Identification and compatibility of suitable natural enemies against *Earias vittella* and *Helicoverpa armigera* in Bhendi ecosystem

Abstract

Aims: The current study was conducted to identify and compatibility of parasitoids against bhendi fruit borers in the bhendi field.

Study design: Randomized Complete Block Design (**RCBD**)

Place and Duration of Study: National Bureau of Agricultural Insect Resources (ICAR NBAIR) Hebbal, Bengaluru and field evaluation at ICAR-NBAIR, Attur Layout, Bengaluru (Latitude: 13.097221 Longitude: 77.568291), during 2019-20 and 2020-21.

Methodology: Two field tests were done at the entomological research farm, NBAIR-ICAR Bengaluru, Karnataka, India, to evaluate the efficiency of the braconid parasitoids, *B. brevicornis* and *C. blackburni*, against *E. vittella* and *H. armigera* on bhendi. Arka Nikita (IIHR) bhendi variety was used for the two trials with different treatments. The release rates of parasitoids were decided based on the parasitic potential studies.

Results: In the first trial, among the treatments, a combination of *T. chilonis* + *C. blackburni* + *B. brevicornis* and *C. blackburni* + *B. brevicornis* recorded the lowest pooled mean larval population reduction over pre-treatment count of *E. vittella* and were on par with each other, recording 40.19 % and 37.27 %, respectively ~~in reduction over pre-treatment count~~ followed by *T. chilonis* + *B. brevicornis* (31.28 %), *C. blackburni* (30.90 %), *B. brevicornis* (28.64 %), Neem oil 0.5% (27.71 %), Neem oil 0.5% + *B. brevicornis* (25.46 %) *and T. chilonis* (25.32 %). A similar trend was also observed in combination of *T. chilonis* + *C. blackburni* + *B. brevicornis* and *C. blackburni* + *B. brevicornis* against *H. armigera*, which accounted~~s~~ for 47.19 % and 37.22 % of reduction over pre-treatment count in the respective treatment combination of parasitoids. The next best treatments ~~are~~ were *T. chilonis* + *B. brevicornis* (28.21 %), *C. blackburni* (26.56 %), *B. brevicornis* (22.21 %), Neem oil 0.5% (20.37 %), *T. chilonis* (17.31 %) and Neem oil 0.5% + *B. brevicornis* (16.07 %)

Conclusion: From this study we recommend that, ~~a~~ for bhendi fruit borers release of parasitoids like *T. chilonis* + *C. blackburni* + *B. brevicornis* at 50,000+1000+1000 adults/ha, respectively in bhendi field starting from 35 DAS at 15 days' intervals of duration, two-time release of parasitoids is enough later, they have augmented and reduce the population of fruit borer larvae effectively.

Key words: *Bhendi, Fruit borer, Pests, Biological control, Natural enemies, parasitoids.*

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Comment [A2]: Is something missing? .... 'efficacy' or 'suitability' ? check & update

Comment [A3]: Braconid? Braconid with trichogrammatid? Or with neem oil? Make it clear & simple

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1. INTRODUCTION

Vegetables are the best sources of vitamins and minerals and it helps to reduce malnutrition problems and provide considerable revenue to farmers in a short period of time. Among the major vegetables, Bhendi or Okra~~,~~ (*Abelmoschus esculentus* (L.) Moench) is one such important crop that belongs to malvaceae~~ous~~ family. It is one of the major contributors to the total global vegetable production and it is believed to have originated from Ethiopia (Joshi and Hardas, 1976).

Among the biological constrains in okra cultivation, pest infestation is a prominent one. ~~There are several~~ As many as 72 insect pests species have been observed on bhendi. ~~*Viz*~~ Among them, Okra shoot and fruit borer~~s~~, *Earias* spp.~~:~~ Okra fruit borer, *Helicoverpa armigera* (Hubner~~),):~~ Aphid~~s~~, *Aphis gossypii* (Glover)~~:~~ Leafhopper~~s~~, *Amrasca biguttula biguttula* (Ishida) and ~~Whiteflies~~Whitefly, *Bemisia tabaci* (Gennadius) which cause considerable crop damage and are regarded serious pests of bhendi (Srinivas Rao and Rajendra, 2002).

