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Evaluation of pesticide tolerant strain of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) on maize stem borer, *Chilo partellus* (Swinhoe)

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Field evaluation of pesticide tolerant strain of *Cotesia flavipes* Cameron, a gregarious larval endoparasitoid of the maize stem borer, *Chilo partellus* (Swinhoe) was carried out and the economics of such evaluation was worked out. Releases of the pesticide tolerant parasitoid strain, prior to application of pesticide or after application resulted in significantly higher net returns and yield, as compared to the releases of susceptible parasitoid strain and untreated control. Four releases of the tolerant parasitoid at 2000/ha at 500 per release registered maximum number of parasitized cocoons (52.6 per larva), higher yields (4019 kg/ha) and net profits (Rs. 7807 kg/ha), followed by two releases of the tolerant strain with two sprays of insecticide (40.8, 3809 kg/ha and Rs. 6847/ha, respectively). The insecticidal treatment gave higher net profits and yields than all other treatments. However, the additional costs, ecological hazards and pesticide residues (likely to occur) do not outweigh the benefits of biological control.

Key words: *Cotesia flavipes, Chilo partellus*, population, pesticide tolerance.

INTRODUCTION

Lepidopteran stem borers are among the most damaging pests of maize in the semi-arid countries and are a major constraint in realizing the yield potential of the crop. In India, the stem borer, *Chilo partellus* (Swinhoe) (Crambidae: Lepidoptera) is one of the most serious insect pests causing 24.3 to 36.3% loss in different agroclimatic regions of the country (Ganguli et al., 1997). A large number of insecticides belonging to different groups viz., the organochlorines, organophosphates, carbamates and synthetic pyrethroids have been widely used to curtail the pest (Dharmasena, 1993; Teli et al., 2007) with limited success. Chemical control is often effective on the neonate instars, before the larvae enter the stem (Reyes, 1987), therefore the timing of application is crucial for the successful management of the pest (Nwanze and Mueller, 1987). Indiscriminate use of pesticides had resulted in several ecological hazards; therefore bio-intensive pest management strategies have been advocated. Among the biocontrol agents, *Cotesia flavipes* (Cameron) (Braconidae: Hymenoptera) a gregarious endo larval parasitoid has been widely used in maize, sorghum and sugarcane ecosystems (Kalra and Srivatsava, 1996; Tanwar and Varma, 2002). The parasitoid is reported to cause 32 to 55% decrease in stem borer densities in various crops (Kfir et al., 2002). Parasitism up to 40% on sugarcane borer in Texas (Setamou et al., 2003), 11 to 21% in Brazil and 17 to 21% in Africa on Maize stalk borer *Busseola fusca* (Ngi-Song et al., 2012) was reported. In India, the extent of parasitism was 36.67%in Pantnagar (Choudhary and Sharma, 1987), 35 to 50% in Himachal Pradesh (Nirmala Devi and Desh raj, 1996), 6.6 to 21% in Assam (Borah

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and Arya, 1995) and 2 to 33% in Haryana (Mohan et al., 1999). The extent of parasitism is often low, due to their susceptibility to insecticidal applications on the target pest (Songa et al., 1999). Therefore, the use of tolerant strain of the parasitoid would result in effective suppression of the pest. A pesticide tolerant population of the parasitoid (*C. flavipes*) was developed and evaluated under the field conditions on the maize stem borer, *C. partellus*. The laboratory experiments were conducted at the Division of insect Molecular Entomology, National Bureau of Agriculturally Important Insects and the field experiments were carried out at the Biocontrol Research Farm of the institute at Attur in Bangalore, India during the year 2009-2010.

MATERIALS AND METHODS

Rearing and maintenance of culture of the stem borer and the parasitoid

Field populations of *C. partellus* were collected from different geographic locations (Aurangabad, Bangalore, Chindwara, Devaganahalli, Dindigul, Hoishiarpur, Malur and Shimla) in India. The populations were maintained in the laboratory on semi-synthetic diets as prescribed by Ballal et al. (1995). The larvae were reared individually in glass vials (10 x 2.5 cm) at ambient temperature of 26 ± 1°C and 65% RH. The populations maintained in the laboratory were utilized for toxicity bioassay, rearing of the parasitoid and field experiments. Fourth-fifth instar larvae of *C. partellus* were offered to two day old adults of the parasitoids kept in plastic jars (10 x 15 cm), covered with muslin cloth. Cotton swab soaked with honey (30%) was placed inside the jar as food for the parasitoids. The larvae were exposed to the parasitoid for two hours and the parasitized larvae were individually transferred into glass vials (10 x 2.5 cm) containing the semi-synthetic diet and were reared till the formation of cocoons. The cocoons were then transferred into another vial for adult emergence.

