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Efficacy of Entomopathogenic Fungi with Insecticides Mixtures against *Oxycarenus hyalinipennis* (Costa) (Lygaeidae: Hemiptera)

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KA performed experiment and wrote the manuscript. SF provided technical assistance, supervision and helped in writing manuscript. RFS and KWA performed statistical analysis, assisted in experiments and manuscript writing.

Key words
***Oxycarenus hyalinipennis*, Entomopathogenic fungi, Microbial control, Insecticides, Dusky cotton bug.**

ABSTRACT

Dusky cotton bug, *Oxycarenus hyalinipennis* has appeared as a serious insect pest of various crops including cotton. The present study was carried out to evaluate the combined effect of some isolates of *Beauveria bassiana, Metarhizium anisopliae* and *Isaria fumosorosea* and six synthetic insecticides *i.e.,* triazophos, imidacloprid, bifenthrin, nitenpyram, pyreproxifin and lambda cyhalothrin against *O. hyalinipennis*. The efficacy of these microbial agents could be enhanced by applying in combination with insecticides. Five concentrations *i.e.,* 1×10^6, 1×10^7, 1×10^8, 2×10^8 and 3×10^8 conidia/ml of each isolate of entomopathogenic fungi were used in combination with insecticides against 5th instar nymphs of *O. hyalinipennis*. The combined treatment of isolates of entomopathogenic fungi and synthetic insecticides caused higher mortality of *O. hyalinipennis*. Moreover, the combined treatments decreased the adult emergence, male and female longevity, while increased the nymphal duration. The results indicated that these entomopathogenic fungi can provide better control in combination with insecticides and can be used in integrated pest management for reducing the population density of *O. hyalinipennis*.

INTRODUCTION

Cotton, *Gossypium hirsutum* L. is a critical fiber crop and exportable produce of Pakistan which contributes 7.0 % to the national economy and 1.7% of the Gross Domestic Product (GDP) of Pakistan (Farooq, 2014). There are numerous factors which contribute for the low cotton production, while insect pests including chewing and sucking are the major factor for lowering the yield of cotton (Shah, 2014; Akram *et al*., 2013; Sammaiah *et al*., 2012; Patil and Rajankanth 2005). Yunus and Yousuf (1979) stated that nearly 93 different insects and mites attack on cotton crop that damage the cotton crop in different ways either by sucking the cell sap or eating different parts of plant, causing 20-40% yield loss (Aslam *et al*., 2004).

The use of insecticides is the common strategy adapted by the farmers for control of *Oxycarenus hyalinipennis*. About 90% farmers use insecticides against insect pests to protect their crops (Prayogo *et al*., 2005). However, the unnecessary use of insecticides create problem including resistance (Wang *et al*., 2011). Owing to these reasons, alternate methods of insect pest's management including biological control using insect pathogenic organisms have several advantages including low costs, safe for beneficial insects, more efficient and eco-friendly than the conventional insecticides (Carruthers and Hural, 1990; Lacey *et al*., 2001; Freed *et al*., 2012). Fungal biocontrol agents have different mode of action than other micro-organisms by invading their hosts directly through cuticle rather than ingestion. This is the reason that insect pathogenic fungi can infect eggs (Ujian and Shahzad, 2007; Anand and Tiwary, 2009) and pupae of the insects (Nguyen *et al*., 2007; Anand *et al*., 2008).

Another strategy to increase the efficacy of entomopathogenic fungi is the combined application with insecticides. The studies report that the insecticides application increased the efficacy of fungus making these bicontrol agents more effective for pest management (Quintela and McCoy, 1998; Dayakar *et al*., 2000; Hiromori and Nishigaki, 2001; Serebrov *et al*., 2005; Purwar and Sachan, 2006; Rachapa *et al*., 2007). Combined use of insecticides and entomogenous fungi can increase the efficacy by reducing the quantity of insecticides, minimizing the environmental hazards and resistance in pests (Moino and Alves, 1998; Quintela and McCoy, 1998). Keeping in view the importance of emerging economic insect pests in cotton, the study was planned to evaluate the efficacy of mixtures of entomopathogenic fungi with insecticides against *O. hyalinipennis*.

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MATERIALS AND METHODS

Oxycarenus hyalinipennis

The nymphs and adults of dusky cotton bug *O. hyalinipennis* were collected from the cotton fields. These insects were reared in plastic jars on cotton leaves in the laboratory. The jars openings were covered with muslin cloth. Cotton seeds were also placed in the jars as egg laying medium. Rearing of insects was carried out at temperature 28±1°C, relative humidity 60-70% and 14:10 light: dark photoperiod.

Fungal culture and concentrations

Isolates of *Metarhizium anisopliae* (Ma-11.1, Ma-2.4, isolated from soil), *Isaria fumosorosea* (If-03, If-02, isolated from soil and Rove beetle) and *Beauveria bassiana* (Bb-01, Bb-10, isolated from soil) were used from the laboratory maintained culture. The required concentrations i.e., 1×10^6- 3×10^8 conidia/ml of each isolate were made by serial dilution method.

Preparation of insecticide concentrations

Insecticides, bifenthrin, lambda cyhalothrin, nitenpyram, imidacloprid, triazophos, and pyreproxifin were used to check the efficacy against *O. hyalinipennis*. Required concentrations of each insecticide *i.e.,* 0.005, 0.0025, 0.00125, 0.000625 and 0.00031ppm were prepared by serial dilution method.

