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# CONSERVATION OF BIO-CONTROL AGENTS IN COTTON, *GOSSYPIUM HIRSUTUM* L. FIELD BY FOOD SUPPLEMENTS FOR INSECT PESTS MANAGEMENT

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The study reports the use of artificial food sprays to conserve the parasitoids and predators for the management of insect pests in cotton field. Cotton crop was treated with bio-control agents, *Chrysoperla carnea* and *Trichogramma chilonis* alongwith different food attractants such as protein hydrolysate and sugar alone and in combination in a randomized complete block design. Each treatment was applied on one-acre field with three replications. Results showed that the chemicals tested helped in increasing the populations of beneficial insects including; *C. carnea*, *T. chilonis* and *Orius* spp., in the field. The populations of *C. carnea* and *T. chilonis* were found the highest in the combined treatment of protein hydrolysate and sugar as compared to other treatments where protein hydrolysate and sugar were used separately. However, the population of *Orius* spp. was higher in the treatment where only sugar solution was sprayed as food supplement. Consequently, incorporation of food supplements in the trial increased the establishment of natural enemies and subsequently the predation/ parasitism percentage enhanced on the insect pests of cotton.

**Keywords:** Parasitoids, Conservation, Cotton, Food sprays, Attractants

## 1. Introduction

Cotton, *Gossypium hirsutum* L., being a non-food cash crop contributes significantly in foreign exchange earning of Pakistan. Cotton accounts for 8.6 percent of the value added in agriculture and about 1.8 percent to GDP. The crop was sown on an area of 3106 thousand hectares and its production estimated at 12.7 million bales for the year 2009-10. However, the cotton production was 5.0 percent less than the target of 13.36 million bales due to wide spread of cotton leaf curl virus in addition to the shortage of irrigation water, high temperature in the month of August resulting in an excessive fruit shedding and flare up of insect pest complexes [1]. Cotton is attacked by a number of insects and diseases from seedling to fruiting stage [2]. Cotton production is adversely affected by many insect pests, which have been traditionally controlled by large quantities of insecticides. Although chemical insecticides provide an excellent control, but, resistance in insect pests of cotton have been reported against many pesticides. This enhanced the use of increased amount of insecticides [3]. The excessive use of chemical insecticides has produced several undesirable effects including development of resistance in insect pests to insecticides, toxic residues in lint and seed, environmental pollution, destruction of beneficial organisms and health risks [4]. Therefore, the use of excessive insecticides is unsustainable [5] and it is imperative to tap alternate methods of insect's control. The concept of Integrated Pest Management (IPM) is also becoming popular because it encourages the use of several components in the pest control system in a harmonious combination, which has minimal impact on the environment [6].

Biological control which is a basic component of any IPM system is being used successfully to combat many insect pests. The biological control alone has managed pests well enough to eliminate the need for further treatment [7]. The use of *Chrysoperla carnea* in conjunction with *Trichogramma* wasp as bio-control agents is a recognized alternative of insecticides [8] and has been applied successfully for the management of many insect pests [9]. *C. carnea*, an effective predator of jassids, whiteflies, thrips, aphids and mites, also feeds on the eggs and tiny larvae of the

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cotton bollworms. It has been reported that *C. carnea* larvae attack about 80 species of insects and 12 species of tetranychid mites [10]. The adults are not predatory and can be easily cultured on relatively simple diets [11]. Parasitoid *T. chilonis* is an effective biocontrol agent against a wide range of lepidopterous insect pests. Similarly, *Orius* spp., are recorded as potential predators against sucking insect pests of cotton. The beneficial insects alone or in adjunct to other management tactics have been successfully manipulated with a variety of augmentation and conservation strategies [12, 13]. The adults of most beneficial insects feed on honeydew, nectars and pollens; as a result, use of artificial food sprays to increase the abundance and impact of natural enemies of arthropod pests is important. Augmentation of parasitoid *T. chilonis* and predator *C. carnea* can successfully control the insect pests of cotton below the economic injury level. However, the establishment of these parasitoids and predators may be very low during the hot growing month that indicates the importance of conservation of bio-control agents, which is an important component of biological control program. The use of supplement food as conservation tool has a great potential to encourage natural enemies. Consequently, the current experiments were conducted to evaluate the effect of protein hydrolysate and sugar on the conservation of natural enemies' especially *C. carnea* and *T. chilonis* in the field for the successful eco-friendly management of cotton insect pests.