Among serious/major insect pests, the bhendi shoot and fruit borers, *Earias* spp. and Okra fruit borer, *Helicoverpa armigera* are the most destructive and primary limiting factor in both the quantity and quality of bhendi fruit harvest. ~~It is a~~These widely spread insect pest that has been found infesting bhendi, cotton, hollyhock, and safflower plants (Khan and Verma, 1946). It is reported that 23-54 percent of losses were observed in marketable yield Rai *et al*~~.~~ (2014).

In order to prevent the losses caused by insects and to produce a quality crop, it is essential to manage the pest population at the appropriate time with ~~these~~ suitable control measures. To mitigate the losses due to major pests of bhendi, a huge quantity of pesticides is used in bhendi and it is ~~un~~usual for the vegetable growers to give 10-12 sprays in a season, thus the fruits harvested at short intervals are likely to retain an unavoidably high level of pesticide residues, which is estimated from okra fruits obtained from non-IPM plots at Pesticide Residue and Food Quality Analysis Laboratory (PRFQAL), University of Agricultural Sciences, Raichur, Karnataka. The residues of imidac~~h~~loprid (6.7 ppm), thiomethoxam (3.8 ppm), flubendiamide (7.9 ppm), chlorantraniliprole (6.5 ppm) were identified in the harvested okra fruits, which are far above the maximum residue limits (MRL), however no pesticides were detected in the okra fruits from IPM plot (Puvvala *et al*., 2020). Which may be highly hazardous to consumers? Further, the excessive reliance on chemicals has led to the health problem in human beings and resistance in insect's pests, resurgence of minor insect's pests, and creation of environmental pollution

Under such circumstances, the use of bio control agents in pest management is considered an ecologically viable option to overcome the above problems. Their greatest strength is their specificity as most are essentially nontoxic and non-pathogenic to animals and humans. This bio control strategy involving potential natural enemies which can be successfully incorporated into a sound Integrated Pest Management (IPM) program.

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Comment [A7]: Do check it refers to all the 3 spp.; if not supplement with relevant references, rephrase the text if necessary.

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Comment [A10]: General statements- retain after revising with proper verbatim only if you can support with facts and figures along with latest references.

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Among the biocontrol agents, braconids are well-known parasitoids for the management of various lepidopteran larvae, notably the okra fruit borer complex. However, evidence on the efficacy of natural enemies on okra pests is scarce in okra ~~feild~~field. Keeping these in view, the current studies were conducted to compare the efficacy of natural enemies with previously advised practises against okra fruit borers ~~in~~ under~~the~~ field conditions.

2. MATERIAL AND METHODS

T~~he methodologies adopted and materials used in t~~his experiment~~s~~ w~~as~~ere carried out at the National Bureau of Agricultural Insect Resources (ICAR- NBAIR) Hebbal, Bengaluru and field evaluation was carriedout at ICAR-NBAIR, Attur Layout, Bengaluru (Latitude: 13.097221 Longitude:77.568291), during 2019-20 and 2020-21.

Culture of parasitoids

The larval parasitoid, *Bracon brevicornis* Wesmael (Braconidae: Hymenoptera)~~,~~ egg larval parasitoid, *Chelonus blackburni* ~~cam~~Cam. (Braconidae: Hymenoptera) and the egg parasitoid, *Trichogramma chilonis* Ishii (Trichogrammatidae: Hymenoptera) maintained in the Biocontrol Laboratory, NBAIR-ICAR Bengaluru, Karnataka, India by following the procedures adopted by Jhansi (1984). These reared parasitoids were utilized for the field experiments.