Table I.- Calculated doses of fungi (conidia/ml) and insecticides (ppm) for binary treatments.

	LC_{40}	LC_{30}	LC_{20}	LC_{10}
Fungal isolates				
Bb-01	2.01×10^7	1.56×10^7	1.22×10^7	1.01×10^7
Bb-10	3.75×10^7	3.18×10^7	2.98×10^7	2.65×10^7
Ma-2.4	4.01×10^8	3.65×10^8	3.22×10^8	2.67×10^8
Ma-1.1	2.01×10^7	1.73×10^7	1.32×10^7	1.01×10^7
If-02	4.67×10^7	4.19×10^7	3.87×10^7	3.12×10^7
If-03	3.43×10^8	3.21×10^8	2.76×10^8	2.18×10^8
Insecticides				
Triazophos	0.00073	0.00067	0.00061	0.0005
Imidacloprid	0.00058	0.0005	0.00044	0.00037
Pyreproxifin	0.00067	0.00064	0.00058	0.00053
Nitenpyram	0.00062	0.00056	0.00051	0.00045
Lambda cyhalothrin	0.00057	0.0005	0.00043	0.00036
Bifenthrin	0.00065	0.00059	0.00056	0.00049

Preparation of binary mixtures of insecticides and fungi

The desired concentrations (LC_{40}, LC_{30}, LC_{20} and LC_{10}) of fungi and insecticides were calculated by conducting preliminary experiments (Table I). Binary mixture of insecticides and entomopathogenic fungi was made by mixing the LC_{10} fungi + LC_{10} insecticides, LC_{20} fungi + LC_{20} insecticides, LC_{30} fungi + LC_{30} insecticides and LC_{40} fungi + LC_{40} insecticides.

Bioassay

The experiment was conducted under complete randomized design with four replications having ten insects per replication. Immersion method was used in which 5th instar nymphs of *O. hyalinipennis* were individually immersed in the combined concentrations of entomopathogenic fungi and insecticides for 8-10 seconds. To soak up the excessive moisture the insects were placed on tissue paper. While, in the control treatment, insects were treated with Tween 80 (0.05%) solution only. These treated insects were then transferred into petri dishes provided with fresh cotton leaves. The mortality data was recorded for consecutive 7 days. After seven days the remaining alive insects were reared again and were kept under examination to assess the effect of binary mixture on the survival and biological parameters of *O. hyalinipennis.*

Data analysis

All the values (LC_{10}, LC_{20}, LC_{30}, and LC_{40}) of insecticides and entomopathogenic fungi were calculated by Probit analysis by using POLO-PC software (LeOra Software, 2003). The means were compared by LSD test at 0.05 probability levels and analyzed by analytical software (Statistix version 8.1).

RESULTS

Percent mortality of O. hyalinipennis

Mortality of 5th instar nymphs of *O. hyalinipennis* after the application of mixtures of entomopathogenic fungi and insecticides are presented in Table II. An increasing trend of mortality was found towards higher concentration of insecticides and fungi combinations in a dose dependent manner. In case of mixtures of insect pathogenic fungi and insecticides, the highest percent mortality (±SE) (90.0± 4.0) was recorded in case of combined use of higher doses of isolate Bb-01 (LC_{40}) and bifenthrin (LC_{40},) and Bb-10 (LC_{40}) with bifenthrin and triazophos followed by the LC_{40} of Bb-01 and LC_{40} of nitenpyram and LC_{40} of isolate Ma-11.1 (LC_{40}) and LC_{40} of triazophos.

The results regarding entomopathogenic fungi (LC_{30}) and insecticides (LC_{30}) mixture showed highest percent mortality (±SE) (82.5 ± 4.7) in Bb-01(LC_{30}) and bifenthrin (LC_{30}) while in case of entomopathogenic fungi (LC_{20}) and insecticides (LC_{20}) mixture, highest percent mortality (75.0 ±6.4) was caused by Bb-01 (LC_{20}) and bifenthrin (LC_{20}). Moreover, in case of LC_{10} highest percent mortality (75.5 ± 4.7) was observed for If-03 (LC_{10}) and bifenthrin (LC_{10}).

Table II.- Percent nymphal mortality of *O. hyalinipennis* after treatment of binary mixture of entomopathogenic fungi and insecticides.

