



Environmental impact and feeding preference of predatory spiders on larval instars of *Helicoverpa armigera* in laboratory and field conditions

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Abstract

Relationship between American Bollworm (*Helicoverpa armigera*, H) and four different species i.e. *Araneus mitificus* (Kidney spider garden), *Plaxipus paykulli* (Jumping spider), *Thomisus pugilis* (crab spider) and *Hippasa holemerae* (wolf spider) was evaluated in laboratory in free choice and no choice feeding assays. In free choice feeding assays, the consumption of fifth larval instar (27.13 ± 2.8) by wolf spider was statistically high followed by crab spider i.e. (12.8 ± 0.39). In no choice assay all spider species in current study preferred to feed on fifth larval instar of American bollworm. Spiders showed positive correlation with wind and humidity i.e. 0.0233 and 0.1619 respectively while it is negatively correlated with temperature (-0.379) in field conditions. Results of present study revealed predatory potential of these spider species against *H. armigera* larval instar, which shows spider could be a best biological control agent for this insect pest.

Keywords: spider, predator, larval instars, biological control, free and no choice feeding assay

Introduction

Pest population density maintained by predators and acting as biological agent in agro-ecosystem (Reigad *et al.*, 2016). Effectiveness of predator as biological control agent is very important to know either predator play an active role in field conditions against prey or not (Stenchly *et al.*, 2012; Macfadyen *et al.*, 2015) [27, 16]. Organism belonging to different arthropods classes are acting as predator on organism of some other classes belonging to same or other phyla (Lefeybre *et al.*, 2017). Spiders are one of the important predators found in higher numbers in fields (Sebastin *et al.*, 2005; Stenchly *et al.*, 2011) [28]. Spiders, as a generalist predator are very helpful to eradicate different harmful insect pests (Malony *et al.*, 2003). It is a need of hour to conserve them as an important biological pest control (Lou *et al.*, 2013) [15], aided in environment friendly cropping practices. Cotton is important lint producing crop and acting as an economic back bone for different countries (Abid *et al.*, 2011) [1]. To improve the cotton yield, introduction of modern machinery, genetically engineered varieties and different agrochemical were used by cultivars. However, intensive use of such measures might affect the predator prey interaction in cropping areas (Lee *et al.*, 2014; Zhao *et al.*, 2015) [14, 33]. Introduction of fertilizers promoted pest population while pesticides not only kill the harmful pest species but also kills the natural enemies (Men *et al.*, 2005; Sherawat and Butt, 2014;) [26]. Predators like spiders were killed by pesticide application and are unable to control the pest population, that reaches to its maximum later in growing season. Furtherly, it is noted that spider population diversity found in fields results in better yield, while spider diversity depends upon different features like as land scape structures, plant community, stages of plant growth and kinds of habitat (Seer *et al.*, 2015; Naranjo *et al.*, 2015) [25, 20]. Bollworms, commonly called as chewing pests, because of having chewing types of mouth parts, attacked on cotton bolls (Sivakof *et al.*, 2012). Common bollworms that

harmed cotton are American bollworm (*Helicoverpa armigera*, H), Spotted bollworm (*Earias vitelli*, F), Pink bollworm (*Pectinophora gossypiella*, S) and Tobacco cutworm (*Spodoptera litura*, F). American bollworm (*H. armigera*) is a polyphagous pest, attacked on bolls to cause severe economic loss in the form of boll damage (Fitt *et al.*, 2009; Ibrahim, 2012) [6, 10]. Bt cotton has largely been used to control *H. armigera* since 1996 (Wilson *et al.*, 2013; Wilson *et al.*, 2018) [6, 32]. As the resistance against pesticide has been developed by *H. armigera*, it is concerned that resistance might developed by *H. armigera* against Bt toxin (Downes and Mahon, 2012; Wilson *et al.*, 2013) [5, 6]. Last larval instar of American bollworm was exposed to ground dwelling predators, which are the vital part of IPM (Integrated pest management) in cotton crops (Parez-Guerreero *et al.*, 2013; Naranjo *et al.*, 2015) [20]. Jumping spider (Araneae: Salticidae) were active hunters and became a successful predator in cotton field (Knutson and Ruberson, 2005) [13]. Jumping spiders have been reported to predate on chewing pest larvae in cotton fields (Johnson *et al.*, 2000) [11]. It is studied that 5th larval instar of *H. armigera* has been predated by spiders as they move towards soil to pupate, so due to this, different spiders have the potential to reduced the population of American bollworm (Rendon *et al.*, 2015) [22]. Therefore, the present research work was conducted to evaluate the biological control of *H. armigera* by different spider species found in study area.

