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Efficacy of *Bracon hebetor* Say on *Spodoptera frugiperda* (J.E. Smith) evaluated with *Helicoverpa armigera* (Hub.), *Spodoptera litura* Fabricius and *Corcyra cephalonica* Stainton as alternate hosts

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Abstract
Bracon hebetor is a common parasitic wasp of Lepidopteran larvae and can attack coleopteran larvae. It is an ecto larval parasitoid belongs to the family *Braconidae* of order *Hymenoptera*. The wasp has wide host range and successful biocontrol agent recommended for the control of lepidopteran larvae in many crops and stored grains. Occurrence of fall army worm, *Spodoptera frugiperda* was reported recently in June 2018 in India. A study on *Bracon hebetor* parasitization effects on *Spodoptera frugiperda* in comparison with *Spodoptera litura, Helicoverpa armigera* and *Corcyra cephalonica* was conducted by providing them as alternate hosts under laboratory conditions at National Institute of Plant Health Management, Rajendranagar, India. The study revealed that when all the hosts were offered to *Bracon hebetor* it is parasitizing in the order of *C. cephalonica* followed by *H. armigera*, and then *S. frugiperda*, there was no parasitization on *S. litura*. When two hosts were offered *Bracon hebetor* preferred *C. cephalonica* over *H. armigera* and *H. armigera* over *S. frugiperda.* When individual hosts were offered *Bracon hebetor* is forming more larvae, pupae and producing more adults on *C. cephalonica* followed by *H. armigera* then on *S. frugiperda*, and no egg laying and pupation was observed on *S. litura.*

Keywords: *Bracon hebetor, Spodoptera frugiperda*, Fall armyworm, Biological control, Parasitoid

Introduction
Bracon hebetor is most widely used gregarious polyphagous ecto parasitoid which parasitizes many lepidopteran larvae. *B. hebetor* females first paralyze the last-stage larvae of their host in a "wandering" phase by injecting paralytic venom and ovipositing variable numbers of eggs on or near the surface of paralyzed host (Mukti and Thomas, 2010) [1]. It attacks many important lepidopterous pests of stored products as well as field crops (Landge *et al.*, 2009 and Dabhi *et al.*, 2011) [2, 3]. The rice moth *Corcyra cephalonica* Stainton is an important insect-pest of different stored products in tropics (Jyoti *et al.*, 2017) [4]. In India, this pest is being utilized in bio-control research developmental units for mass production of number of natural enemies which includes both parasitoids and predators (Jalali and Singh, 1992 [5], Jyoti *et al.*, 2017 [4]). *Spodoptera litura* is a serious pest of various economically important crops such as cotton, tomato, groundnut, chilli, tobacco, castor, okra and pulses in India, China and Japan (Promod *et al.*, 2015) [6]. *Helicoverpa armigera* is a major pest of many economically important crops including cotton, pigeon pea, chickpea, sunflower, tomato, sorghum, millet, okra, and corn in India, (Manjunath *et al.*, 1989) [7].
Spodoptera frugiperda (J. E. Smith) (Insecta: Lepidoptera: Noctuidae), commonly known as fall armyworm (FAW) is a notorious pestiferous insect with high dispersal ability, wide host range and high fecundity that makes it one of the most severe economic pests (Shylesha *et al.*, 2018) [8]. The FAW has been restricted to the American countries and in 2016 reported from various countries in Africa, posing a serious challenge of sustainability of maize production in Sub-Saharan African countries. The occurrence of the FAW on maize in various districts of Karnataka state, India has been reported in 2018 (Sharanabasappa *et al.*, 2018) [9]. Later it was also reported in Tamil Nadu, Telangana and Maharashtra (The Hindu 20-08-2018) [10] states of India. Further it was reported from many places of Gujarat, Madhya Pradesh, and Bihar including north east states like Mizoram.

Invasiveness of a pest species e.g., fall armyworm (FAW) into new geographies in the absence of biotic regulatory factors often results in the disruption of natural control, leading to devastating outbreaks (Prasanna *et al.*, 2018) [11]. WTO expressed threat to Indian food production due to FAW. In this context the present study is carried under laboratory conditions with FAW as host for *B hebetor* to study its parasitizing ability in comparison with other lepidopterous pests *H. armigera*, *S. litura* and *C. cephalonica.*

Materials and Methods

The laboratory studies were carried out in Biocontrol laboratory at National Institute of Plant Health Management, Rajendranagar Hyderabad during 2018-19 (https://niphm.gov.in/).