	Triazophos	Imidacloprid	Pyreproxifen	Nitenpyram	Lambda cyhalothrin	Bifenthrin
LC_{10}+ LC_{10}						
Bb-01	50.0±7.1a	52.5±4.8a	45.0±9.6a	52.5±9.5ab	62.5±8.5a	65.0±8.7a
Bb-10	60.0±0.0a	52.5±2.5a	52.5±2.5a	47.5±4.8b	52.5±4.8a	60.0±0.0ab
Ma-11.1	50.0±7.1ab	50.0±0.0a	45.0±6.5a	50.0±7.1ab	50.0±4.1a	57.5±4.8ab
Ma-2.4	60.0±5.8a	50.0±0.0a	50.0±0.0a	57.5±4.8a	45.0±5.0a	50.0±0.0ab
If-03	57.5±2.5a	45.0±2.9a	40.0±4.1ab	62.5±7.5a	50.0±9.1a	75.5±4.8b
If-02	62.5±2.5a	52.5±6.3a	52.5±2.5a	50.0±4.1ab	55.0±6.5a	50.0±4.1b
Control	2.5±2.5b	2.5±2.5b	2.5±2.5b	2.5±2.5c	2.5±2.5b	2.5±2.5c
LSD	8.2	7.9	8.4	7.1	9.1	8.7
F-value	23.4	15.2	19.8	16.2	10.1	21.8
P-value	0.0	0.0	0.0	0.0	0.0	0.0
LC_{20}+ LC_{20}						
Bb-01	57.5±4.8ba	65.0±6.5a	55.0±2.9a	65.0±2.9a	67.5±2.5a	75.0±6.5a
Bb-10	67.5±4.8a	60.0±0.0a	50.0±4.1ab	60.0±0.0a	62.5±2.5a	67.5±4.8ab
Ma-11.1	60.0±5.8a	60.0±4.1a	52.5±6.3ab	60.0±7.1a	57.5±2.5a	65.0±2.9ab
Ma-2.4	70.0±4.1a	55.0±2.9ab	62.5±6.3a	67.5±4.8a	57.5±6.3a	57.5±7.5ab
If-03	67.5±8.5a	52.5±4.8ab	50.0±4.1ab	70.0±7.1a	57.5±2.5a	70.0±8.2b
If-02	70.0±5.8a	62.5±4.8a	57.5±4.8a	60.0±4.1a	67.5±4.8a	55.0±2.9b
Control	2.5±2.5b	2.5±2.5b	2.5±2.5b	2.5±2.5b	2.5±2.5b	2.5±2.5c
LSD	10.2	8.4	6.9	8.5	9.1	5.8
F-value	22.8	15.8	18.2	22.4	38.8	20.2
P-value	0.0	0.0	0.0	0.0	0.0	0.0
LC_{30}+ LC_{30}						
Bb-01	67.5±7.5a	72.5±8.5a	62.5±10.3a	75.0±5.0a	77.5±7.5a	82.5±4.8a
Bb-10	77.5±4.8a	70.0±7.1a	60.0±0.0a	70.0±4.1a	70.0±5.8a	77.5±4.8ab
Ma-11.1	72.5±2.5a	67.5±4.8a	65.0±5.0a	70.0±4.1a	67.5±2.5a	75.0±2.9ab
Ma-2.4	75.0±2.9a	62.5±4.8a	70.0±4.1a	77.5±6.3a	70.0±4.1a	67.5±8.5ab
If-03	75.0±2.9a	60.0±4.1a	60.0±4.1a	77.5±2.5a	65.0±5.0a	77.5±2.5b
If-02	77.5±2.5a	67.5±4.8a	65.0±6.5a	70.0±5.8a	75.0±2.9a	65.0±6.5b
Control	2.5±2.5b	2.5±2.5b	2.5±2.5b	2.5±2.5b	2.5±2.5b	2.5±2.5c
LSD	8.2	9.4	7.8	10.3	7.9	8.8
F-value	18.2	25.5	23.2	28.8	31.5	29.8
P-value	0.0	0.0	0.0	0.0	0.0	0.0
LC_{40}+ LC_{40}						
Bb-01	77.5±6.3a	80.0±7.1a	75.0±6.5a	87.5±6.3a	82.5±4.8a	90.0±4.1a
Bb-10	90.0±4.1a	80.0±8.2a	72.5±4.8a	77.5±6.3a	80.0±4.1a	90.0±4.1a
Ma-11.1	87.5±4.8a	77.5±4.8a	75.0±2.9a	80.0±4.1a	77.5±4.8a	82.5±7.5a
Ma-2.4	85.0±2.9a	70.0±4.1a	77.5±2.5a	85.0±2.9a	80.0±4.1a	80.0±9.1a
If-03	82.5±7.5a	70.0±4.1a	72.5±2.5a	85.0±6.5a	72.5±2.5a	82.5±2.5a
If-02	82.5±4.8a	75.0±5.0a	77.5±2.5a	82.5±4.8a	80.0±0.0a	75.0±5.0a
Control	2.5±2.5b	2.5±2.5b	2.5±2.5b	2.5±2.5b	2.5±2.5b	2.5±2.5b
LSD	9.3	8.7	11.9	9.5	11.7	10.8
F-value	43.4	35.8	48.3	35.8	64.2	32.0
P-value	0.0	0.0	0.0	0.0	0.0	0.0

Table III.- Nymphal duration of *O. hyalinipennis* after treatment of binary mixture of entomopathogenic fungi and insecticides.