Material and Methods

Present research work was carried out in cotton growing area of Multan, Pakistan during 2016. Coordinate for the study area is 30°05'N 71°40'E with 215meter i.e. 740 feet of sea level, consist of wide plain areas divided by rivers and canals. Extreme climatic conditions are found in study area which reaches to 49°C in Summer and minimum temperature in winter i.e. 1°C with average rain fall of 127mm and having fertile land for cultivation.

Field work

Approved Government cotton variety was sown in study area. Drilling method was used to sow the seed by keeping 20cm distance among two plants. 50 different plants were used to select for recording spider population density. Data were collected from July 2016 – October 2016, after every ten days of interval. Different cotton spider species (larger in size) were collected by using hand net of 14cm diameter. While to collect smaller sized spiders, handmade vacuum pump was used. After that these spiders were transferred to the laboratory. The meteorological data were taken from the meteorological department. The spiders were identified not only by using key formulated by Wagner (2011) but online data base was also used i.e. <https://spiderid.com/spider>.

Spider (Predator) collection

Spiders were collected manually from study area and put them in a plastic container. Larger spiders were chosen because smaller one does not feed on fifth larval instar of *H. armigera*. After collection, put these spiders in clear plastic container with controlled environmental condition i.e. 25±2°C.

Prey collection

Eggs of *H. armigera* were collected from the research site and placed it in temperature 25±2°C and 62±5% relative humidity. These eggs were hatched into larvae which after molting converted into different larval instars.

No choice assays

Fully grown spiders of four different species were placed in separate porcelain cup with whatman® qualitative filter paper, grade 1. Counted number of *Helicoverpa* larval instar i.e. 3rd, 4th & 5th were provided in every cup. Predator potential was noted by counting the remaining larval instar in each cup until pupation. Average consumption was obtained by using formula

$$\text{Average rate} = \frac{\text{Larvae consumed}}{\text{Larvae offered}} \times 100$$

Free choice assay

Each larval instar was provided to spider by using free choice feeding test. In this test, single instar was offered to spider in plastic jar lined with cotton cloth for ventilation, and kept these jars in maintained laboratory conditions i.e. 25±2°C temperature and 62±5% of relative humidity. A mixture of 300 larval instar was presented in each jar and cotton bolls for larval feeding purpose. Daily consumption

was planned to calculate by subtracting the number of larvae from number of individuals offered in jar. The number of larvae consumed by spiders were counted after every 24 hours till the end of each larval instar.

Statistical Analysis

Before statistical analysis, the normality of data was checked by using Shapiro-wilk test. The data about predator (spider) and prey (Larvae of *Helicoverpa*) were analyzed by two-way analysis of variance (ANOVA) by using statistics 8.1 computer-based software, and to separate means, least significant difference test (LSD) was employed. Pearson correlation coefficient was calculated by using following formula.

$$r = \frac{n(\sum xy) - (\sum x)(\sum y)}{\sqrt{[n\sum x^2 - (\sum x)^2][n\sum y^2 - (\sum y)^2]}}$$