Field evaluation parasitoids

Two field tests were done at the entomological research farm, NBAIR-ICAR Bengaluru, Karnataka, India, to evaluate the efficiency of the braconid parasitoids, *B. brevicornis* and *C. blackburni*, against *E. vittella* and *H. armigera* on bhendi. Arka Nikita (IIHR) bhendi variety was used for the two trials with different treatments (Table.1). The release rates of parasitoids were decided based on the parasitic potential studies.

Table 1.Treatment details of field evaluation of selected natural enemies against bhendi fruit bores

Sl.No	Treatments	Dosage
1	*B. brevicornis*	2000 adults/ha
2	*Trichogramma chilonis*	1,00,000 adults~~-~~/~~-~~ha
3	*C. blackburni*	2000 adults/ha
4	Neem oil 0.5%	5ml/l~~it~~
5	*T. chilonis* +*B. brevicornis*	50,000 + 1000 adults/ha
6	*C. blackburni* + *B. brevicornis*	1000 + 1000 adults/ha
7	Neem oil 0.5% + *B. brevicornis*	2.5 ml/ l~~it~~+ 1000 adults/ha
8	*T. chilonis* + *C. blackburni* + *B. brevicornis*	50,000+1000+1000 adults/ha
9	Untreated check.	-

Comment [A12]: Support with references and extent of protection/management offered if not in Okra, try with any other vegetable or malvaceous crop (cotton)

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Comment [A18]: What about T. chilonis?Is the study aim was to check the compatability with this? Poorly presented.

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The treatments were replicated thrice with a plot size of 4 x 5 m. Treatments were imposed at 15-day intervals three times. The sachet made up of tissue paper containing ready to emerge cocoons of *B. brevicornis* and adults of *C. blackburni* were tied individually in the middle of the treatment plots. The mouth of the sachet was tied after inserting a small piece of straw to facilitate the exit of adults. ~~For~~ *Trichogramma chilonis* ~~tricho~~ egg card was stapled in treatment block plants. Neem oil 0.5% is also used in this experimental study to check their efficiency along with natural enemies against target pests. In between each treatment and each replication, a buffer plot sprayed with chemicals at weekly intervals was maintained to restrict the movement of the parasitoids from the released plots. The treatments were imposed starting from pests infestation i.e. 35 DAS (Days after sowing).

Observations on the population of *E. vittella* and *H. armigera* were recorded in 15 randomly selected plants from each plot before and after 4, 8, and 12 days of treatment, and the pooled mean was worked out after three rounds of release /spray.

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Statistical analysis:

The data was statistically ~~evaluated~~ analyzed for randomized full block design (RCBD), Duncan's multiple range test (DMRT), and one factor ANOVA, and the results were interpreted.

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3. RESULTS

~~**Field evaluation of parasitoids**~~

Observations on the larval population showed that the larvae of *E. vittella* preferred to feed on freshly formed and tender fruits, while, *H. armigera* did not show any preference for the stage of fruits. However, the larvae of both species were rarely observed together in a single fruit. Results of both the trials showed that a combination of two braconid parasitoids *viz*., *C. blackburni* + *B. brevicornis* were more effective and were on par with ~~a~~ the combination of *T. chilonis* + *C. blackburni* + *B. brevicornis* which ~~is~~ was a most superior combination ~~-~~of parasitoids in reducing the *E. vittella* and *H. armigera* larval population (Table 2).

In the first trial, among the treatments, ~~a~~ combinations of *T. chilonis* + *C. blackburni* + *B. brevicornis* and *C. blackburni* + *B. brevicornis* recorded the lowest pooled mean larval population reduction over pre-treatment count of *E. vittella* and were on par with each other, recording 40.19 % and 37.27 %, respectively ~~in reduction over pre-treatment count~~ followed by *T. chilonis* + *B.*

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brevicornis (31.28 %), *C. blackburni* (30.90 %), *B. brevicornis* (28.64 %), Neem oil 0.5% (27.71 %), Neem oil 0.5% + *B. brevicornis* (25.46 %) *and T. chilonis* (25.32 %). A similar trend was also observed in combination of *T. chilonis* + *C. blackburni* + *B. brevicornis* and *C. blackburni* + *B. brevicornis* against *H. armigera* ~~accounts for~~with 47.19 % and 37.22 % of reduction over pre-treatment count in the respective treatment combination of parasitoids. The next best treatments ~~are~~ were *T. chilonis* + *B. brevicornis* (28.21 %), *C. blackburni* (26.56 %), *B. brevicornis* (22.21 %), Neem oil 0.5% (20.37 %), *T. chilonis* (17.31 %) and Neem oil 0.5% + *B. brevicornis* (16.07 %) (Table 2).

