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Assessment of Binary Mixtures of Entomopathogenic Fungi and Chemical Insecticides on Biological Parameters of *Culex pipiens* (Diptera: Culicidae) under Laboratory and Field Conditions

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RFS and KWA performed experiment, analysed the data and wrote the manuscript. SF provided technical assistance, supervision and helped in writing the manuscript. AR compiled the manuscript.

Key words
***Culex pipiens*, Mosquito, Larvicides, Biological parameters, Entomopathogenic fungi, Progeny.**

ABSTRACT

Southern house mosquito, *Culex pipiens* (Diptera: Culicidae), is most common agent for transferring a mass of pathogens *i.e.,* West Nile Virus (WNV), avian pox virus (APV) etc. in rural and urban areas. To mitigate these problems study was conducted by using binary mixtures of entomopathogenic fungi, *Beauveria bassiana* (isolates Bb-01, Bb-10), *Metarhizium anisopliae* var. *anisopliae* (isolate Ma-11.1, Ma-2.4) and *Isaria fumosorosea* (isolates If-2.3, If-02) and chemical insecticides *i.e.,* bifenthrin, lambda cyhalothrin, imidacloprid, triazophos, spinosad, pyriproxyfen and nitrinpyrum, as larvicides against *C. pipiens*. Highest larval percent mortality (73.3 ± 4.7) was observed after application of Ma-11.1(LC_{40}) + nitenpyram (LC_{40}) mixtures under laboratory and field conditions (71.5 ± 7.4). The results showed a significant effect of binary treatments of fungi and chemical insecticides on biological parameters of *C. pipiens* and its progeny ($P<0.05$). The insect pathogenic fungi showed compatibility with insecticides and the combined application can improve the management program of *C. pipiens*.

INTRODUCTION

Culex pipiens (Diptera: Culicidae), is the most common mosquito in rural and urban areas which in capable of transferring a number of pathogens *i.e.,* West Nile Virus (WNV) (CDC, 2002; Turell *et al.*, 2001; Alouani *et al.*, 2009), avian pox virus, bird malaria pathogen, dog heartworm, *Dirofilaria immitis*, filarial nematode, *Wuchereria bancrofti* (Vinogradova, 2000) and St. Louis encephalitis (Eldridge *et al.*, 2000). One million people around the world are suffered annually from mosquito transmitted diseases (Kager, 2002). Mostly, synthetic pyrethroids, organophosphates and insect growth regulators are used for the control of adults and larvae of mosquito (Zaim *et al.*, 2000; Hougard *et al.*, 2002; Cui *et al.*, 2006; WHO, 2006).

Over the last five decades relatively large number of problems have been raised due to the misuse of chemical insecticides namely, insecticide resistance, environmental and water pollution, toxic hazards to humans and other non-target organisms (Jirakanjanakit *et al.*, 2007; Seccacini *et al.*, 2008; Al-Sarar, 2010; Khandagle *et al.*, 2011). These problems require the development of effective, easy to apply and cheap substitutes by using eco-friendly products to control the mosquitoes (Liu *et al.*, 2004, 2013; Nielsen and Lewis, 2012). In order to mitigate these problems, a major emphasis has recently been given by using insect pathogenic fungi (IPF) as larvicides (Howard *et al.*, 2010; Mahesh *et al.*, 2012). In general, mosquitoes show susceptibility towards pathogenic fungi and its derived products. The pathogenic fungi being low toxic to non-target organisms and its activity as larvicides proves to be a promising approach for the biological control of insect pests (Soni and Soam, 2010; Bukhari *et al.*, 2011; Freed *et al.*, 2012). In addition to this, entomopathogenic microorganisms have the advantage to be generally specific without affecting other natural enemies as compared to most of chemical insecticides (Perinotto *et al.*, 2012).

Beauveria bassiana and *Metarhizium anisopliae* var. *anisopliae* are two of the most common cosmopolitan fungi which are pathogenic to a number of insect pests

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(Nussenbaum and Lecuona, 2012). The objective of current study was to investigate the efficiency of individual and combined application of fungi and insecticides for developing an eco-friendly approach and to investigate the effect of sub-lethal doses on the biological parameters of *C. pipiens*.

MATERIALS AND METHODS

Mosquito collection and rearing

Larval collections (all instars) were carried out from Multan, Punjab, Pakistan from different sites and were pooled per locality. Larvae were brought to the Laboratory of Insect Microbiology at B.Z. University (BZU) Multan, where these were reared under standard controlled conditions (26±2°C, 80±10% relative humidity (RH) and 12:12 L-D photoperiod). Larvae were fed daily with baby fish food. The adult emergence was morphologically identified using identification keys and shifted in disinfected plastic cages (1.5 × 2 ft). Adult males were feed on 10% sugar solution, while females were given blood meal by feeding on laboratory white albumen mice. Female mosquitoes laid eggs in the form of patches in glass petri dishes containing 10ml water placed in plastic cages. Larvae on hatching were shifted in glass jars (10 ×15cm) having 500 ml tap water and were feed on fish food till pupation. Water and diet of each jar was changed after every 48 h.