## 2. Materials and Methods

The experiment was conducted at farmer's field near Tandojam on cotton variety "Chandi-95"in May 2005 in three blocks each measuring an area of 5 acres having a distance of at least 200 meters between each block. Experimental field was direct seeded, and plants spaced at 75 cm between rows and 30 cm between plants within a row. The crop was fertilized and cultivated according to local commercial practices and irrigated with water when required. Out of the five acres, a block of four acres were treated with bio-control agents, *T. chilonis* and *C. carnea* alongwith different treatments. The acre one and two were supplemented with sugar and protein hydrolysate alone, respectively. The concentrated 10% solutions of sugar and protein hydrolysate, were applied using hand knack sprayer at fortnightly intervals as food supplements/ attractants. The solutions of sugar and protein hydrolysate were sprayed separately in spots of 2 meters grid at ten uniformly distributed locations in the respective treatments at fortnightly intervals. The third acre of cotton was treated with bio-control technology and supplemented/ sprayed with 1:1 ratio of protein hydrolysate and sugar solution as a diet for the released bio-control agents on the same date when natural enemies applied in the first or second numbers of acre. The fourth acre was treated with bio-control agents only and nothing was supplemented as food for the released insects. Whereas, the fifth acre in each block was kept as control, where no treatment was applied except farmer's own practice who applied four sprays of insecticides. Similar trials were repeated in the other replicates of two five acres blocks having the same treatments.

Biological control agents (*C. carnea* and *T. chilonis*) were obtained from the mass rearing laboratory running at Nuclear Institute of Agriculture (NIA), Tando Jam and were reared on the eggs of angoumois grain moth, *Sitotroga cerealella,* in two separate laboratories maintained at 25± 2 degree Celsius and 60-70% relative humidity. For the parasitoid, the eggs of angoumois grain moth *S. cerealella* were glued on white paper cards and exposed to parasitoids for 24 hours. The parasitoids prior to adult emergence were released by attaching the paper cards on the lower side of the cotton leaves at fortnightly intervals at the rate of 10,000 parasitoids/ acre in each of the four acres of a block. The predator was released at the mature egg stage by gluing them on paper cards and the cards were attached to the lower surface of the leaves at fortnightly interval at the rate of 500 eggs/ acre in the respective treatments. Data on the establishment of the bio-control agents released and those present naturally in the cotton field were recorded weekly from each treatment. Populations of the predators were determined by counting their numbers per 40 randomly selected cotton plants from each treatment of the three replicates. The establishment/ conservation of the *T. chilonis* was surveyed by placing 2000/ acre fresh eggs of angoumois grain moth glued on paper cards in the cotton field of each treatment. These cards were brought into the laboratory after 24 hours exposure in the differently treated cotton plots and parasitoid emergence was recorded. The data were analyzed statistically by following Steel and Torrie [14], and all the tests were set at the significant level of $P \leq 0.05$.

Table 1. Population of *C. carnea* in treated and untreated cotton fields.

| Month | Mean population per treatment | | | | |
|---|---|---|---|---|---|
| | Bio-control & Sugar alone | Bio-control & Protein hydrolysate alone | Bio-control & Sugar+ Protein hydrolysate | Bio-control alone | Control |
| June | 1.20 cd | 2.10 bc | 2.00 d | 0.76 c | 0.00 |
| July | 2.50 bc | 2.84 bc | 7.00 c | 1.30 bc | 0.00 |
| Aug. | 3.80 b | 4.60 b | 11.00 bc | 2.70 b | 0.89 ab |
| Sep. | 5.86 ab | 7.20 ab | 16.00 b | 4.50 a | 1.20 a |
| Oct. | 8.40 a | 10.30 a | 19.00 a | 5.30 a | 2.00 a |
| Nov. | 3.00 c | 5.00 b | 7.00 c | 2.40 b | 1.50 a |
| Mean | 4.12 B | 5.34 B | 10.33 A | 2.82 C | 0.93 D |
| LSD Value | 2.57 | 2.64 | 5.79 | 1.93 | 1.13 |

Means sharing similar letters have non-significant differences ($P \leq 0.05$).