***B. hebetor* Culture:** Initial *B. hebetor* mother culture was procured from coconut research station, Ambajipet, Andhra Pradesh, India. The culture used for experiments was maintained by tub method of *B. hebetor* rearing procedure developed by NIPHM. In this method a clean dry tub was taken and a cotton swab dipped in honey was placed inside the tub on one side. Then 50 g of broken sorghum grain was taken in the tub and 400 *C. cephalonica* larvae were placed and 50 *B. hebetor* adults were released. Tub was covered with muslin cloth and kept it for 30-40 days. Cotton swab with honey was changed once in two days and sorghum grain was turned once in a week. The adults coming after 30-40 days were collected and used to conduct experiment.

***H. armigera* culture:** Larvae of *H. armigera* were obtained from a laboratory culture maintained at NIPHM. The culture was established from NIPHM red gram field and regularly supplemented with field-collected larvae. Larvae were reared on a chickpea based diet (Armes *et al*., 1993) [12] at 27˚C.

***S. frugiperda* culture:** Larvae of *S. frugiperda* were obtained from a laboratory culture maintained at NIPHM. The initial culture was established from NIPHM maize filed, and regularly supplemented with field-collected larvae. Each larva was reared separately in individual vial as cannibalism was observed during rearing. Tender maize leaves were collected from organic maize field of NIPHM, cleaned and provided as food. Every day the larvae were shifted to sterilized vials with fresh food. Later the larvae reaching pupation stage were transferred to glass jars containing soil to pupate. The soil in glass jar with pupae was moistened till the adult emergence and the emerging adults were transferred to oviposition cages and provided with 50 % honey diluted with water and added with 1 vitamin E tablet/10 ml. The egg masses laid on paper in oviposition cages were transferred to Petri dish and kept in incubator for hatching. The hatched larvae were fed with maize leaves for 5 days commonly and then shifted to individual vials on 6th day.

***S. Litura* culture:** Larvae of *S. litura* were reared on castor leaves at NIPHM and used for experiment.

***C. cephalonica* culture:** Larvae of *C. cephalonica* were reared on regular sorghum diet at NIPHM biocontrol laboratory.

Preliminary screening was done with 3rd, 4th and 5th instar larvae of all 4 host insects and comparative efficacy studies were made with 5th instar larvae of host insects as described below.

The method followed for efficacy studies was sandwich method developed for *B. hebetor* rearing at NIPHM. In this method for efficacy studies of 4 hosts, individual experiments were conducted with 3 replications. In the first experiment for each replication 20 *B. hebetor* adults were released in 4 jars and cotton dipped in 10% honey solution was placed to one side on inner wall of the jar to provide nutrition for the adult *B. hebetor.* The jars were covered on the top with a muslin cloth. In the first jar 10 *C. cephalonica* larvae were placed on the cloth and again it was covered on the top with a muslin cloth tightly so that larvae cannot move. The host larvae were sandwiched between 2 muslin cloth layers. The adult *B. hebetor* placed in the jar were fed on the honey solution and parasitized the larva by inserting ovipositor through muslin cloth. In the second jar 20 adults of *B. hebetor* were released and 10 *S. litura* larvae were taken for parasitization. In the third jar 20 adults of *B. hebetor* were released and 10 *S. frugiperda* larvae were taken for parasitization. In fourth jar 10 *H. armigera* larvae were placed. The muslin cloths were tied tight because if the larvae were allowed to move due to high rate of cannibalism *H. armigera* and *S. frugiperda* larvae may consume or damage the other larvae in the on the cloth. After 24 hours of exposure individual host larvae were transferred to Petri plates to observe number of *B. hebetor* larvae, pupae and adults per host larva. Number of *B. hebetor* larvae formed on each host larva was observed under magnifying glass one day after transfer to petri plate and *B. hebetor* pupae separated from host larva and formed on filter paper were counted daily for 3 days and total was calculated. *B. hebetor* adults emerging from each host larva during 8 to 14 days after parasitization were counted daily and total adults emerged were calculated.

The second experiment was conducted as mentioned above and along with S. *frugiperda* an alternate host was also provided to *B. hebetor*. In first jar a combination of S. *frugiperda* and *C. cephalonica* each 5 larvae were exposed for oviposition by 20 *B. hebetor*. In 2nd jar *S. frugiperda* with *S. litura* and in 3rd jar *S. frugiperda* with *H. armigera* larvae were exposed for oviposition. The host insects were exposed to *B. hebetor* for oviposition for 24 hours and the parasitized host larvae were transferred individually to petri plates with filter paper to note larval and pupal counts and adult emergence.