	Triazophos	Imidacloprid	Pyreproxifen	Nitenpyram	Lambda cyhalothrin	Bifenthrin
LC_{10}+ LC_{10}						
Bb-01	8.2±0.0a	9.1±0.5a	7.2±0.5a	7.8±0.5a	8.2±0.1a	7.8±0.9ab
Bb-10	8.8±0.9a	7.1±0.5a	8.3±0.5a	8.1±0.1a	9.7±0.4a	8.6±0.9a
Ma-11.1	7.1±0.7ab	9.9±0.4a	7.9±0.7a	8.8±0.4a	7.8±0.6a	8.5±0.7a
Ma-2.4	7.6±0.2a	8.8±0.6b	6.8±0.8ab	9.9±0.5a	9.9±0.5a	9.7±0.5a
If-03	9.3±0.4a	8.5±0.3a	8.9±0.9a	9.2±0.2a	7.1±0.6a	9.3±0.2a
If-02	8.0±0.7a	8.0±0.7a	7.6±0.5a	7.7±1.0a	8.9±0.2a	8.9±0.1a
Control	6.4±0.7ab	6.1±0.4ab	6.4±0.3ab	6.0±0.7ab	6.1±0.3ab	6.6±0.6b
LSD	3.8	2.2	3.8	1.8	4.1	1.4
F-value	3.6	4.8	4.0	3.9	4.8	3.2
P-value	0.0	0.0	0.0	0.0	0.0	0.0
LC_{20}+ LC_{20}						
Bb-01	11.3±0.3a	11.0±0.4a	9.9±0.5a	8.1±1.0b	9.3±0.9a	8.9±1.0ab
Bb-10	8.9±0.7a	8.9±0.1ab	9.7±0.3a	9.2±0.3ab	11.5±0.7a	9.7±0.8ba
Ma-11.1	9.0±0.4a	11.3±0.7a	10.1±0.5a	11.9±0.7a	9.4±0.9a	10.4±0.5a
Ma-2.4	10.6±0.3a	9.5±0.5a	7.3±0.3ab	10.6±0.9a	10.0±0.5a	9.3±0.3a
If-03	9.3±0.8a	9.1±0.5ab	8.6±0.9a	10.0±0.1ab	9.9±0.4a	11.0±0.7a
If-02	10.0±0.5a	10.6±0.9a	8.0±0.8ab	8.6±0.4b	8.3±0.5ab	10.0±0.6a
Control	6.3±0.2b	5.3±0.8b	6.2±0.5b	6.1±0.9c	6.5±0.8b	7.0±0.4b
LSD	2.2	0.9	2.8	1.5	2.1	2.0
F-value	4.9	3.2	4.2	2.9	3.8	4.2
P-value	0.0	0.0	0.0	0.0	0.0	0.0
LC_{30}+ LC_{30}						
Bb-01	12.3±0.5a	11.0±0.4a	10.0±0.4a	10.9±.0.7ab	11.7±0.5a	11.9±1.0a
Bb-10	10.1±0.9ab	11.4±0.7a	9.0±0.9ab	13.8±0.9a	12.3±0.4a	9.8±0.5ab
Ma-11.1	12.0±0.6a	12.7±0.8a	10.5±0.8a	12.8±0.7a	9.9±0.9ab	11.1±0.7a
Ma-2.4	9.7±0.5ab	9.9±0.4ab	11.3±0.4a	13.1±0.8a	10.2±0.6a	11.0±0.9a
If-03	10.9±1.0a	10.5±0.8a	9.0±0.2ab	12.4±0.4a	12.9±0.4a	10.6±0.4a
If-02	11.9±0.6a	12.1±0.5a	12.6±0.3a	11.2±0.5a	12.1±0.4a	12.7±0.8a
Control	5.9±0.5b	6.0±0.5b	6.5±0.3b	6.0±0.9b	5.5±0.4b	5.9±0.7b
LSD	0.9	2.5	1.7	3.1	2.8	3.6
F-value	3.2	3.0	3.8	4.2	3.9	4.0
P-value	0.0	0.0	0.0	0.0	0.0	0.0
LC_{40}+ LC_{40}						
Bb-01	14.2±0.3a	13.3±1.7a	11.0±1.8a	11.8±0.5ab	12.8±0.7a	12.2±0.4ab
Bb-10	13.2±1.0a	12.7±0.3a	11.8±0.5a	12.2±0.3a	13.9±0.2a	12.0±0.5ab
Ma-11.1	11.9±1.9a	12.0±0.2a	12.1±0.2a	13.9±0.7a	12.1±0.9ab	12.6±0.9a
Ma-2.4	13.7±1.4a	12.9±0.3a	13.7±0.6a	14.2±0.4a	15.0±0.4a	15.1±0.3a
If-03	13.0±0.6a	11.2±0.4ab	12.8±0.3a	12.8±0.5a	12.9±0.8a	14.8±0.6a
If-02	10.5±0.3ab	12.3±0.6a	12.8±0.5a	13.2±0.6a	13.1±0.4a	13.8±1.0a
Control	6.6±0.3b	6.9±0.5b	7.1±0.3b	6.6±0.6b	6.0±0.9b	6.3±0.5b
LSD	1.4	3.5	3.4	1.9	4.2	1.0
F-value	4.2	2.9	4.0	3.5	4.9	3.6
P-value	0.0	0.0	0.0	0.0	0.0	0.0

Table IV.- Percent adult emergence of *O. hyalinipennis* after treatment of binary mixture of entomopathogenic fungi and insecticides.

