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OPTIMIZATION OF REARING PROTOCOL FOR EGG AND LARVAL PARASITOIDS WITH THEIR ASSOCIATED HOSTS AND EVALUATING THE EFFICACY OF *BRACON HEBETOR* AGAINST FALL ARMYWORM IN MAIZE

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ABSTRACT

The Fall Armyworm (FAW), *Spodoptera frugiperda,* poses a significant threat to maize production in Patuakhali and other regions globally. To mitigate this problem, the present study focused on developing a rearing protocol for two potential biological control agents: the larval parasitoid, *Bracon hebetor*, and the egg parasitoid, *Trichogramma chilonis* and the efficacy of *Bracon hebetor* was carried out in the bio-control laboratory of Entomology department, PSTU during October 2022 to September 2023. Laboratory trials were conducted to evaluate the efficacy of the reared parasitoids against FAW larvae of different instars. The rearing protocols of *B. hebetor* were established utilizing the associated hosts, *Galleria mellonella* (wax moth) and *Corcyra cephalonica* (rice moth), respectively. The rearing protocol development involved optimizing environmental conditions, nutritional requirements, and host-rearing methods to ensure large parasitoid populations. Observations on parasitism rates, and FAW population suppression were recorded to assess the effectiveness of the biological control strategy. The results demonstrated the potential for *Bracon hebetor* as biological control agent against FAW. The highest parasitism was found in 5th instar larvae which was about 96% while the lowest parasitism was found in 3rd instar larvae which was about 33%. In 4th instar mean parasitization was 6.33±1.52 which is about 63%. The parasitism of FAW by *B. hebetor* ranged from 20-40 % in net cage trial. The length and breadth of 6th instar larva of wax moth were 1.215 ± 0.13cm and 0.371 ± 0.03cm, respectively while the length and breadth of pupa were 2.13 ± 0.14cm and 0.33 ± 0.03cm, respectively. The length and breadth of 5th instar larva of fall armyworm were 3.66 ± 0.23cm and 0.372 ± 0.02cm while the length and breadth of pupa were 1.46 ± 0.05cm and 0.5 ± 0.00cm, respectively. Overall, the protocol development for rearing of larval and egg parasitoids and their efficacy against FAW provide valuable biological control strategies for managing FAW in maize fields and offer a sustainable alternative to chemical pesticides.

Keywords: Fall Armyworm, *Bracon hebetor, Corcyra cephalonica, Galleria mellonella,* maize, *Trichogramma chilonis*

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INTRODUCTION

Fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae), is an indigenous to the tropical and subtropical continents of South and North America. It was first discovered outside of its natural range in January 2016 in the Nigeria region of West Africa (Goergen *et al*. 2016). It is highly polyphagous insect pest that has invaded over 40 countries in Africa and Asia since 2016. FAW damaged 353 host plant species from 76 different plant families, with the Poaceae family being its preferred host (Montezano *et al.* 2018). The first report of FAW in Bangladesh was in November 2018. FAW larvae were first observed in a cabbage field on November 8, 2018 and adults were trapped in a maize field on November 23, 2018 (Alam *et al*. 2018). In recent years, the fall armyworm (FAW) has become a significant threat to maize production in Bangladesh. It is highly invasive pest that can cause significant damage to maize crops, leading to substantial economic losses. The FAW infestation had a devastating impact on maize production. The larvae feed on the leaves and stalks of maize plants, reducing yield and quality. In some cases, entire fields have been destroyed. The infestation has also led to increased costs for farmers, who have to spend more on pesticides and other control measures. Farmers are facing a number of challenges in controlling the pest infestation. In the absence of pesticides, FAW reduces maize production's profitability by an average of 37.7%. Bangladesh intends to stop importing maize by 2030, although its output still trails that of imports because of the growth of Bangladesh's chicken feed business, around 74% of the country's maize is currently farmed locally, with the remaining 26% imported at an annual cost of USD 0.4 billion to meet demand (Ullah *et al*. 2023). Pesticides are used by all farmers to suppress this pest. The use of chemical pesticides is one of the most common methods for controlling FAW. But the effectiveness of chemical pesticide is too much poor and there is no well-known chemical pesticide for controlling this pest in Bangladesh. However, use of chemicals has several drawbacks, including the development of resistance in pests, environmental pollution, and health hazards for humans and animals. These moth species have developed resistance to pesticides. In addition, because they leave residue in food products and in processing facilities where employees are exposed, pesticides pose a direct risk to human health and the environment (Fields and White 2002).