Laboratory evaluation of pesticide tolerant strain of *C. flavipes*

In vitro toxicity bioassays were carried out with the widely used insecticides in maize ecosystem (fenvalerate and endosulfan), on field populations of *C. flavipes* obtained from different geographic populations of the *C. partellus* (as mentioned above), Laboratory bioassays were carried out by dry film method (Kao and Tzeng, 1992; Keimeesuke et al., 1994). The insecticidal spray was prepared by taking dosages higher than field recommended dosages and corresponding serial dilutions were made to test the level of toxicity to the insecticides. Thus, the concentrations for endosulfan were: 21.88, 43.75, 87.50, 175.0, 300.0 and 750 ppm and for fenvalerate the dosages were 1.26, 2.5, 5.0, 10.0, 25 and 40 ppm. The assays were carried out following the IOBC protocols (Hassan et al., 1985).

The insecticides were prepared as aqueous solutions based on the dosages and sprayed with the help of atomizer. Modified glass tube with both sides open was used as testing unit. The sprayed glass tubes were dried in the shade. After drying, one end of tube was covered with double-layered black cloth and was fastened with rubber bands. From the other side, about 50 adults were released in each vial in each concentration. There were 10 replications for each treatment.

The mortality of the parasitoid was recorded after 15 and 30 min and after 1, 2, 4, 6 and 24 h of constant exposure. The data obtained on mortality were subjected to probit analysis by statistical program SPSS version 8.0. The data were transformed to log base 10 before probit analysis and antilog of calculated values gave actual LC_{50} and LC_{90}. The fiducial limits slope and χ^2 values were also calculated.

Field evaluation of pesticide tolerant strain of *C. flavipes*

A field experiment was conducted during 2009-10, rabi season (October to February) to evaluate the pesticide tolerant strain of *C. flavipes* (Shimla population) at the Biocontrol Research Farm, Attur (Bangalore). A composite maize (variety: Varalakshmi), was sown in randomized block design following the recommended package of agronomic practices. There were eight treatments and three replications with a plot size of 5 x 4 m each. The following treatments were imposed; T1, Four sprays of fenvalerate (0.1 ml/L); T2, Four releases of PTS of *C. flavipes* (Shimla population); T3, four releases of susceptible *C. flavipes* population; T4, two sprays of fenvalerate + 2 releases of tolerant *C. flavipes;* T5, two sprays of fenvalerate + 2 releases of susceptible *C. flavipes*; T6, two releases of tolerant *C. flavipes* + 2 sprays of fenvalerate; T7, two releases of susceptible *C. flavipes* + 2 sprays of fenvalerate and T8, untreated check (control).

Release of the stem borer and parasitoids

The population of *C. partellus* maintained in the laboratory was utilized for the field experiments. The different treatments were created by releasing 10 first instar larvae into the leaf whorl of maize plants with the help of "0" sized camel hair brush. Five plants (tagged) were chosen at random from three central rows of each plot. Application of the insecticide and releases of parasitoid as per the treatments scheduled were done after 3 to 4 days of larval implantation as advocated by Teli et al. (2007). The parasitoids were released in the field as adults in release stations as prescribed by Songa et al. (2001). The treatment of release of parasitoid following the insecticidal sprays was done immediately after the scheduled spray. Darkened cocoon masses (likely for adult emergence) obtained from the laboratory culture (Shimla population) were placed in plastic Petri plates (9 x 1.5 cm) lined with moist filter paper and then placed in the centre of each plot. The parasitoids were released at 2000 cocoons/ha which works out to 500 cocoons per release, for an individual plot size of 5 x 4 m replicated thrice. There were two release stations per plot. The various treatments were far apart by a distance of 3 m on each side. Releases were not a onetime application since the insect pest, *C. partellus* has over lapping generations and the parasitism is more effective on the 2-3rd instar larvae of the host. The releases commenced at about three weeks after emergence of the maize plants to synchronise the host larval stages (2nd to 3rd instar larva). The study included the releases of the parasitoid to be made either prior to the application of the insecticide and vice versa, to generate information on the pesticide tolerant strain of the parasitoid and its efficiency in terms of parasitism as compared to the susceptible strain.

The parasitism was noted by destructive sampling. Observations were recorded on percent dead hearts, number of cocoons formed per parasitized larvae and yield (kg/ha) in each of the treatments and compared with the untreated control. Parasitized larvae obtained from the different treatment plots were collected, taken to the laboratory and reared individually in glass vials containing artificial diets. The results after angular transformation were subjected to statistical scrutiny by ANOVA. The cost economics of the different treatments was worked out.