In 3rd experiment multiple hosts were offered to *B. hebetor*. Initially all 4 host species *S. frugiperda, S. litura, H. armigera and C. cephalonica* each 3 larvae exposing to 20 *B. hebetor* adults in 3 replications and repeated with 3 hosts *S. frugiperda, H. armigera* and *C. cephalonica* excluding *S. litura* in 4th experiment.

Statistical analysis: After transferring individual host larvae to petri plates the data from each petri plate was noted. From each host larva the number of *B. hebetor* larvae, pupae and adults emerging were noted. Each experiment was repeated 3 times. Mean of total data of particular treatment and standard deviation as a measure of data variability around mean (the average squared deviation from the mean) of a treatments was calculated and represented as mean $\pm$ SD.

Results

During initial screening experiments on 3rd instar larvae with all 4 host species there was no parasitization, 4th instar larvae were partially preferred and maximum parasitization was

observed from 5th instar onwards.

When single host insect C. *cephalonica* was offered to *B. hebetor*, mean number of larvae, pupae and adults of *B. hebetor* were 20.6 ± 5.2, 19.3 ± 4.8 and 17.7 ± 4.2 respectively per single C. *cephalonica* larva. Mean number of larvae, pupae and adults of *B. hebetor* were 7.7 ± 4.2, 16.5 ± 5.4 and 12.6± 4.7 per larva respectively when *H. armigera* alone was offered. Mean number of larvae, pupae and adults of *B. hebetor* were 15.9 ± 7.7, 13.4 ± 6.9 and 8.9 ± 5.4 on *S. frugiperda.* Larvae of *S. litura* were not parasitized by *B. hebetor* and they became black, dead and dried within 24 hours after exposure to *B. hebetor* (Table 1).

In second experiment when *S. frugiperda* and *C. cephalonica* were offered to *B. hebetor* less number of larvae, pupae and adults of *B. hebetor/*host larva were recorded on *S. frugiperda* i.e. 15.5 + 7.1, 14.0 + 5.7 and 12.2 + 6.1 respectively compared to *26.2 + 3.9, 25.2 + 2.8 and 23.8 + 2.5* respectively *on C. cephalonica.* When the choice was given between *S. frugiperda* and *H. armigera,* higher number of larvae, pupae and adults of *B. hebetor*/ host larva 17.6 ± 6.3, 15.3 ± 5.2 and 14.3± 4.8 respectively were observed on *H. armigera* compared to 14.9 ± 8.7, 13.5 ± 7.3 and 11.8 ± 6.2 respectively on *S. frugiperda.* When the choice was given between *S. frugiperda* and *S. litura* there was no parasitization on *S. litura* but number of larvae, pupae and adults of *B. hebetor*/ *S. frugiperda* larva 16.7± 9.8, 12.8 ± 7.3 and 11.5 ± 7.4 respectively were observed (Table 2).

When all the 4 hosts *S. litura, S. frugiperda, H. armigera* and *C. cephalonica* were offered to *B. hebetor* highest number of larvae, pupae and adults of *B. hebetor*/host larva 25.6± 4.2, 22.2± 4.2 and 21.0± 4.9 respectively were observed on *C. cephalonica* followed by 22.3± 5.5, 19.5± 4.4 and 15.5± 5.6 respectively on *H. armigera* and then 20.2 ± 6.1, 15.6 ± 6.3 and 12.7 ± 5.1 respectively on *S. frugiperda*. No parasitization on *S. litura*. (Table 3).

When 3 hosts *S. frugiperda, H. armigera* and *C. cephalonica* were offered highest number of larvae, pupae and adults of *B. hebetor*/host larva 24.5± 4.3, 23.9± 4.8 and 21.5±4.7 respectively were formed on *C. cephalonica* followed by 22.3±5.5, 20.3± 4.1and 15.5± 5.6 on *H. armigera* then 21.7 ± 4.4, 17.8 ± 5.0and 15.6 ± 4.5 respectively on *S. frugiperda* (Table 4).