Thus, for sustainable pest management, tried-and-true agroecological techniques and bio-control agents are required. Biological control is an alternative method that has been gaining popularity in recent years. The use of parasitoids is one such method that has shown promising results in controlling FAW. Natural enemies like parasitoids are essential for controlling insect populations. The larval parasitoid *Bracon hebetor* and the egg parasitoid *Trichogramma chilonis* are two significant parasitoids are using in the management of lepidopteran pests. The need for pest management strategies that are environmentally benign was driven by the rising concern for environmental safety and the desire for pesticide-free food on a global scale. As a result of these varied pesticide-related environmental effects and emerging global trends that support conservation and the sustainable use of biological resources, interest in biological control has significantly increased (Farag *et al*. 2015). The development of non-chemical insect control strategies, such as cultural, physical, biological, varietal, biorational, and genetic control measures in place of conventional pesticides for the management of field pests and stored product insects, has received a lot of attention in recent years (Phillips 2006). The employment of natural enemies, such as parasitoids and predators, is one of these tactics that is crucial to IPM and offers numerous benefits over chemical control (Scholler and Flinn 2000). One of the potential biological control agents is *Bracon hebetor*, an ectoparasitoid that is gregarious and feeds primarily on Pyralidae (Lepidoptera) larvae to complete its larval development. A highly polyphagous parasitoid known as *B. hebetor* is found worldwide. It is simple to grow in a lab on fictitious hosts like *galeria menolella*. It has been successfully incorporated into IPM programmes. A productive gregarious larval parasitoid of numerous lepidopteran storage and vegetable pests is *Bracon hebetor* Say (Hymenoptera: *Bracon*idae). This parasitoid can be artificially released to control a variety of destructive pests, including *Leucinodes orbonalis*, *Spodoptera* sp., *Helicoverpa armigera*, *Maruca vitrata*, *Corcyra cephalonica*etc (Thanavendan and Jeyarani 2010, Amir-Maafi and Chi 2006) but can also parasitize hosts that are phylogenetically distant, like Coleoptera (Lopez *et al*. 2011). This parasitoid species can also parasitize a variety of pyralids from the family Crambidae and subfamily Galleriinae, such as the grass moth *Laetitia coccidivora*, the greater wax moth *Galleria mellonella*, and the rice moth *Corcyra cephalonica*, as well as non-pyralid moth larvae like the angoumois grain moth *Sitotroga cerealella* and the potato tuberworm (Krombein *et al*. 1979).

The greater wax moth (GWM) and other pyralid moth larvae in their fourth and fifth stages are attacked by the ecto-parasite *Bracon hebetor* Say (Hymenoptera: *Braconidae*). *Galleria mellonella* (L.) Pyralidae Lepidoptera (Awadallah *et al*.

1985). Variable quantities of eggs are then placed on or near the surface of the paralysed hosts by *Bracon hebetor* females after they have first paralyzed their host larvae by stinging them (Antolin *et al*. 1995). For the parasitoid to be released at the appropriate moment, pupae must be stored. This knowledge is particularly beneficial for mass-producing insects, especially for bio-control agents (Ashfak *et al*. 2011). So, there is need to be developed a rearing protocol of larval parasitoid, *Bracon hebetor* and its host wax moth, *Galaria melonella*. Considering above facts, this research work has been undertaken to develop rearing protocols for *B. hebetor* and *Trichogramma chilonis* with their associated host, wax moth (*Galeria mellonella*) and Rice moth (*Corcyra cephalonica)* and to evaluate the efficacy of *B. hebetor* against FAW.

MATERIALS AND METHODS

Mass rearing of larval parasitoid, *Bracon hebetor*: Techniques such as controlled temperature and humidity, suitable host provision, and monitoring of parasitoid development were implemented to enhance rearing efficiency. Mass rearing of *B. hebetor* parasitoid was done at Biocontrol lab, Entomology Department of Patuakhali Science and Technology and 5th to 6th instars wax moth, *Galleria mellonella* larvae were used as the host of *B. hebetor*.

Procedure of wax moth rearing: At first a parent stock of wax moth was developed in honeycomb in glass jars. Honeycomb wax was sterilized by electric autoclave at 125°C at 15 PSI for 60-70 minutes. The autoclaved wax is placed on a tray for cooling, then warm wax was given in ball shape and kept this ball for room temperature. About 200 to 250 g wax was taken in a glass jar and 20 to 30 adult male and female wax moth was released on this wax containing jar for egg laying then closed its mouth by black cloth. Adult wax moth was mate in jar and lay egg on wax. After 8 to 10 days first to second instar larvae of wax moth were release in the artificial diet (made with definite proportion of wheat flour, maize flour, milk, animal fat, sugar and yeast (Table 1) and autoclaved at 125°C and 1.5 PSI for 70 min).