Table 2. Parasitism (percent) of the *T. chilonis* in treated and untreated cotton fields.

| Month | Mean population per treatment | | | | |
|---|---|---|---|---|---|
| | Bio-control & Sugar alone | Bio-control & Protein hydrolysate alone | Bio-control & Sugar + Protein hydrolysate | Bio-control alone | Control |
| June | 1.50 d | 1.00 d | 1.80 d | 0.00 | 0.00 |
| July | 2.60 cd | 2.30 cd | 10.95 cd | 1.20 b | 0.58 b |
| Aug. | 3.75 bc | 2.60 c | 16.55 bcd | 2.75 b | 0.95 b |
| Sep. | 5.96 b | 4.20 b | 20.45 bc | 4.95 b | 1.80 ab |
| Oct. | 9.95 a | 9.35 a | 35.85 b | 8.95 a | 2.75 a |
| Nov. | 11.50 a | 11.00 a | 55.95 a | 9.57 a | 3.50 a |
| Mean | 5.87 B | 5.07 B | 23.59 A | 4.57 C | 1.95 C |
| LSD Value | 2.26 | 2.04 | 8.91 | 3.47 | 1.76 |

Means sharing similar letters have non-significant differences ($P \leq 0.05$).

## 3. Results and Discussion

As shown in Table 1, the mean monthly population of *C. carnea* per 40 cotton plants per treatment was observed highest (10.33) in the field where the bio-control agent was supplemented with the protein hydrolysate plus sugar followed by the treatment where the protein hydrolysate (5.34) was applied alone in combination with bio-control agent. The supplementation of sugar solution only (4.12) also indicated positive effect on population of the predator as compared to non provision of the food (2.82). On the other hand, population of the bio-control agent was higher in the field where both sugar and protein hydrolysate were sprayed as supplemental food. The populations of bio-control agent were very low in the untreated control field (0.93).

The parasitization on angoumois grain moth's eggs by *T. chilonis* (Table 2) indicated that the mean percent parasitism of the parasitoid increased in all the fields, where food was supplemented as conservation tool. However, the parasitism of the *T. chilonis* was significantly higher (23.59%) in the field treated with both protein hydrolysate and sugar alongwith the bio-control technology followed by the field where concentrated sugar solution was sprayed (5.87) alone. The parasitism by the *T. chilonis* was significantly higher in the field where only protein hydrolysate solution was supplemented (5.07) along with the release of bio-control agents as compared to fields with biocontrol agent used alone (4.57) or non-treated control (1.95).

Table 3. Population of *Orius* spp., in treated and untreated cotton fields.

| Month | Mean population per treatment | | | | |
|---|---|---|---|---|---|
| | Bio-control & Sugar alone | Bio-control & Protein hydrolysate alone | Bio-control & Sugar + Protein hydrolysate | Bio-control alone | Control |
| June | 2.00 c | 0.00 | 1.50 c | 0.00 | 0.00 |
| July | 3.30 bc | 1.30 d | 2.50 bc | 1.20 c | 0.00 |
| Aug. | 4.30 b | 2.60 c | 3.80 b | 0.89 d | 0.74 c |
| Sep. | 8.10 a | 3.40 b | 9.10 a | 3.60 b | 3.20 b |
| Oct. | 9.40 a | 6.30 a | 9.50 a | 6.50 a | 6.10 a |
| Nov. | 6.40 ab | 2.50 c | 5.00 b | 2.40 bc | 2.30 b |
| Mean | 5.58 A | 2.58 B | 5.23 A | 2.43 B | 2.06 B |
| LSD Value | 2.83 | 1.21 | 1.54 | 1.66 | 0.95 |

Means sharing similar letters have non-significant differences ($P \leq 0.05$).

Table 4. Population/ Infestation of insect pests in treated and untreated cotton fields.

| Food attractants | Jassids/ leaf | Thrips/ leaf | Whiteflies/ leaf | PBW infest. (%) | SBW infest. (%) |
|---|---|---|---|---|---|
| Bio-control & Sugar alone | 1.35 bc | 2.38 cd | 0.89 bc | 5.72 b | 6.23 bc |
| Bio-control & Protein hydrolysate alone | 0.86 cd | 1.82 de | 0.73 c | 4.52 c | 5.98 c |
| Bio-control & Sugar+ Protein hydrolysate | 0.50 d | 1.42 e | 0.36 d | 2.04 c | 3.18 d |
| Bio-control alone | 1.91 ab | 3.45 b | 0.73 c | 5.52 bc | 7.98 b |
| Control | 2.27 a | 5.25 a | 1.88 a | 9.28 a | 12.45 a |
| LSD Value | 0.49 | 0.68 | 0.43 | 1.77 | 1.85 |