	Triazophos	Imidacloprid	Pyreproxifen	Nitenpyram	Lambda cyhalothrin	Bifenthrin
LC_{10}+ LC_{10}						
Bb-01	50.0±2.5ab	47.5±1.8b	55.0±5.3ab	47.6±3.7b	37.5±6.3b	35.0±3.1bc
Bb-10	50.0±3.3ab	47.5±4.9b	60.0±5.9ab	52.5±5.8b	47.5±5.5b	40.0±3.5b
Ma-11.1	50.0±5.3ab	50.0±3.7b	55.0±4.9ab	50.0±1.9b	50.0±8.2b	42.5±4.8b
Ma-2.4	40.0±2.3b	50.0±7.1b	50.0±3.5b	42.5±2.8b	55.0±4.9ab	50.0±2.5b
If-03	42.5±5.6b	55.0±4.6ab	60.0±7.5ab	37.5±4.8b	50.0±3.9b	42.5±3.9b
If-02	37.5±3.5b	47.5±5.6b	47.5±4.8b	50.0±4.5ab	45.0±2.9c	50.0±4.6b
Control	97.5±2.5a	97.5±2.5a	97.5±2.5a	97.5±2.5a	97.5±2.5a	97.5±2.5a
LSD	20.3	27.8	25.0	32.1	29.5	26.4
F-value	52.5	43.8	45.2	40.5	42.8	35.9
P-value	0.0	0.0	0.0	0.0	0.0	0.0
LC_{20}+ LC_{20}						
Bb-01	42.5±3.3ab	35.0±5.3b	45.0±2.3ab	35.0±5.2b	32.5±7.1b	25.0±2.8b
Bb-10	37.5±5.8ab	40.0±3.6b	50.0±4.9a	40.0±1.9b	37.5±7.5b	32.5±5.4b
Ma-11.1	40.0±4.9ab	40.0±2.9b	47.5±1.9ab	40.0±2.5b	42.5±4.9b	35.0±3.8b
Ma-2.4	30.0±2.7b	45.0±4.8ab	37.5±2.8ab	32.5±4.5b	42.5±5.3b	42.5±2.5b
If-03	32.5±4.5b	47.5±3.9ab	50.0±3.2a	30.0±2.5bc	42.5±3.5b	30.0±3.9b
If-02	30.0±5.4b	37.5±5.2b	42.5±4.8ab	40.0±3.6b	32.5±2.9b	45.0±4.2ab
Control	97.5±2.5a	97.5±2.5a	97.5±2.5a	97.5±2.5a	97.5±2.5a	97.5±2.5a
LSD	33.5	32.8	29.6	35.2	30.4	28.5
F-value	65.0	55.5	60.8	40.6	45.8	50.0
P-value	0.0	0.0	0.0	0.0	0.0	0.0
LC_{30}+ LC_{30}						
Bb-01	32.5±5.0ab	27.5±2.6b	37.5±7.1ab	25.0±5.7b	22.5±4.8b	17.5±5.7b
Bb-10	27.5±3.8b	30.0±7.5b	40.0±6.8ab	30.0±5.4b	30.0±4.3b	22.5±4.3b
Ma-11.1	27.5±5.5b	32.5±4.7b	35.0±5.6ab	30.0±4.8b	32.5±6.7ab	25.0±3.7b
Ma-2.4	25.0±2.4b	37.5±6.5b	30.0±2.9b	23.5±5.3b	30.0±4.9b	32.5±3.3b
If-03	25.0±3.7b	40.0±7.6ab	40.0±3.4ab	22.5±3.9b	35.0±2.6ab	22.5±2.3b
If-02	22.5±4.6b	32.5±8.5b	35.0±4.5ab	30.0±4.7b	25.0±3.3b	35.0±5.5b
Control	97.5±2.5a	97.5±2.5a	97.5±2.5a	97.5±2.5a	97.5±2.5a	97.5±2.5a
LSD	18.7	22.3	19.5	15.8	20.5	23.4
F-value	64.1	58.0	65.6	60.0	66.2	60.7
P-value	0.0	0.0	0.0	0.0	0.0	0.0
LC_{40}+ LC_{40}						
Bb-01	22.5±4.6b	20.0±3.3bc	25.0±3.3b	12.5±2.9c	17.5±5.2b	10.0±2.6bc
Bb-10	17.5±2.7bc	20.0±2.5bc	27.5±6.6b	22.5±3.5bc	20.0±4.8b	10.0±1.8bc
Ma-11.1	12.5±2.0bc	22.5±4.5b	25.0±5.7b	20.0±4.9bc	22.5±2.6b	17.5±4.6b
Ma-2.4	15.0±1.6bc	30.0±5.9b	22.5±3,4b	15.0±4.5c	20.0±5.6b	20.0±3.8b
If-03	17.5±3.6bc	30.0±4.9b	27.5±5.5b	15.0±3.5c	27.5±3.6b	17.5±5.6b
If-02	17.5±4.9bc	25.0±5.3b	22.5±4.3b	17.5±3.3bc	20.0±3.8b	25.0±4.9b
Control	97.5±2.5a	97.5±2.5a	97.5±2.5a	97.5±2.5a	97.5±2.5a	97.5±2.5a
LSD	34.2	28.8	19.7	30.2	27.4	22.8
F-value	47.7	55.4	50.6	40.5	49.7	56.4
P-value	0.0	0.0	0.0	0.0	0.0	0.0

Table V.- Male longevity of *O. hyalinipennis* after treatment of binary mixture of entomopathogenic fungi and insecticides.