Milk 1L, sugar 800gm, fat 250g and wax 300g were boiled together and made syrup. This solution was then mixed to the mixture of wheat 2kg and maize flour 2kg and

Table 1. Preparation of artificial diet for rearing wax moth

Name of ingredients	quantity
Wheat flour	2 kg
Maize flour	2 kg
Milk	1 L
Fat (mutton)	250 g
Sugar	800 g
Wax	300 g
Yeast	1.5 tea spoon full

yeast 1.5 tea spoon. Then the whole mixtures were autoclaved at 125°C temperature and 15 PSI for one hour. Then this diet kept in a bowl for room temperature then 1st to 2nd instar wax moth larvae were released on this.

Described amount of milk, sugar, fat and wax were boiled together and made syrup. This solution was then mixed to the mixture of wheat and maize flour and yeast. Then the whole mixtures were autoclaved at 125°C and 15 PSI for one hour. Then this diet kept in a bowl for room temperature then 1st to 2nd instar wax moth larvae were released on this. The larvae will attain full growth at 20 to 25 days later.

Procedure for mass rearing of *Bracon hebetor*: The full-grown larvae were transferred in a sterilized 3-4 litters plastic bottle (200 larvae /bottle) with a corrugated paper sheet. The full-fed larvae took position on the corrugated paper sheet for pupation. Then 100 adult *B. hebetor* (60 female and 40 male) were released in the plastic bottle with 10% honey solution as food. The opening end of the jar was closed with black cloth by a rubber band. Then the whole pot was covered with a black cloth to increase its parasitizing capacity. The wax moth larvae and *B. hebetor* were then kept in rack for 8-10 days for parasitism, egg laying, pupation and adult emergence of *B. hebetor*. The female parasitoids lay 8-12 eggs on ventral surface of the host larvae after paralyzing by stinging. It was observed that 100% parasitism of wax moth larvae occurred by *B. hebetor*. The duration of the egg stage of *B. hebetor* on host larva was 3-4 days, larval stage inside the host larva was 6-8 days, pupal stage outside the host larva was 5-6 days. Total adult longevity in the laboratory was 20-25 days (with honey as food). However, in nature adult *B. hebetor* can survive up to 45 days.

Mass rearing of egg parasitoids, *Trichogramma chilonis*: In the laboratory Rice moth *Corcyra cephalonica* (Table 2) were used as the host for mass rearing of *Trichogramma.* For mass rearing of *Trichogramma* spp. The following steps are followed

Table 2. Mass rearing of host insect rice moth, *Corcyra cephalonica*

At first artificial diet was made for rice moth *C. cephalonica* larvae.

Ingredient	Quantity
Maize flour	1 kg
Wheat flour	1 kg
Pea-flour (bason)	500 g

Described quantity of ingredient are mixed together then whole mixture was autoclaved at 125°C temperature at 15 PSI for 1 hour. After autoclaved this diet was kept at a bowl for room temperature. Then 2g or 3g eggs of rice moth were spread over the diet and covered the bowl with 64 meshes net.

This bowl was kept at untouched condition until adult emergence. After 4 to 5 days hatched and 28 to 33 days old adult rice moth come out (depends on temperature) then adult containing bowl kept inside a small net box for adult collection. 30 to 35 adult rice moths kept in a special plastic jar bottom of which was covered by a net. Then adult containing jar was kept on another jar, female moth lay eggs and these eggs fall down through the net and store at the jar. Eggs were collected and cleaned and were ready for rearing of *Trichogramma* spp.

Mass rearing of *Trichogramma* spp. preparation of trichocards: A coat of 10% gum arabic was applied on the cards and the eggs of *Corcyra* were sprinkled uniformly in a single layer with the aid of a strainer. The excess eggs pasted are removed gently by passing a brush over the card after sufficient air drying under fan. Label information on the species of the parasitoid and date of parasitizing are given in the leftover spaces. Very small amount of honey was placed on card for supplying food. The egg cards were placed into test tube/glass cylinder of suitable size and the nucleus card of *Trichogramma* was introduced in it. *Trichogramma* adults emerge from this card in 7-8 days after parasitization.