Table 1. Field evaluation of tolerant strain of *C. flavipes* on *C. partellus*.

Treatment	Per cent dead hearts	(No. of parasitized cocoons/larva)	Yield (kg/ha)
Four sprays of Fenvalerate	5.72[a]	---	4280[f]
Four releases of tolerant *C.flavipes*	27.5[c]	52.6[g]	4019[e]
Four releases of susceptible *C.flavipes*	22.2[bc]	21.8[c]	3719[c]
Two sprays of Fenvalerate + two releases of tolerant *C. flavipes*	18.6[b]	28.3[d]	3768[c]
Two sprays of Fenvalerate + two releases of susceptible *C. flavipes*	26.2[c]	17.2[b]	3702[c]
Two releases of tolerant *C. flavipes* + two sprays of Fenvalerate	32.6[d]	40.8[f]	3809[d]
Two releases of susceptible *C. flavipes* two sprays of Fenvalerate	38.4[e]	33.2[e]	3692[b]
Untreated check (Control)	79.98[f]	10.6[a]	2448[a]
CD (5%)	5.22	3.57	107.89
SEM	2.283	1.17	67.51
CV (%)	12.9	7.98	4.73

Mean of three replications.

RESULTS

The laboratory bioassays indicated that population of the parasitoid from Shimla was more tolerant (with LC_{50} 2.77 ppm) as compared to others (Coimbatore LC_{50} 2.24 ppm, Chindwara LC_{50} 1.98 ppm). The population from Shimla cons obtained from different locations, the population tolerant was evaluated under field conditions

Efficacy of pesticide tolerant strain (PTS) of *C. flavipes* on maize stem borer

The efficiency of PTS of the parasitoid on the stem borer *C. partellus* in the various treatments revealed that higher percentage of dead hearts (dead central shoot of the plant was formed as a result of feeding by the stem borer) was observed in the releases of the susceptible parasitoid strains than releases of tolerant strains of the parasitoid, either alone or in combination with pesticides. Releases of the susceptible strains of the parasitoid followed by insecticidal sprays recorded 38.4% dead hearts, while releases made after the insecticidal sprays registered 26.2% dead hearts. Interventions with insecticides alone recorded the lowest percent of dead hearts (5.72%).

Effective parasitism of the stem borer was observed in the treatments, where releases of the parasitoid were followed by insecticidal sprays in both the tolerant and susceptible populations of the parasitoid. Exclusive releases of the tolerant parasitoid population (T2), however recorded the maximum number of parasitized cocoons (52.6 per larva). Two releases of the tolerant parasitoids followed by insecticidal sprays recorded 40.8 cocoons/larva, while a low parasitism (17.2 cocoons/ larva) was recorded in the treatment of insecticidal spray followed by releases of the susceptible parasitoid populations (Table 1).

The treatment with two sprays of fenvalerate followed by two releases of the tolerant parasitoid population recorded lower proportion of dead hearts (18.6), while releases of the susceptible parasitoid followed by insecticidal sprays registered the maximum percentage of dead hearts (38.4) (Table 1). However, spray of insecticide alone recorded the least percent dead hearts.

Cost Economics of the field evaluation of PTS

Significant differences in the yields obtained were observed in the different treatments. Releases of the susceptible parasitoid population followed by insecticidal sprays recorded low yield (3692 kg/ha). Four releases of the tolerant population of the parasitoid alone gave higher yield (4019 kg/ha) followed by two releases of the tolerant parasitoid populations with two sprays of insecticides (3809 kg/ha), while the control plots registered the minimum yield (2448 kg/ha).

The cost economics of the field evaluation of the tolerant strain of *C. flavipes* was worked out considering the market value of the produce, cost of the inputs and labour to arrive at the incremental cost-benefit ratio (ICBR) for the various treatments. Among the different treatments, four releases of *C. flavipes* recorded higher net profits (Rs. 7807 or 4,21,578 USD), additional returns (Rs. 10,997 or 5,92,758 USD) and yields (4019 kg/ha), followed by the treatment with two releases of tolerant *C. flavipes* along with two sprays of fenvalerate where the

Table 2. Economics of field evaluation of tolerant strain of *C .flavipes* on *C. partellus.*