Results

In the present study, four predatory spiders i.e. *Araneus mitificus*, *Plaxipus paykulli*, *Thomisus pugilis* and *Hippasa holemerae* were used to test against larval instars of *Helicoverpa armigera* in the laboratory environment at 25±2°C and 62±5% relative humidity and a 12D:12L photoperiod. Prey preference and predator potential was observed on daily basis after every 24 hours. In no choice assay the consumption rate of different larval instars by different spider is similar. Furtherly, it is observed that 2nd, 3rd and 4th larval instar needs three to four days to complete its stage and converted into next instar, while 5th and 6th larval instar too three and five days respectively. As the size of spider species increases consumption is also increased, while it is detected that 5th larval instar consumption was higher by spiders as compared to first four larval instars (Table 1). In free choice feeding test higher consumption of larval instar was observed by *H. holomerae* (wolf spider); while consumption of 5th larval instar was statistically higher for all spider species i.e. 3.12 ± 0.72 for *A. mitificus* (Kidney garden spider), 6.23±1.52 for *P. paykulli* (Jumping spider), 12.8± 0.39 for *T. pugilis* (Crab spider), 27.13±2.8 for *H. holemerae* (wolf spider) depicted in table 2. In fields, spider population (13.82±3.04) was recorded while average temperature, humidity and wind observed during data collection was 36.88±3.04, 41±2.59 and 11.47±0.89 respectively. Correlation between spider population and environmental factor was displayed that spider population was negatively correlated with temperature (-0.379), while it was positively correlated with humidity (0.161) and wind (0.023) as shown in Fig. 1.

Table 1: Average feeding of spider species on bollworm

Spider Species	<i>H. armigera</i> (American Bollworm)					
	1 st Larval instar	2 nd Larval instar	3 rd Larval instar	4 th Larval instar	5 th Larval instar	6 th Larval instar
<i>Araneus mitificus</i> (Kidney garden Spider)	1.23±2.49	1.69±2.12	1.40±0.51	1.80±0.94	2.80±0.32	1.60±0.64
<i>Plaxipus paykulli</i> (Jumping Spider)	1.14±2.82	1.32±2.10	1.20±0.42	1.70±0.89	3.10±0.29	1.70±0.89
<i>Thomisus pugilis</i> (Crab Spider)	1.13±2.12	1.39±2.02	1.02±0.49	1.70±0.89	4.70±0.31	1.80±0.94
<i>Hippasa holemerae</i> (Wolf Spider)	1.02±2.02	1.56±2.00	1.38±0.48	1.58±0.78	3.80±0.32	1.60±0.64

Table 2: Average feeding of spider species on bollworm

Spider Species	<i>H. armigera</i> (American Bollworm)			
	2 nd Larval instar	3 rd Larval instar	4 th Larval instar	5 th Larval instar
<i>Araneus mitificus</i> (Kidney garden Spider)	1.62±0.48	2.00±0.48	1.50±0.61	3.12±0.72

<i>Plaxipus paykulli</i> (Jumping Spider)	4.02±0.30	4.02±0.30	3.00±0.54	6.23±1.52
<i>Thomisus pugilis</i> (Crab Spider)	6.30±1.21	3.90±0.50	6.30±1.02	12.8±0.39
<i>Hippasa holemerae</i> (Wolf Spider)	7.82±2.10	4.12±1.65	7.50±1.50	27.13±2.8