To delay the emergence of *Trichogramma*, these parasitized cards can be stored in refrigerator at 5-10°C and 65-70% RH for 10-15 days. On removing the cards to room temperature, the parasitoids emerge normally.

Maize flour 1 kg, wheat flour 1 kg, pea-flour 500 g are mixed together then whole mixture was autoclaved at 125°C temperature at 15 PSI for 1 hour. After autoclaved this diet was kept in a bowl for room temperature. Then 2 g or 3 g eggs of rice moth were spread over the diet and cover the bowl with 64 meshes net.

Female moth lay eggs, this egg falls down through the net and store at the jar. Eggs are collected and cleaned. thick color papers used as trichocard and the size of trichocard should fit to the test tube. Date and label are noted at one end of trichocard. A coat of 10% gum arabia was applied on the cards.

Eggs of Corcyra are sprinkled uniformly in a single layer with the aid of a strainer. The excess eggs pasted were removed gently by passing a brush over the card after sufficient air drying under fan. Label information on the species of the parasitoid and date of parasitization were given in the left-over spaces. Very small amount of honey was placed on the card for supplying food. The egg cards were placed into test tube/glass cylinder of suitable size and the nucleus card of *Trichogramma* was introduced in it. Black coloration of eggs indicated the presence of parasitoid.

Efficacy of *Bracon hebetor* against Fall Armyworm: Initial *B. hebetor* mother culture was procured from Patuakhali Science and Technology University laboratory, Dumki, Patuakhali. The method followed for efficacy studies was developed for *B. hebetor* rearing at PSTU. In this method for efficacy studies of 1 host, individual experiments were conducted with 3 replications. In the first experiment for each replication 20 *B. hebetor* adults were released in 3 jars and cotton dipped in 10% honey solution was placed at 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 black cloth. In each jar 20 adults of *B. hebetor* were released and 10 *S. frugiperda* larvae were placed in corrugated paper sheet for parasitization. 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. Each host larva was observed under magnifying glass one day after transfer to petri plate.

The second and third experiment was conducted as mentioned above and along with *S. frugiperda*. 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.

Statistical analysis: 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 treatment was calculated and represented as mean ± SD.

RESULTS AND DISCUSSION

Optimized protocol for Mass rearing of larval parasitoid, *Bracon hebetor* in the bio-control laboratory: In this protocol development, controlled environment conditions were maintained for insect, like lab temperature was 26-28°C and relative humidity was 70-75%. Steps are given bellow (Fig. 1):

Parent stock of wax moth developed in honeycomb in glass jar
↓ First to second instars larvae of wax moth are reared in artificial diet
↓ 18-20 days later, full-fed 200 larvae transferred in a plastic bottle with a corrugated paper sheet
↓ 100 adults *B. hebetor* (60 female and 40 male) released in the plastic bottle with honey socked cotton pieces
↓ The wax moth larvae and *B. hebetor* containing whole bottle covered the with black cloth and kept on rack for parasitism, egg laying, pupation and adult emergence of *B. hebetor*
↓ 8-10 days later, adults B. hebetor emerged from the parasitized larvae and ready for field release.

Fig. 1. Mass rearing protocol of *B. hebetor*

Optimized protocol for Mass rearing of egg parasitoid, *Trichogramma chilonis* in the bio-control laboratory: In this protocol development, controlled environment conditions were maintained for insect, like lab temperature at 26-28°C and relative humidity of 70-75%. Steps are given bellow (Fig. 2)

Sterilized artificial diet infested with older eggs of *Corcyra cephalonica* and kept for 28-30 days

↓

After 28-30 days, adults were collected and kept in a jar for egg laying

↓

Eggs were brush up from the cylinder and sieved to get fresh eggs. Fresh eggs used for *Trichogramma* rearing while older eggs used for *Corcyra rearing*

↓

Eggs were spreads over the gum containing trichocard, then cards placed in a *Trichogramma* containing test tube for patasitization

↓

Next day parasitized trichocard replaced in a new test tube, after 7-8 days new adult was emerged and ready for release.