Means followed by similar letters are not significantly different ($P \leq 0.05$)

Unlike *C. carnea* released, the population of the *Oius* spp., was present naturally in the cotton field, which was observed significantly higher (5.58) in the treatment where, only sugar solution was sprayed as food supplements followed by the fields supplemented with sugar plus protein hydrolysate (5.23), in comparison to protein hydrolysate alone (2.58), biocontrol agent alone (2.43) and untreated control fields (2.06) (Table 3). The results revealed that protein hydrolysate was less preferable food attractant for the *Orius* spp., than the sugar solution.

The impacts of food sprays through natural enemies were positive on the cotton pests control and significantly lowered the populations of white flies, jassids and thrips recorded in the fields treated with protein hydrolysate plus sugar followed by the separate treatments of protein hydrolysate and sugar. Although the spray of protein hydrolysate in combination with sugar reduced the population of thrips, jassids and white flies but protein hydrolysate spray as food attractant alone, also gave very good results to reduce the populations of bollworms indicating its preference for the bio-control agents. The infestations of pink and spotted cotton bollworms were significantly less in the fields sprayed with protein hydrolysate alone or in combination with sugar as compared to control (Table 4).

The present study suggested that providing supplemental foods in the form of sugar and protein hydrolysate sprays have great potential for enhancing the bio-control agent's populations in the cotton field. The bio-control agents ultimately played a vital role to increase predation on different life stages of pests by *C. carnea* and parasitism in the eggs of cotton bollworms by *T. chilonis* resulting an increased numbers of predators and parasitoids in the target area. These food sprays may satisfy metabolic and reproductive needs of bio-control agents [15].Thus, the availability of food is an important factor in the successful application of biological control agents [16]. In the cotton field, conservation of natural enemies was increased in the fields, supplemented with food sprays (sugar and protein hydrolysate) indicating the significance of this factor. Similar results have been documented for many hymenopteran parasitoids [17-19]. Carbohydrates, the main energy source for most insects, are obtained by the insects from floral nectars or honeydew produced by aphids and scale insect [20-23], while, such food sources are unavailable in the field, thus artificial food sprays are needed as supplement to enhance the effectiveness of natural enemies in the field [20-24]. Therefore, food availability and internal nutritional status can determine whether a natural enemy forages for hosts or food [25].

The results of the experiment illustrated the importance in understanding food-foraging behavior of parasitoids/ predators when attempting to enhance biological control through addition of supplemental food sources. Parallel to our findings, feeding of *T. vernalis* on honeydew deposits from soft scales or aphids have frequently been observed and hundreds of the wasps by spraying sugar water on tree foliage have been collected [26, 27]. Hagen *et al*. [28] demonstrated that predators (adult lacewings) were arrested, not attracted, by sucrose sprays alone and that addition of an attractant such as triptophan to sucrose solutions was necessary to recruit them from a distance [29]. Many species of parasitoid *Tiphia* imported and released for biological control of *Popillia japonica* failed to become establish [30]. Some of these failures were attributed to lack of suitable food for adult wasps. Indeed, food availability may be the most important factor limiting the establishment and distribution of the parasitoid *Tiphia* spp., [31]. Application of supplemental food sprays to the cotton foliage where host insect pests are abundant may facilitate the establishment of parasitoid (*T. chilonis*) and also increase the populations of the predators (*C. carnea* and *Orius* spp.) in the cotton field. Such food may also prove useful to prolong the longevity of the parasitoids and predators in the field. This has implications not only for conserving native populations of natural enemies but also for introduction and establishment of bio-control agents from the adjacent areas. However, to achieve greater success with artificial food sprays and to overcome a major impediment to their adoption, this technique should be used in combination with other compatible elements of an integrated pest management strategy.

## 4. Conclusion

It is concluded from the present study that supplementation of food to biocontrol agents under natural field conditions has increased the efficacy and abundance of biocontrol agents for the effective management of cotton insect pests. Until these outcomes are demonstrated, it is envisaged that artificial food sprays will form a small component of future conservation biological control programs. Consequently, it is appropriate to adopt this technology more widely. Long term studies that incorporate such food supplements at multiple locations across a range of habitats are needed to fully evaluate the effectiveness of this tactic for biological control of insect pests of cotton.

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