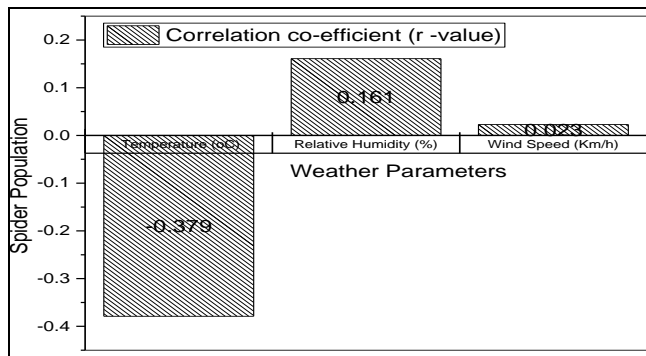


Fig 1: Correlation between spider population and environmental factor

Discussions

Present study was conducted to know the environmental impact on predatory spider of cotton bollworm (*H. armigera*) and feeding preference on larval instars of American bollworm. There are four species of spiders which are common in cotton fields of study area i.e. *Araneus mitificus* (Kidney spider garden), *Hippasa holemerae* (wolf spider), *Plaxipus paykulli* (Jumping spider) and *Thomisus pugilis* (Crab spider), belonging to different families, Araneidae, Gnaphosidae, Salticidae and Thomisidae respectively. Information on predators displayed that various spiders found in cropping system having a potential to predate on bollworms. If someone wants, spiders to be an active predator against chewing worms, then avoid the pesticide application as pesticide not only harmful for unwanted pests but it is very dangerous for wanted insect pests as well like as spiders and enhance the economic loss (Ghafoor *et al.*, 2011) [7]. Present investigation showed that temperature is having negative correlation which means that as the temperature increases spider number decreases which are contrary to the work done by Ghafoor *et al.*, 2002 [8], their investigation on predatory spider explained that as the temperature increases their numbers also increases. Spider utilized all types of hosts pests because they are voracious and polyphagous predators, which are harmful for insect pest but don't damage the plants (Rajeswarm *et al.*, 2005) [21]. Al-Heneidy *et al.*, (1996) demonstrated that predatory spiders found to be highest during growing season in an unsprayed cotton field. Tahir *et al.* (2015) [1] proposed that Spiders play a vital role in keeping pests to a threshold level. Good ability of prey capturing and better body parts proved spiders a very important spiders in all environmental conditions (Hooks *et al.*, 2003) [19]. Biotic and abiotic factors are the key factors in an ecosystem, however abiotic factor also known as non- living one directly impacted on population dynamics of living ones that form biotic factors (Arshad *et al.*, 2015) [3]. Predator prey relationship is determined by noting the number of preys killed by prey (Messina and Sorenson, 2001) [18]. Result of present study revealed that different species have great potential against American bollworm. Predation study showed that all tested species readily fed *H. armigera* especially wolf spider. Our findings are related with the scientist working on spider species (Rendon *et al.*, 2016) [22]. A gradual increase in consumption was observed with increasing larval instar.

This difference in consumption might be due to the availability and recognition of last larval instar (Dixon, 2000) [4]. Different organisms found on different trophic levels were fed by spiders. They feed on herbivores and detritivore organisms (Oelbemann *et al.*, 2008). Prey consumed in no-choice feeding assay was higher as compared to choose feeding assay indicated non-availability of other food sources (Walker and Rypstra, 2001) [30]. Mohsin *et al.* (2015) [19] also investigated about spiders as predators and concluded that higher rate of predation was found in no choice assay as compared to free choice assay. Furthermore, predation also vary regarding different stages of spider. Young spiders are active and have better body parts than the older spider (Sebastian and Sudikumar, 2003). Among all four species of spiders in current study showed better predatory efficacy in choice and no choice feeding assays, this study was similar to the research conducted by Khuhro *et al.*, 2012 [12] who used to study seven predatory spider species in cotton field. The current research work is reinforced that spider can be used in integrated pest management (IPM) by which we can minimize the harmful effect of insecticide and pesticide. These spider species used in current study might be better choice for controlling the American bollworm.

Conclusion

It is indicated from the present study that spiders had significant predation on fifth larval instars of *H. armigera* as compared to other larval instars. Hence it is suggested to used spiders as biological control agent on American bollworm.

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Author's Contribution

MNA conducted, compiled and interpret results of the present study. AF compiled give technical support, critically reviewed and helped in data analysis. SH and QA make final corrections while approval for publication was given by AM.

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