	Triazophos	Imidacloprid	Pyreproxifen	Nitenpyram	Lambda cyhalothrin	Bifenthrin
LC_{10}+ LC_{10}						
Bb-01	9.8±0.9ab	9.9±0.3ab	9.1±0.5ab	9.0±0.3a	8.4±1.0b	8.8±0.4ab
Bb-10	9.0±0.3b	9.1±0.2ab	9.8±0.8ab	9.2±0.5a	9.1±0.4ab	7.8±0.4b
Ma-11.1	8.8±0.2b	8.1±0.4b	8.4±0.8b	8.6±0.9ab	8.6±0.4ab	8.1±0.8b
Ma-2.4	8.2±0.8b	8.8±0.8ab	9.5±0.7ab	9.0±0.5a	8.0±0.3b	7.1±0.4b
If-03	10.0±0.8a	9.9±0.6ab	8.9±0.8b	8.2±0.8ab	9.5±0.5ab	9.7±0.5a
If-02	9.2±0.4ab	9.4±0.4ab	10.2±0.6b	9.7±0.7b	10.0±0.7ab	10.0±0.8a
Control	11.2±0.3a	12.5±0.7a	11.6±0.5a	11.0±0.9a	12.7±0.6a	12.5±0.5a
LSD	0.9	1.9	1.5	2.7	1.4	2.9
F-value	2.1	3.8	2.6	3.2	4.2	3.8
P-value	0.0	0.0	0.0	0.0	0.0	0.0
LC_{20}+ LC_{20}						
Bb-01	8.1±0.5a	9.0±0.9ab	7.2±0.4b	7.1±0.7b	8.2±0.8ab	8.0±0.ab
Bb-10	7.2±0.4ab	8.6±1.0b	8.2±0.6b	7.0±0.8b	7.0±0.3b	6.4±0.5b
Ma-11.1	7.8±0.7ab	6.3±0.4c	7.3±0.5b	7.6±0.3b	7.2±0.7b	7.1±0.7b
Ma-2.4	6.1±0.3b	6.0±0.7c	6.0±1.0bc	6.2±0.5b	6.0±0.5b	6.6±0.6b
If-03	7.5±0.8ab	6.8±0.9bc	7.7±0.7b	8.1±0.6ab	7.9±0.2ab	8.7±0.4ab
If-02	6.9±0.7ab	7.1±0.8b	7.0±0.3b	6.9±0.7b	7.0±0.7b	7.8±0.6ab
Control	10.6±0.9a	11.3±0.7a	12.0±0.7a	11.7±0.7a	10.8±0.6a	11.5±0.3a
LSD	1.6	0.8	2.5	1.2	2.1	1.9
F-value	3.8	2.4	3.8	4.1	3.4	4.2
P-value	0.0	0.0	0.0	0.0	0.0	0.0
LC_{30}+ LC_{30}						
Bb-01	4.9±0.5b	6.8±0.4b	6.9±0.7b	5.4±0.8b	6.0±0.3bc	6.2±0.5b
Bb-10	4.1±0.4bc	5.7±0.9bc	5.1±0.5bc	4.9±0.4b	5.1±0.8c	4.7±0.8b
Ma-11.1	4.5±0.8b	5.2±0.6c	5.6±0.9b	5.8±0.6b	5.7±1.0bc	5.1±0.6b
Ma-2.4	4.0±0.3bc	4.2±0.2c	4.8±0.7bc	4.0±0.5bc	4.3±0.2c	4.1±0.5b
If-03	6.4±0.5ab	5.8±0.7bc	6.1±0.6b	5.1±0.9b	5.9±0.6bc	4.9±0.5b
If-02	5.3±0.8b	6.2±0.9bc	5.5±0.3b	4.6±1.0b	4.9±0.8c	5.9±0.8b
Control	10.6±0.7a	12.9±0.6a	12.3±0.3a	11.6±0.4a	13.5±0.2a	12.1±0.5a
LSD	2.0	1.7	3.2	2.5	2.9	4.0
F-value	4.3	3.7	5.0	4.8	4.2	5.5
P-value	0.0	0.0	0.0	0.0	0.0	0.0
LC_{40}+ LC_{40}						
Bb-01	4.3±0.5b	4.5±0.5b	4.9±0.7b	4.1±0.6bc	5.8±0.7ab	5.9±0.7b
Bb-10	3.6±0.7b	4.6±0.4b	4.3±0.9b	3.7±0.1bc	5.0±0.5b	4.2±0.7b
Ma-11.1	4.0±0.3b	3.8±0.4b	5.1±0.6b	5.0±0.2b	5.5±0.8ab	5.5±0.8b
Ma-2.4	3.0±0.5bc	2.9±0.4bc	3.9±0.5bc	3.2±0.4bc	4.0±0.4b	5.0±0.3b
If-03	4.3±0.6b	4.9±0.5b	5.6±0.4b	4.7±0.6b	5.3±0.7ab	6.2±0.5b
If-02	5.0±0.4b	4.3±0.6bc	4.6±0.6b	4.1±1.0bc	4.3±0.5b	5.2±0.4b
Control	10.5±0.8a	12.3±0.6a	12.1±0.8a	12.6±0.5a	11.2±0.7a	11.5±0.3a
LSD	3.5	2.8	4.1	3.6	2.2	5.9
F-value	4.8	5.2	5.8	5.0	6.1	5.2
P-value	0.0	0.0	0.0	0.0	0.0	0.0

Nymphal duration

The binary treatment of fungi and insecticides significantly prolonged nymphal duration in all treatments. The maximum prolongation of nymphal duration (days) (15.1 ± 0.3) was observed in treatment with Ma-2.4 and bifenthrin (LC_{40}). In case of LC_{30}, nymphal duration (13.1 ± 0.8) days was recorded in treatment with LC_{30} of Ma-2.4 and nitenpyram. On the other hand, the nymphal duration of 11.8±0.6 days was observed in the treatment of combined mixture of Ma-11.1 (LC_{20}) and nitenpyram (LC_{20}), while 9.9±0.4 days were recorded by the treatment of combined mixture of LC_{10} of Ma-2.4 and lambda cyhalothrin (Table III).