Fig. 2. Mass rearing protocol of *Trichogramma chilonis*

Efficacy of *Bracon hebetor* against different instar of FAW larvae: When the 3rd instar larvae of host insect was offered to *B. hebetor* mean ± SD of parasitized host larvae was 3.33 ± 0.57 and SE 0.33. Mean ± SD of egg, larvae, pupae and adults of *B. hebetor* were 14.67±1.52, 14.33±1.53,11±1 and 8.66±0.577, respectively per single *S. frugiperda* larva in jar 1. Mean ± SD number of eggs, larvae, pupae and adults of *B. hebetor* were 16.5±2.08,15±0.816,11.25±0.95 and 8±1.41 per larva, respectively in jar 2. Mean ± SD of egg, larvae, pupae and adults of *B. hebetor* were 15.33±2.52, 14±1.73, 9.33±2.15 and 6±1 on *S. frugiperda* larvae in jar became black, dead within 24 hours after exposure to *B. hebetor* (Table 3, Fig. 3).

Table 3. Parasitizing of *B. hebetor* on 3rd instar larvae of *Spodoptera frugiperda* under laboratory conditions

Experimental jar	Number of host (*Spodoptera frugiperda*)/ jar	Number of *B. hebetor*/ jar	Number of parasitized larvae/jar	No. of *B. hebetor* egg formed/ host larva (Mean±SD)	No. of *B. hebetor* larvae formed/ host larva (Mean ± SD)	No. of *B. hebetor* pupa formed/ host larva (Mean ± SD)	No. of *B. hebetor* adult formed/ host larva (Mean ± SD)
jar 1	10	20	3	14.67±1.52	14.33±1.53	11±1	8.66±0.577
jar 2	10	20	4	16.5±2.08	15±0.816	11.25±0.95	8±1.41
jar 3	10	20	3	15.33±2.52	14±1.73	9.33±2.15	6±1
Mean ± SD			3.33 ± 0.57				
SE			0.33				

Fig. 3. Efficacy of *B. hebetor* on 3rd instar larvae in three jars

When the 4th instar larvae of host insect was offered to *B. hebetor* mean ± SD of parasitized host larvae was 6.33 ± 1.53 and SE 0.88. Mean ± SD of egg, larvae, pupae and adults of *B. hebetor* were 18.6±1.67, 15.6±1.67,10.67±1.14 and 9.8±1.30, respectively per single S. *frugiperda* larva in jar 1. Mean ± SD of egg, larvae, pupae and adults of *B. hebetor* were 18.67±2.25,14.16±2.13,10±2.19 and 9.33±2.33 per larva, respectively in jar 2. Mean ± SD of egg, larvae, pupae and adults of *B. hebetor* were 18.85±3.23, 14±2.82, 12±2.38 and 11.28±2.28 on *S. frugiperda* larvae in jar became black, dead within 24 hours after exposure to *B. hebetor* (Table 4, Fig. 4).

Fig. 4. Efficacy of *Bracon hebetor* on 4th instar larvae in three jars

Table 4. Parasitization of *Bracon hebetor* on 4th instar larvae of *Spodoptera frugiperda* under laboratory conditions

Experimental Jar	No. of host (*Spodoptera frugiperda*)/ jar	No. of *Bracon hebetor*/jar	No. of parasitized larvae/jar	No. of *B. hebetor* egg formed/ host larva (Mean ± SD)	No. of *B. hebetor* larvae formed/ host larva (Mean ± SD)	No. of *B. hebetor* pupa formed/ host larva (Mean ± SD)	No. of *B. hebetor* adult formed/ host larva (Mean ± SD)
Jar 1	10	20	5	18.6±1.67	15.6±1.67	10.6±1.14	9.8±1.30
Jar 2	10	20	6	18.67±2.25	14.16±2.13	10±2.19	9.33±2.33
Jar 3	10	20	8	18.85±3.23	14±2.82	12±2.38	11.28±2.28
Mean ± SD			6.33 ± 1.53				
SE			0.88				

When the 5th instar larvae of host insect were offered to *B. hebetor* had mean ± SD of parasitized host larvae was 9.66 ± 0.57 and SE 0.33. Mean ± SD of egg, larvae, pupae and adults of *B. hebetor* were 22.44±2.79, 18.44±2.06,11.55±2.08 and 10.88±2.31, respectively per single *S. frugiperda* larva in jar 1. Mean ± SD of egg, larvae, pupae and adults of *B. hebetor* were 23.4±4.03,21±3.50,13.6±4.06 and 12.6±3.97 per larva, respectively in jar 2. Mean ± SD of egg, larvae, pupae and adults of *B. hebetor* were 25±4.54, 22.5±4.47, 17.9±4.33 and 16.1±4.04 in *S. frugiperda* larvae, respectively in jar where eggs became black, dead within 24 hours of exposure to *B. hebetor* (Table 5, Fig. 5).

