 3).

Predation by immature stages (2nd to 8th instars) of three Pardosa spider species feeding on 1st instars of H.armigera

The three *Pardosa* spider species (2nd to 8th Instars) had significant difference for the predation on 1st instars of *H. armigera*. Seven Instars of these species had significant difference for the predation of 1st instars of *H. armigera*. The interaction between species and instars were non significantly different for the predation of 1st instars of *H. armigera* (Table 4).

Table 3. ANOVA for the predation by different sex of three spider species feeding on 2nd instar of *Helicoverpaarmigera*.

S.O.V	d.f	S.S.	M.S.	F.Value	Pr(>F)
Spider species	2	0.3596	0.17982	3.291	0.0546
Sex	1	0.0151	0.01508	0.276	0.6042
Species: Sex	2	0.0060	0.00300	0.055	0.9467
Residual	24	1.3114	0.05464		

NS = Non significant at P>0.05; * =significant at P<0.05; **= highly significant at P<0.01.

Table 4. ANOVA for the predation by immature stages (2nd to 8th instar) of three spider species feeding on 1st instar of *Helicoverpaarmigera*.

S.O.V	d.f	S.S.	M.S.	F.Value	Pr(>F)
Spider species	2	40.93	20.47	10.665	7.46e-05 ***
Instars	6	240.91	40.15	20.923	7.43e-15 ***
Species: Instars	12	28.80	2.40	1.251	0.264
Residual	84	161.20	1.92		

NS = Non significant at P>0.05; * =significant at P<0.05; **=highly significant at P<0.01.

The three *Pardosa* species viz., *P. birmanica*, *P. leucopalpis, P. oakleyi (*2nd to 8th instars) per day consumption rate on 1st instar of *H. armigera* was followed as the 2nd instars of *P. birmanica* had 1.2±0.089/day and 3rd, 4th, 5th ,6th ,7th, 8th had 2.2±0.225/day, 2.4±0.136/day, 3.4±0.186/day, 4.4±0.384, 6.6±0.583, 7±0.634/day respectively. The 2nd instars of *P. leucopalpis* had 0.8±0.374/day and 3rd, 4th, 5th, 6th, 7th and 8th had 1.8±0.374, 2±0.707, 2.8± 0.583/day, 3±0.707/day, 3.4±0.927/day, 6±0.707/day respectively.

The 2nd instars of *P. oakleyi* had 1±0.316 and 3rd, 4th, 5th, 6th, 7th, 8th, had 1.2±0.374/day, 1.8±3.74/day, 2.6±0.509/day, 2.8±0.734/day, 3.2±0.969/day, 4.2±0.969/day consumption rate respectively. *P. birmanica* had higher per day consumption rate which was followed by *P. leucopalpis* and *P. leucopalpis* followed by *P. oakleyi* (Fig 4).

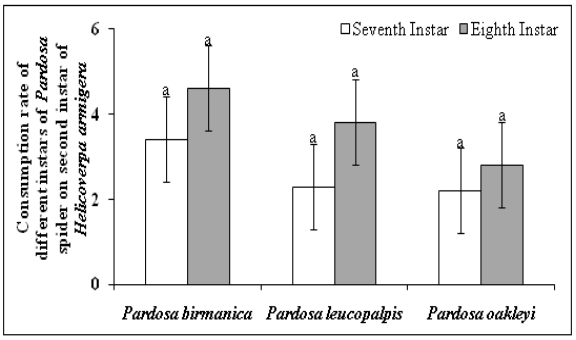

Fig. 1. Mean (SEM) of the consumption rate of seventh and eight instars of *Pardosa* spider on 2nd instars of *Helicoverpa armigera*.

The adult male *P. birmanica*5.25±0.692/day consumption rate on 2nd instar of *H. armigera* and female had 7.4±0.663. The female *P. birmanica* had higher per day consumption rate as compared to that to male. The male *P. leucopalpis* had 7.62±0.678/day

consumption rate on 2nd instars of *H. armigeria* and female had 8.9±0.871/day. Female *P. leucopalpis* had higher consumption rate as compared to tha of male. Male *P. oakleyi* had 4.7±9.27/day comsumption rate on 2nd instar of *H. armigera* and female had a 5.78±0.812/day. The female *P. oakleyi* had higher/day consumption rate on 2nd instar of *H. armigera* as compared to male. The female of all three *Pardosa* species had higher consumption rate as compared to that of male (Fig 5).

Discussion

Predatory efficacy of three selected Pardosa species

This study was formulated to test the predatory efficacy of three most dominant species of genus *Pardosa* viz., *P. birmanica*, *P. leucopalpis* and *P. oakleyi* on the *H. armigera* in the laboratory conditions (27±2°C and 70±5% RH).Present investigation showed that the all the tested spiders of the genus consumed major pests in laboratory conditions. These results are in lined with the findings of Gustavo and Joao (2003) that showed spider predation on arthropods of several groups but preference was for the wingless prey. Therefore spider could be used as an important predator in the IPM.