Treatment	Yield (kg/ha)	Market value (Rs.700/Q)	Addl. returns Over control	Total cost/ha (Rs.)	Net profit/ha (Rs)	ICB ratio
Four sprays of Fenvalerate	4280	29960 (16,17,840)	12824 (6,92,696)	3254 (1,75,716)	10650 (5,75,100)	1:3.27
Four releases of tolerant *C. flavipes*	4019	28133 (15,19,182)	10,977 (5,92,758)	3170 (1,71,180)	7807 (4,21,578)	1:2.46
Four releases of susceptible *C. flavipes*	3819	26773 (14,45,742)	9597 (5,18,238)	3170 (1,71,180)	6427 (3,47,058)	1:2.02
Two sprays of Fenvalerate + two releases of tolerant *C. flavipes*	3768	26376 (14,24,304)	9240 (4,98,860)	2680 (1,44,720)	6560 (3,54,240)	1:2.44
Two sprays of Fenvalerate + two releases of susceptible *C. flavipes*	3702	25914 (13,99,356)	8778 (4,74, 012)	2680 (1,44,720)	6098 (3,29,292)	1:2.27
Two releases of tolerant *C. flavipes* + two sprays of Fenvalerate	3809	26663 (14,39,802)	9527 (5,14,458)	2680 (1,44,720)	6847 (3,69,738)	1:2.55
Two releases of susceptible *C. flavipes* two sprays of Fenvalerate	3692	25884 (13,97,736)	8748 (4,72,392)	2680 (1,44,720)	6068 (3,27,672)	1:2.26
Untreated check (Control)	2448	17136 (9,25,344)	-	1598 (86,892)	-	-

Figures in parenthesis correspond to value in US $).

net profits, additional returns and yields were Rs. 6847, Rs. 9527 and 3809 kg/ha, respectively. However, ICBR (1:2.55) was higher in the latter. Exclusive insecticidal applications with four sprays of fenvalerate resulted in higher yields (4280 kg/ha), and additional returns (Rs. 12,824 or 6, 92,496 USD) with highest ICBR (1:3.27) over the rest of the treatments (Table 2).

DISCUSSION

Our studies have indicated that pesticide tolerant strain of the braconid parasitoid, *C. flavipes* proved more effective in the suppression of the maize stem borer, *C. partellus*. Utilisation of the parasitoid for the management of the pest was carried out with releases made either prior to or after the application of insecticidal sprays. The parasitism was more effective when releases of the parasitoid were made preceeding spray application of insecticides.

Parasitization by *Cotesia* spp. induces host immune suppression in the sugarcane borer (*Diatraea saccharalis*) and the Diamond back moth *Plutella xylostella*. The immune suppression could impair insect defence capacity against pathogens and insecticides (Mahmoud et al., 2012). The immune suppression factors include the polyadnavirus, venoms, proteins and teratocytes. The parasitism may cause changes in insecticide-susceptibility of the parasitized host larvae, and that insecticide tolerance may result from changes in metabolic activities of enzymes involved. The enhanced activity of detoxifying enzymes such as glutathione-*S*-transferase (GST), cytochrome P450 monooxygenase (CYP) and carboxyesterase (CE) were proven to be responsible for many cases of alteration in insecticide-susceptibility (Tomoko et al., 2006).

In addition, the effective host searching ability, establishment and development of the parasitoid in a pesticide free habitat coupled with tolerance to the pesticide applied later, had played role in the reduction in the percentage dead hearts and increased number of parasitoid larvae than in other treatments. Further, changes in the host selection behavior (either due to repelling odors or avoidance) by the parasitoid due to application of the insecticide prior to their releases (treatments T4 and 5), would have resulted in reduced parasitism. Successful parasitisation includes host finding based on the frass excreted by the tunnelling larvae and the probe and biting strategy by the parasitoid (White et al., 2004). The chemical cues present in stemborer larvae are also involved in host recognition and acceptance (Meshack et al., 2010). The inability of the parasitoid to properly discriminate the cues due to the altered receptivity, after the application of the insecticide had contributed to the decreased parasitism.

Higher parasitism (parasitized cocoons/larva) due to *C. flavipes* contributed to higher yields in the various treatments. Low percent dead hearts was observed with the use of synthetic pyrethroids (Deltamethrin) in maize (Deepti and Sharma, 2004; Teli et al., 2007), in sweet sorghum (Deepti and Patil, 2008) and sugarcane (Tanwar and Varma, 2002),

In all the treatments, releases of the susceptible strain of the parasitoid either alone or in combination with insecticide registered lower net profits, returns and yield as compared to those with releases of the tolerant strain. All the treatments were superior to the control. The additional costs and net returns obtained in the insecticidal treatment, coupled with environmental hazards and residues, do not outweigh the benefits of ecofriendly biocontrol agents in our attempts to manage the insect pest in a sustainable manner. Although, the releases of the parasitoid either alone or in combination with pesticides resulted in a relatively lower accrued benefits, the increased parasitism in the absence of the insecticide or its reduced quantum, could eventually contribute to higher net returns and profits over a period of time.

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