In the second trial, observations of the population of *E. vittella* and *H. armigera* revealed similar trends as that of the first trial (Table.2). A significantly lower population of *E. vittella* and *H. armigera* was recorded in the treatments involving the combination of *T. chilonis* + *C. blackburni* + *B. brevicornis* and *C. blackburni* + *B. brevicornis*.

In previous study by Sangwan (1972) reported ~~the~~ higher efficacy of *Bracon kirkpatricki* at 3000 adults/~~-~~ha and *T. brasiliensis at* 1,50,000 adults/~~-~~ha under field conditions against *H. armigera* and *Earias* spp. on cotton. Efficacy of *C. blackburni*, *B. kirkpatricki* and *Trichogramma* spp. against *Earias* spp. and *H. armigera* on cotton was also reported by earlier workers (Pawar and Prasad, 1988) in agreement with the present findings. Agarwal and Gupta (1986) and Forehand *et al.* (2006) reported that mass releases of egg parasitoids, *T. chilonis* and *T. acheae*~~;~~ egg larval parasitoid, *C. blackburni* and larval parasitoid, *B. kirkpatriki* during square formation stage, reduced the incidence of all three species of bollworms in cotton.

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Table 2. Efficacy of braconid parasitoids against the larval population of bhendi fruit borers

Tr. No.	Treatment*	No. of larvae/10 plants											
		E. vittella						*H. armigera*					
		Trail 1		% ROPTC**	Trail 2		% ROPTC**	Trail 1		% ROPTC**	Trail 2		% ROPTC**
		PTC	T*		PTC	T*		PTC	T*		PTC	T*	
T_1	*B. brevicornis*	11..25 (3.50)^{a}	8.03 (3.00)^{bcd}	28.64	8.15 (3.03)^{a}	5.33 (2.52)^{cd}	34.56	12.02 (3.61)^{a}	9.35 (3.22)^{abc}	22.21	11.52 (3.56)^{a}	6.82 (2.80)^{cd}	40.81
T_2	*T. chilonis*	11.15 (3.48)^{a}	8.33 (3.05)^{b}	25.32	8.20 (3.06)^{a}	6.33 (2.71)^{b}	22.76	12.44 (3.66)^{a}	10.28 (3.36)^{ab}	17.31	11.48 (3.53)^{a}	8.19 (3.03)^{b}	28.66
T_3	*C. blackburni*	11.35 (3.51)^{a}	7.84 (2.97)^{bcd}	30.90	8.15 (3.03)^{a}	5.16 (2.48)^{de}	36.71	12.41 (3.65)^{a}	9.12 (3.18)^{bc}	26.56	11.44 (3.51)^{a}	6.68 (2.77)^{de}	41.64
T_4	Neem oil 0.5%	11.25 (3.50)^{a}	8.13 (3.02)^{bc}	27.71	8.17 (3.05)^{a}	5.67 (2.58)^{c}	30.60	12.14 (3.62)^{a}	9.67 (3.27)^{ab}	20.37	11.33 (3.46)^{a}	7.33 (2.89)^{c}	35.30
T_5	*T. chilonis* + *B. brevicornis*	11.01 (3.47)^{a}	7.56 (2.92)^{cd}	31.28	8.12 (3.01)^{a}	4.82 (2.41)^{ef}	40.64	12.40 (3.64)^{a}	8.90 (3.15)^{bc}	28.21	11.12 (3.30)^{a}	6.41 (2.72)^{def}	42.34
T_6	*C. blackburni* + *B. brevicornis*	11.65 (3.56)^{a}	7.31 (2.88)^{de}	37.27	8.13 (3.02)^{a}	4.43 (2.33)^{fg}	45.55	12.63 (3.69)^{a}	7.93 (2.99)^{bc}	37.22	11.73 (3.82)^{a}	6.15 (2.67)^{ef}	47.59
T_7	Neem oil 0.5% + *B. brevicornis*	10.57 (3.40)^{a}	7.88 (2.98)^{bcd}	25.46	8.10 (3.00)^{a}	4.59 (2.37)^{fg}	43.27	12.57 (3.68)^{a}	10.55 (3.40)^{ab}	16.07	11.27 (3.38)^{a}	6.32 (2.71)^{def}	43.97