Table 1: Parasitization of *Bracon hebetor* on *S. litura, S. frugiperda, H. armigera* and *C. cephalonica* under laboratory conditions, NIPHM, Rajendranagar, 2018-19

Experimental Jars	Host Insect	*B. hebetor* adults/jar	No. of *B. hebetor* larvae formed/ host larva (Mean ± SD)	No. of *B. hebetor* pupae formed/host larva (Mean ± SD)	Total No. of *B. hebetor* adults emerged/host larva (Mean ± SD)
Jar1	*C. cephalonica*	20	20.6 ± 5.2	19.3 ± 4.8	17.7 ± 4.2
Jar 2	*S. litura*	20	0	0	0
Jar 3	*S. frugiperda*	20	15.9 ± 7.7	13.4 ± 6.9	8.9 ± 5.4
Jar 4	*H. armigera*	20	19.4 ± 6.2	16.5 ± 5.4	12.6± 4.7

Table 2: Parasitization of *Bracon hebetor* on *S. frugiperda* with *S. litura, H. armigera* and *C. cephalonica* as alternate hosts in laboratory, NIPHM, Rajendranagar, 2018-19

Exp. Jars	*B. hebetor* adults/jar	Host Insects	No. of *B. hebetor* larvae formed/ host larva (Mean ± SD)	No. of *B. hebetor* pupae formed/ host larva (Mean ± SD)	No. of *B. hebetor* adults emerged/host larva (Mean ± SD)
Jar 1	*20*	*S. frugiperda*	*15.5 + 7.1*	*14.0 + 5.7*	*12.2 + 6.1*
		C. cephalonica	*26.2 + 3.9*	*25.2 + 2.8*	*23.8 + 2.5*
Jar 2	20	*S. frugiperda*	16.7± 9.8	12.8 ± 7.3	11.5 ± 7.4
		S. litura	0	0	0
Jar 3	20	*S. frugiperda*	14.9 ± 8.7	13.5 ± 7.3	11.8 ± 6.2
		H. armigera	17.6 ± 6.3	15.3 ± 5.2	14.3± 4.8

Table 3: Parasitization of *Bracon hebetor* on *S. litura, S. frugiperda, H. armigera* and *C. cephalonica* multiple hosts under laboratory conditions, NIPHM, Rajendranagar, 2018-19.

Host Insect	No. of *B. hebetor* larvae formed/ host larva (Mean ± SD)	No. of *B. hebetor* pupae formed/ host larva (Mean ± SD)	No. of *B. hebetor* adults emerged/host larva (Mean ± SD)
S. litura	0	0	0
S. frugiperda	20.2 ± 6.1	15.6 ± 6.3	12.7 ± 5.1
H. armigera	22.3± 5.5	19.5± 4.4	15.5± 5.6
C. cephalonica	25.6± 4.2	22.2± 4.2	21.0± 4.9

Table 4: Parasitization of *Bracon hebetor* on *S. frugiperda, H. armigera* and *C. cephalonica* with multiple hosts under laboratory conditions, NIPHM, Rajendranagar, 2018-19

Host Insect	No. of *B. hebetor* larvae formed/ host larva (Mean ± SD)	No. of *B. hebetor* pupae formed/ host larva (Mean ± SD)	No. of *B. hebetor* adults emerged/host larva (Mean ± SD)
S. frugiperda	21.7 ± 4.4	17.8 ± 5.0	15.6 ± 4.5
H. armigera	23.9 ±4.6	20.3± 4.1	15.5± 5.6
C. cephalonica	24.5± 4.3	23.9± 4.8	21.5± 4.7

Discussion

Invasive alien species pose a serious threat to agriculture and cost billions of dollars in terms of reduced production and productivity (Paini *et al.,* 2018) [13]. In this regard FAW reported recently in India is a notorious insect with high dispersal ability, wide host range and high fecundity that

make it one of the most severe economic pests. Braconids like egg-larval endoparasitoid *Chelonus insularis* Cresson (Braconidae, Cheloninae), and the larval endoparasitoids *Cotesia marginiventris* (Cresson) (Braconidae, Microgastrinae) were effective against FAW. Ashley 1979 [14a], Ashley *et al.*, 1982 [14b] & 1983 [14c]; Pair *et al.*, 1986 [15]. Worldwide Braconids play crucial role in controlling fall armyworm. Egg Larval parasitoid *Chelonus spp*, Larval parasitoids like *Agathis stigmatera*, *Cotesia marginiventris* were reported from many FAW infested countries. Two larval parasitoids *Habrobracon hebetor* in Nigeria and *Cotesia* spp. in Kenya that can attack FAW larvae were also reported. Another parasitoid, *Bracon mellitor* was introduced into Egypt to control *Spodoptera littoralis* also attack FAW (Heinrichs *et al.*, 2017) [16]. Parasitism by gregarious larval parasitoid *Glyptapanteles creatonoti* (Viereck) (Hymenoptera: Braconidae) in India was reported by Shylesha *et al.*, 2018 [8].