Table 5. Parasitization of *B. hebetor* on 5th instar larvae of *Spodoptera frugiperda* under laboratory conditions

Experimental Jar	No. of host (*Spodoptera frugiperda*)/ jar	No. of *B. hebetor*/ jar	No. of parasitized larvae/jar	No. of *B. hebetor* egg formed/ host larva (Mean ± SD)	No. of *B. hebetor* larvae formed/ host larva (Mean ± SD)	No. of *B. hebetor* pupa formed/ host larva (Mean ± SD)	No. of *B. hebetor* adult formed/ host larva (Mean ± SD)
Jar 1	10	20	9	22.44±2.79	18.44±2.06	11.55±2.08	10.88±2.31
Jar 2	10	20	10	23.4±4.03	21±3.50	13.6±4.06	12.6±3.97
Jar 3	10	20	10	25±4.54	22.5±4.47	17.9±4.33	16.1±4.04
Mean ± SD			9.66 ± 0.57				
SE			0.33				

Fig. 5. Efficacy of *B. hebetor* on 5th instar larvae in three jars

Efficacy of *B. hebetor* on FAW larvae in net cage: The percent parasitism of FAW by *B. hebetor* ranged 20-40 % among three net cage trials where the highest percent was recorded in cage no. 2 and the lowest in cage no. 3 (Fig. 6, Plate 1). Causes of lowest parasitism in case no.3 may due to hiding of larvae inside maize plant and under soil clods.

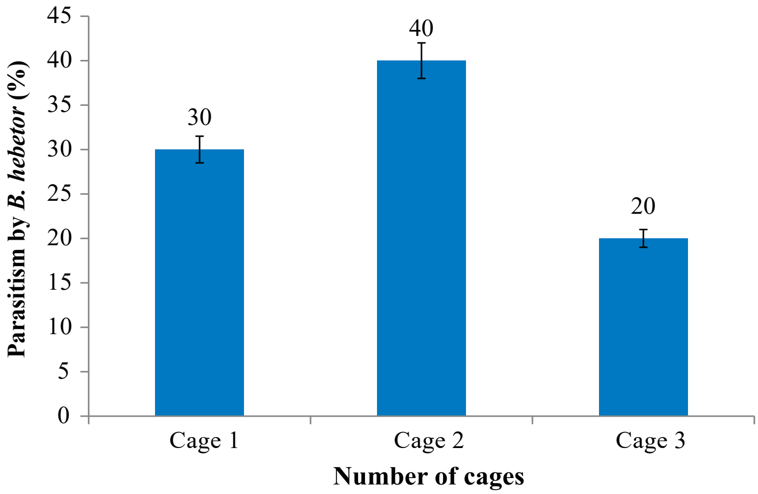

Fig. 6. Percentage of parasitized larvae of FAW by *B. hebetor* in net cage conditions

Plate 1 (A&B). Efficacy of *B. hebetor* on FAW larvae in net cage

The findings of the present study are supported by Ashley *et al*. (1982), Ashley (1983) and Pair *et al*. (1986). FAW reported recently in Bangladesh found as 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. 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. According to Brower *et al*. (1996), *B. hebetor* is a highly effective biological control agent for stored-product insects of the Pyralidae family. *B. hebetor* females sting and inject paralytic venom to paralyze their host, which are often final stage larvae in a "wandering" phase. They then oviposit varying numbers of eggs on or around the paralyzed host. Paralyzed host larvae serve as food for growing wasp larvae and adult females. 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). Heimpel *et al*. (1997) suggest that the wasp species described in earlier research may be a different species than *B. hebetor*, capable of attacking Noctuidae moth larvae in the wild.

CONCLUSION

protocol for mass rearing of egg parasitoid, *Trichogramma* spp. and larval parasitoid *Bracon hebetor* has been optimized in the bio-control laboratory, PSTU. The larvae of *Spodoptera frugiparda* were parasitized after 24 hours of releasing *Bracon hebetor*. The recommended release rates for *Bracon hebetor* and *Trichogramma* spp. will vary depending on the specific pest species, crop, and environmental conditions. Using biological control agents like *Bracon hebetor* and *Trichogramma* can help reduce reliance on chemical insecticides, which can be harmful to human health and the environment. Both *Bracon hebetor* and *Trichogramma* spp. are sensitive to temperature and humidity. It's important to release them under environmental conditions that are favorable for their survival and activity. *Bracon hebetor* and *Trichogramma chilonis* may be incompatible with certain insecticides. It's important to check for compatibility before using them together.

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