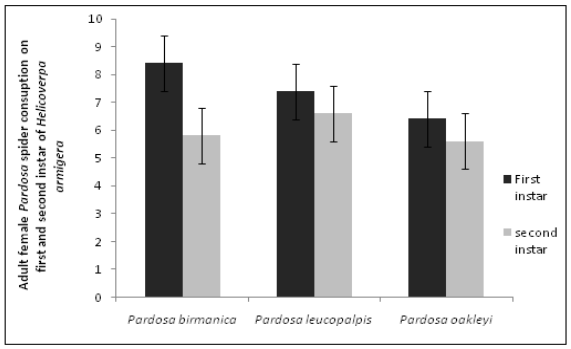

Fig. 2. Mean (SEM) of the consumption rate of adult female *Pardosa* spider on 1st and 2nd instars.

Comparison of effectiveness of male and female Pardosa species

The results of present study are in lined with the finding of Rajeswaran *et al.* (2005). They described spiders were carnivorous and used a large number of prey but were not harmful to the plants. In the present investigation it is observed that in laboratory conditions the mature females were very effective predator than the mature male. These findings were also corroborated with the finding of Sebastian and Sudhikumar (2003). They reported that adult female were aggressive to their prey and had a higher predatory efficacy than that of the adult male of family Lycosidae.

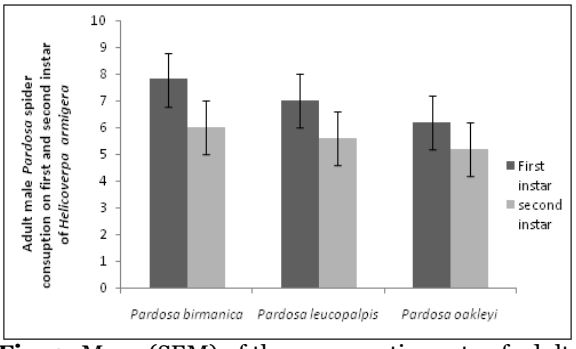

Fig. 3. Mean (SEM) of the consumption rate of adult male *Pardosa* spider on 1st and 2nd instars.

Per day consumption rate

The observations indicated that *P. birmanica* had higher per day consumption rate. These findings are in line with the findings of Sebastian *et al.* (2001) who also reported that *P. birmanica* was one of the most leading spiders. Theywere also recorded the feeding perspective of this spider on various pests and the percent of predation was varied on different developmental periods and between sexes.

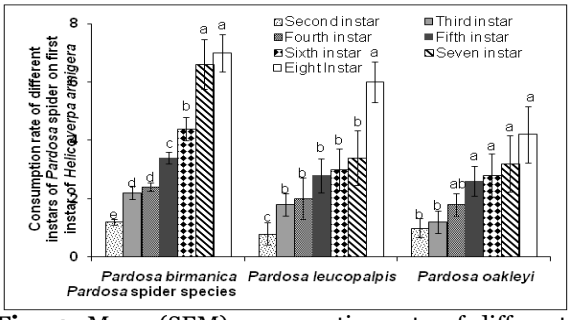

Fig. 4. Mean (SEM) consumption rate of different instars of *Pardosa* spider on 1st instars of *Helicoverpa armigera.*

The immature and mature females have higher feeding ability on four insect pests (*Aphis craccivora, Amarascabi guttula, Tricentrus bicolor, Helicoverpa armigera*) of cotton. The adult female *P. birmanica* consumed 8.4±0.447 (*H. armigera of first instar*) per day. The results were similar to the results of Pearce *et al* (2004), who tested the potential of various spiders of family lycosidae. In this study the eggs and larvae of *H. armigera* were used for predatory

efficacy. The collected spider from the fields belonged to family lycosidae, consumed 90% (9.4±0.1/day) of 1st instar of *H. armigera*.

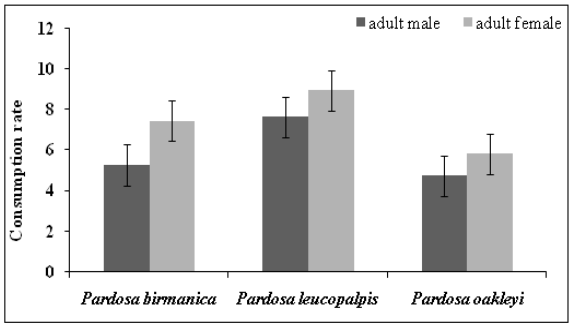

Fig. 5. Mean (SEM) consumption rate of three adult's male and female *Pardosa* spider on 2nd instars of *Helicoverpa armigera*.