The parasitization effects of *B. hebetor* were studied on different hosts *S. frugiperda*, *H. armigera*, *C. cephalonica* and *S. litura* in laboratory conditions. When single host was offered *B.hebetor* larvae, pupae and adults were formed on C. *cephalonica* followed by *H. armigera* and *S. frugiperda.* Whereas *S. litura* was not parasitized by *B. hebetor* and the larvae became black, dried and dead within 24 hours. It is in accordance with Mukti and Thomas 2010 [1] studies that the *B. hebetor* cannot necessarily develop and reproduce on all host species that it can paralyze and oviposit on, and optimum reproduction is with the stored-product pyralid hosts.

When two hosts *S. frugiperda* and *C. cephalonica* were offered, less number of larvae, pupae and adults of *B. hebetor*/host larva was observed on *S. frugiperda* compared to *C. cephalonica*. When the choice was given between *S. frugiperda* and *H. armigera*, higher number of larvae, pupae and adults of *B. hebetor* / host larva was observed on *H. armigera* compared to *S. frugiperda.* These results were similar with the findings of Muhammad *et al.*, 2016 [17] who reported that the intermediate biological activity (parasitizing effect) of *B. hebetor* were found on *H. armigera and S. litura* as the plasticity of this species could also represent an important fitness cost difference in performance, which is discussed in terms of phylogenetic distance of the host species particularly Pyralidae.

When multiple hosts *S. frugiperda*, *H. armigera* and *C. cephalonica* were offered to *B. hebetor* highest number of larvae, pupae and adults of *B. hebetor* were formed on *C. cephalonica* larva followed by *H. armigera* and then *S. frugiperda*. Zain ul abdin *et al.*, 2017 [18] reported that the lethal effects of crude venom extracted from the ectoparasitic wasp *B. hebetor* were examined with cultured insect cell lines of *S. frugiperda.* The results of the study clearly demonstrated that the venom from *B. hebetor* is more effective against the cell line derived from *S. frugiperda*.

Nikam and Pawar, 1993 [19] reported that *Bracon hebetor* Say (Hym., Braconidae) population can be increased naturally on *C. cephalonica* Staint. (Lep., Pyralidae) and *B. hebetor* acts as key parasitoid of *H. armigera* Hbn. The results are in line with Dabhi *et al*, 2011 [3] who stated that *C. cephalonica* was the best host for mass rearing of *B. hebetor*. In the current study *B. hebetor* preferred both *C. cephalonica* and *H. armigera* so *B. hebetor* reared in laboratory can be released in field to control *H. armigera.*

Conclusion

Based on the present laboratory study results it was evident that the *B. hebetor* was an effective parasitoid of invasive pest *S. frugiperda* and further need to be tested for its efficacy in field conditions. If it proves as effective biological control agent of *Spodoptera frugiperda* under field conditions, it will reduce the threat of damage to many crops especially maize in India. Use of biocontrol agents like parasitoids reduces the pesticide usage and environmental pollution.

Life cycle of *B. hebetor* has 4 stages and completes in 20 days during warm weather and extends to 60-70 days during winter. Egg period is 1-2 days, larval period 2-4 days and pupal period 3-7 days. Larval stage is parasitic and rearing in laboratory is easy using *C. cephalonica* as host. Adult is free living with average pre oviposition period of 3 (2-5) days, oviposition period 37.7 (22-55) days and post oviposition period 4.4 (1-8) days, the fecund female live for 45 (20-63) days. Pupal cards or adults @ 5000 adults/hectare or 4000-5000 pupae/hectare need be released in the field. Adult takes shelter on flowering plants and consumes nectar of small flowers. So along with parasitoids flowering plants are also to be recommended for their shelter and food. NIPHM maintains biological control laboratory with various parasitoids and predators for the purpose of training of on farm production of biocontrol agents and maintains ecological engineering organic polyculture field for the demonstration of these biocontrol agents role in pest management. This is a great combination for bio intensive pest management through combination of release and conservation of parasitoids for sustainable agriculture.

Research Category: Biological control

Abbreviations: FAW Fall Armyworm, SED Standard Error of Deviation, NIPHM National Institute of Plant Health Management.

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