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Comment [A29]: If you are confident about E. vitella, do mention the same in methods & objective too in place of E. spp.

Comment [A30]: Give the pooled analysis for both the trails for both the pests

T_8	***T. chilonis + C. blackburni + B. brevicornis***	11.13 (3.48)[a]	6.66 (2.76)[e]	40.19	8.17 (3.05)[a]	4.27 (2.30)[g]	47.76	12.51 (3.67)[a]	6.61 (2.76)[c]	47.19	11.64 (3.75)[a]	5.94 (2.64)[f]	48.96
T_9	**Untreated check**	10.55 (3.40)[a]	10.55 (3.40)[a]	-	8.26 (3.09)[a]	8.58 (3.10)[a]	-	12.50 (3.68)[a]	12.54 (3.68)[a]	-	11.62 (3.70)[a]	12.96 (3.74)[a]	-
C.D @ 0.05 %		NS	0.05		N/A	0.04		N/A	1.13		N/A	0.07	
SE(m)±		0.24	0.02		0.07	0.01		0.23	0.37		0.16	0.02	
C.V.		3.79	7.33		1.52	7.45		3.19	6.84		2.41	7.50	

[*] **Values in the parenthesis are square root transformed**

T*(Treatment): No. of larvae/10 plants (pooled mean of three rounds of releases)

PTC: Pre-treatment count

ROPTC **: Reduction over pre-treatment count due to parasitization

4. DISCUSSION

A field test of braconid parasitoids against the larval population of fruit borers such as *E. vittella* and *H. armigera* revealed that a combination of two braconid parasitoids was effective. ~~*viz., C*~~*hilonis. blackburni + B. brevicornis* were more effective and were on par with the~~a~~ combination of *T. chilonis + C. blackburni + B. brevicornis* which ~~is~~ was a most superior combination of parasitoids in parasitizing the fruit borers larval population.

Due to overlapping generations, pest stages will appear in a staggered manner in the field condition. As a result, releasing a single parasitoid may not produce the desired results ~~because~~ as they exclusively target a specific stage of the pest. Indeed, releasing the combination of two or three parasitoid species, they will attack the pests at different stages and it will be more effective. In the present finding, combination of *T. chilonis + C. blackburni + B. brevicornis* recorded significantly lower

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pest larval population and higher percent parasitization in fruit borer larvae than the combination of *C. blackburni* + *B. brevicornis*. The combined release of *C. blackburni* and *B. brevicornis* at fortnightly intervals, starting from flower initiation to pod development stage may give long term benefits in the bio suppression of the fruit borers ~~in~~ of okra in an ecofriendly manner.

Bhendi is an important medicinal value vegetable; now a day's demand for this crop is more. From this study we recommend that, ~~a~~ for bhendi fruit borers release of parasitoids ~~like~~ *viz., T. chilonis* + *C. blackburni* + *B. brevicornis* at 50,000+1000+1000 adults/ha, respectively twice in bhendi field starting from 35 DAS at 15 days' interval~~s of duration, two time release of parasitoids is enough later, they~~ which will be ~~have~~ augmented on their own and reduce the population of fruit borers ~~larvae~~ effectively.

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REFFERENCE

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Comment [A35]: What it is? MSc/PhD Dissertation?

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