In the present study the three selected *Pardosa* species consumed only 1st and 2ndinstar was consumed by 7th and 8thinstar of the spider species and also by the adult. Similar findings were observed by Chauhan *et al.* (2009). They reported that spider selected soft body of the 2ndinstar of *H. armigera* while the 3rdinstar was not consumed due to the hard cuticle. These findings are also with the findings of Sivasubramanian *et al.* (2009) who described that Caterpillars (*H. armegera*) were utilized by delayed instars and mature spiders.

Data on the per day consumption rate of different instar of *H.* armigera were given in (Figs1-4). The results showed that the consumption was only on 1st and 2nd instar of *H. armigera*. Female *P. birmanica* showed consumption on 1st and 2nd instar 8.4±0.447/day, 5.8±0.663/day respectively, and female *P. leucopalpis* showed consumption rate 7.4±0.678/day, 6.6 ±0.678/day, when fed on 1st and 2nd instar of *H. armigera*. Female *P. oakleyi* showed 6.4±0.927/day, 5.6±0.812/day on 1st and 2nd instar respectively and male *P. birmanica* showed 7.8±0.357/day,6±0.509/day on 1st and 2nd instar of *H. armigera,* respectively. The male *P. leucopalpis* showed 7±0.707/day, 5.6±0.707/day respectively. Male *P. oakleyi* showed 6.2±0.583/day, 5.20±0.583/day on 1st and 2nd instar of *H. armigera*. The results of these researches are in line with the studies of Pearce *et al.* (2004) who found 99% consumption of 1st instars of *H. armigera*. Difference in consumption rate of larvae may be due to specific spider species. Data on predatory efficacy showed that consumption rate of larvae decreased with the increased size of larvae.

In the present study three *Pardosa* species viz., *P. birmanica, P. leucopalpis and P. oakleyi* did not consume egg *of H. armigera* in laboratory condition contrary to the findings of Agnew & Sterling (1982) who showed that laboratory feeding tests suggested that the spider assemblage as a whole may potentially be able to devour 2-5 *H. armigera* eggs per day per spider depending on level of starvation. Insect predators are known to feed on varying numbers of *Helicoverpa* spp. eggs under similar conditions. For example, *Solenopsis invicta* Buren (red imported fire ant) consumed 1.5 eggs 0.2 in 24 hours. In another field study by Agnew & Smith (1989) estimated that almost 8% of the diet of lycosids consisted of Lepidoptera in comparison to the laboratory study of the present project where almost 80 % of the 1st instars of *H. armigera* were consumed. In the present study *Pardosa* species viz., *P. birmanica, P. leucopalpis and P. oakleyi* showed significant difference for predatory efficacy on *H. armigera* in the laboratory conditions. In another study Spider families that also showed significant predatory potential against *Helicoverpa* spp. are discussed below. Fourteen (14) morphospecies of Lycosidae from which majority belonging to the genera *Venatrix* and *Artoria,* from soybean fields and one species *Venoniami carioides*, and one as *Lycosa godeffroyi* (Pearce *et al.* 2004).

In previous studies by Bishop and Blood (1977); Room, (1979); Bishop (1980) and Evans (1985) in Australian agroecosystems only a single genus, *Lycosa* has been recognized. The taxonomy of the Lycosidae in Australia is only now under active scrutiny (Framenau &Vink 2001) and, hence, the higher generic diversity was not recognized in previous studies. All above researchers noted this group as known predators of *Helicoverpa* spp. Larvae having significant difference on the predation of *H.*

armigera. In the field, the above contradiction may be due to many factors, such as area and complexity of search ground, movement of the prey and escape behaviours, predator-species interactions and weather will also impact on predation rates of spiders as also described by the Johnson (1999). Until such factors have been investigated further, these results will have limited application to field situations. Our results may be better for application and to emphasize spider families that should be targeted for further research. In the present project the *Pardosa* spiders tested are in a state of food limitations and they might have ability to consume nine *H. armigera* first-instar larvae per day per spider (85% of those accessible) corroborating the findings of previously acclaimed studies (Pearce *et al.* 2004) as they also demonstrated that the field-collected spider collection ate on average 2.4 to 5.0 eggs per 24 h per spider (10-25% of those available), depending on level of hunger. Clubionidae were the only spiders to readily consume eggs in the laboratory (mean of 18.4 eggs per starved spider and 8.2 per non-starved spider after 24 h). Starved spiders consumed 9.4 first-instar larvae per 24 h per spider (90% of those available).

It is clear from the above discussion that some spider groups found within Agro-ecosystem are competent of feeding on eggs and larvae of *Helicoverpa* spp. in the laboratory and may show such behavior in the field. The impact of spiders on populations of *Helicoverpa* spp. in the field must be calculated before they can be fully included into Integrated Pest Management projects. The mortality that they compel on other predaceous arthropods is statically unknown and may counteract some of their potential as biological control agents and so further investigations are still recommended.

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