Adult emergence

High doses of fungi and insecticides mixture decreased the adult percent emergence to a greater extent, while significant decrease was also recorded in other doses. The highest percent emergence *i.e.,* 30.0±5.8 and 30.0±4.9 after application of LC_{40} of Ma-2.4 + imidacloprid and LC_{40} of If-03 + imidacloprid was recorded, respectively. While the lowest percent emergence *i.e.,* 10.0±2.5 and 10.0±1.8 with the mixture of LC_{40} of Bb-01 and bifenthrin and Bb-10 and bifenthrin, respectively. In case of LC_{30} lowest adult emergence (17.5±5.6) was observed in the mixture of LC_{30} of Bb-01 and bifenthrin. While for LC_{20} and LC_{10} lowest adult emergence *i.e.,* 25.0±2.7 and 35.0±3.1 were recorded, respectively in combination with Bb-01 and bifenthrin (Table IV).

Adult longevity

The combinations of fungi and insecticides greatly shortened the male longevity. At LC_{40} and LC_{20} of Ma-2.4 and imidacloprid reduced the male longevity to 2.8±0.3 and 5.9±0.7 days, respectively. The minimal male longevity, 3.9±0.3 days was observed in combination of LC_{30} of Ma-2.4 and triazophos, while at LC_{10} the minimum male longevity 7.1±0.4 days was recorded in combination of LC_{10} of Ma-2.4 and bifenthrin mixture (Table V).

The combined treatments of fungi and insecticides greatly affected the female longevity of *O. hyalinipennis*. The results showed that the combined mixture of Ma-2.4 and bifenthrin at LC_{40}, LC_{30} and LC_{20} reduced female longevity to 3.1±0.8, 5.0±0.7 and 10.0±0.9 days, respectively. While the mixture of LC_{10} of Ma-2.4 and triazophos reduced female longevity to 12.6±0.8 days.

DISCUSSION

There is a great interest all over the world for utilizing and manipulating entomopathogenic fungi for biological control of insects. Entomopathogenic fungi are just like parasite which kill or disable the insects. Unlike other pathogens like bacteria and viruses which need to be ingested for their action these fungi require contact with cuticle under favorable temperature and humidity (Dhaliwal and Koul, 2007). In order to increase their effectiveness, these can be mixed with insecticides (Pachamuthu and Kamble, 2000). These fungi and selective insecticides may synergize the effect of each other thus increasing the efficiency of control, preservation of natural enemies, reducing insecticides usage, environmental risks and resistance in pests (Quintela and McCoy, 1998; Dayakar *et al*., 2000; Ambethgar, 2003; Rachapa *et al*., 2007). Combination of imidacloprid and fungal pathogen showed the synergistic effect for the control of insect pests (Kaakeh *et al*., 1997; Quintela and McCoy, 1998; Ramakrishnan *et al*., 1999; Lacey *et al*., 1999; Ying *et al*., 2003).

The findings of current study depicted that the binary mixtures of fungi and insecticides at higher doses showed higher mortality than alone application. Highest percent mortality i.e., 90.0 was caused by the mixture of isolate Bb-01 and bifenthrin. It confirms the findings of Purwar and Sachan (2006) that the mixture *B. bassiana* and endosulfan proved to be more toxic and caused higher mortality in *Spilarctia oblique* (Walker). Identical results were reported by Dayakar *et al*. (2000) when combined mixture of *B. bassiana* and insecticide was applied to S*podoptera litura* (Fabricius). *B. bassiana* is highly compatible with avermectin and pyrethroids than any other insecticide (De Olivera and Neves, 2004). Synergistic effect of *B. bassiana* and imidacloprid, *M. anisopliae* and fenitrothion or teflubenzuron were recorded against termite *Reticulitermes flavipes* (Kollar) and scarab beetle larvae (Boucias *et al*., 1996; Hornbostel *et al*., 2005). Previous studies focused on the potential use of combination of insecticides and fungi (Pachamuthu and Kamble, 2000; Zurek *et al*., 2002; Jaramillo *et al*., 2005; Thompson *et al*., 2006; Ericsson *et al*., 2007; Sharififard *et al*., 2011). In addition to the direct effect on insect mortality, the current study also studied the effects on the biological parameters including adult emergence and adult longevity. Binary mixture of entomopathogenic fungi and insecticides greatly reduced the percent adult emergence and adult longevity at higher doses while on other doses there was significant difference in the percent adult emergence and adult longevity than normal which is in accordance to study of Ekesi *et al*. (2002) which showed low adult emergence in *S. litura* when treated with fungus. The results of current research are in accordance to the findings of Pelizza *et al*. (2013), who validated the decrease in

the survival rate and lower fecundity in dipterans when treated with the EPF. The present research shows that the mixture of pathogenic fungi and insecticides increased the management; however, more work is needed to study the compatibility of these fungi and insecticides under field conditions.

Table VI.- Female longevity of *O. hyalinipennis* after the treatment of binary mixture of entomopathogenic fungi and insecticides.

	Triazophos	Imidacloprid	Pyreproxifen	Nitenpyram	Lambda cyhalothrin	Bifenthrin
LC_{10}+ LC_{10}						
Bb-01	12.9±0.4a	14.3±0.5a	14.3±0.8a	14.1±0.6a	13.9±0.4a	14.1±0.2a
Bb-10	13.3±0.6a	13.4±0.3a	13.6±0.3a	14.7±0.8a	14.5±0.8a	14.7±1.0a
Ma-11.1	13.9±1.2a	12.8±0.8ab	13.0±0.7ab	13.9±0.8a	13.7±0.8a	13.5±0.8a
Ma-2.4	12.6±0.8a	13.0±0.6ab	12.8±0.8b	12.3±0.7ab	13.0±0.7ab	12.8±0.6ab
If-03	14.2±0.6a	14.4±1.0a	14.0±0.8a	14.3±0.6a	15.0±0.7a	14.9±0.5a
If-02	13.0±0.4a	14.0±0.7ba	13.0±0.5ab	13.5±0.3a	14.0±0.2a	13.9±0.7a
Control	14.9±1.2a	15.3±1.7a	15.3±1.0a	15.1±0.4a	15.2±1.0a	15.3±0.3a
LSD	0.8	2.6	8.1	12.5	7.4	10.8
F-value	5.6	3.2	2.5	4.3	2.8	3.1
P-value	0.0	0.0	0.0	0.0	0.0	0.0
LC_{20}+ LC_{20}						
Bb-01	12.2±0.4b	12.2±0.9a	13.2±0.7a	13.4±0.5a	12.2±0.8a	12.5±0.5a
Bb-10	13.2±0.7a	12.0±0.7a	12.8±0.7a	12.7±0.5a	12.0±0.7a	12.0±0.6a
Ma-11.1	13.0±0.3ab	12.9±0.3a	13.0±0.6a	12.2±0.7ab	11.0±0.5ab	11.2±0.1ab
Ma-2.4	11.5±0.6b	11.7±0.6ab	11.6±0.8ab	11.5±0.7b	10.6±0.9ab	10.0±1.0b
If-03	13.8±0.5a	13.2±0.6a	12.2±0.3a	13.1±0.9a	12.9±0.5a	11.9±0.4a
If-02	12.1±0.9b	12.6±0.3a	12.0±0,8a	12.3±0.9ab	11.6±0.7ab	12.8±0.7a
Control	15.1±0.3a	14.9±0.4a	14.7±0.7a	15.5±0.3a	14.2±0.9a	14.9±0.4a
LSD	3.5	5.1	3.0	6.3	12.1	10.3
F-value	2.8	4.7	4.6	5.2	3.9	4.1
P-value	0.0	0.0	0.0	0.0	0.0	0.0
LC_{30}+ LC_{30}						
Bb-01	8.0±0.4b	8.2±0.5b	8.7±0.6b	8.1±0.8b	8.8±0.7b	8.0±0.9b
Bb-10	6.4±0.bcl	7.1±0.3bc	8.0±0.7bc	7.2±0.3b	7.8±0.4b	7.3±0.2b
Ma-11.1	7.1±0.4b	7.9±0.5b	8.1±0.5b	7.7±0.8b	7.5±0.5bc	7.8±0.4b
Ma-2.4	5.9±0.1bc	6.8±0.3bc	7.7±0.5bc	6.0±1.0bc	6.1±0.9bc	5.0±0.7bc
If-03	8.2±0.8b	8.3±0.8b	8.4±0.4b	8.7±0.4b	8.4±0.3b	7.4±0.3b
If-02	7.6±0.7b	7.5±0.2b	7.5±0.8c	8.0±0.3b	7.0±0.5bc	6.3±0.7bc
Control	14.3±0.5a	14.8±0.8a	15.1±0.5a	15.2±0.4a	14.4±0.4a	15.1±0.5a
LSD	7.5	12.0	21.4	15.3	14.5	17.1
F-value	5.2	4.7	3.9	6.0	4.7	5.8
P-value	0.0	0.0	0.0	0.0	0.0	0.0
LC_{40}+ LC_{40}						
Bb-01	5.1±1.0b	4.9±0.5b	5.0±0.9b	4.9±0.9b	5.0±0.6b	5.4±0.1b
Bb-10	4.1±0.8b	4.3±0.6bc	4.6±0.5b	3.9±0.7bc	3.6±0.5b	3.5±0.8bc
Ma-11.1	4.5±0.3b	4.2±1.0bc	4.4±0.6b	4.4±0.8b	4.5±0.4b	4.6±1.0b
Ma-2.4	3.8±0.8bc	3.7±0.1c	4.0±0.8bc	3.2±0.5bc	3.9±0.4b	3.1±0.8bc
If-03	5.4±0.9b	5.2±0.5bc	5.3±0.2b	4.8±0.4b	4.1±0.4b	4.0±0.4b
If-02	4.9±0.1b	4.0±0.2bc	4.1±0.3bc	3.7±0.5bc	3.3±0.9bc	3.8±0.7bc
Control	15.1±0.5a	14.3±0.9a	15.1±0.3a	14.2±0.2a	14.6±0.8a	15.3±0.1a
LSD	12.4	9.8	13.5	10.6	15.3	23.0
F-value	7.3	5.7	8.2	6.9	9.0	8.6
P-value	0.0	0.0	0.0	0.0	0.0	0.0

CONCLUSION

The present research shows that the mixture of pathogenic fungi and insecticides increased the management by increasing mortality and decreasing the development time of different stages of *O. hyalinipennis*; however, more work is needed to study the compatibility of these fungi and insecticides under field conditions.

Statement of conflict of interest

The authors declare no conflict of interest.

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