Fungi culture

Different isolates of *B. bassiana* (Bb-01, Bb-10), *M. anisopliae* var. *anisopliae* (Ma-11.1, Ma-2.4) and *I. fumosorosea* (If-2.3, If-02) (Table I) already present in the Laboratory of Insect Microbiology, BZU, Multan were used. Monoconidial cultures of the isolates grown on PDA were used. For further propagation rice media was used. Stock solutions were made and stored at 4°C for further use.

Insecticides

Commercial formulations of different chemical insecticides were purchased for bioassay from the pesticide market of Multan, Pakistan (Table I).

Preparation of concentrations of fungi and insecticides

The spore concentrations of entomopathogenic fungi were measured using the haemocytometer and the desired concentrations (4×10^8, 3×10^8, 2×10^8, 1×10^8 and 1×10^7 spores/ml) and (0.005, 0.0025, 0.00125, 0.000625, 0.00031 ppm) of fungi and insecticides were prepared for bioassay. On the other hand, the binary mixtures were made by using the following scheme *e.g.,* fungi (LC_{40}) + insecticide (LC_{40}), while similar scheme was adopted for other concentrations.

Table I.- List of the isolates of entomopathogenic fungi and insecticides used in the experiments.

Fungi	**Isolate**	**Source**	**Location**
Beauveria bassiana	Bb-01	Cotton Field	Makhdoom Rasheed, Multan, Pakistan
Beauveria bassiana	Bb-10	River side soil	Naran, Mansehra, Pakistan
Metarhizium anisopliae var. *anisopliae*	Ma- 2.4	Barseen field	Tawakal Town, Multan, Pakistan
Metarhizium anisopliae var. *anisopliae*	Ma-11.1	Cotton field	Makhdoom Rasheed, Multan, Pakistan
Isaria fumosorosea	If -02	Rove beetle	Multan, Pakistan
Isaria fumosorosea	If -2.3	Vegetable Field	Makhdoom Rasheed, Multan, Pakistan
Insecticides	**Trade name**	**Manufacturer**	**Formulation**
Bifenthrin	Maya®	Alfa agro chemicals	10EC
Imidacloprid	Confidor®	Bayer	20SL
Lambda cyhalothrin	Karate®	Syngenta	2.5EC
Nitenpyram	Sparks®	FMC	10SL
Pyriproxyfen	Admiral®	FMC	10EC
Spinosad	Tarcer®	Arysta life sciences	24SC
Triazophos	Triazophos®	United distributers	40EC

Work layout and data analyses

The experiment was conducted under the Completely Randomized Design (CRD) with four replications in each treatment. All fungi and insecticides were screened to calculate LC_{50}, LC_{40}, LC_{30}, LC_{20} and LC_{10} values for both laboratory and field experiments. Three fungal isolates from the above mentioned fungi were selected with lowest LC_{50} values. Mixtures of different concentrations of fungi + insecticides was applied on both laboratory and field populations. Small plastic containers with a capacity of 450 ml water were used for experimentation. In each container, 250 ml serially diluted suspension was poured and 15 larvae of same age belonging to late 3rd instar were released and provided with sufficient fish food. The containers were labelled and placed under laboratory condition (temperature, 25±1°C, RH. 75±2% and Photo period 10L-14D h) in Laboratory of Insect Microbiology, BZU, Multan, while for field experiments; these were placed under shady places (Temperature, ≈ 29±2°C, RH. 55±2%) in fields of BZU, Multan. Data of mortality was taken for seven days in case of fungi (Khan *et al*., 2014), three days in case of insecticides and seven days in case of binary mixtures. The data of precent pupation, pupal duration, precent emergence and sex ratio was recorded from beginning to end of experiment (Sivagnaname and Kalyanasundaram, 2004). LC_{50}, LC_{40}, LC_{30}, LC_{20} and LC_{10} values of all fungi and insecticides were calculated by using POLO-PC software (LeOra Software, 2003). The means were analyzed by analytical software (Statistix version 8.1) and compared by LSD test at 0.05 probability levels.

RESULTS

Pre-experimentation was conducted on laboratory and field populations of *C. pipiens* for determining LC_{50}, LC_{40}, LC_{30}, LC_{20} and LC_{10} values of six isolates of fungi and seven chemical insecticides. Later three fungi were selected on the basis of least LC_{50} values and sub-lethal doses were used in binary mixtures to check the mortality and after effects on the progeny (Table II).

Larval mortality after fungi + insecticide treatment

The mortality of *C. pipiens* in laboratory population after the application of fungi + insecticide is shown in Table III. Highest percent mortality (73.3 ± 4.7) was observed when mixture containing Ma-11.1 (LC_{40}) + spinosad (LC_{40}) was applied followed by (71.7 ± 4.1) with the application of Ma-11.1 (LC_{40}) + nitenpyram (LC_{40}). The results showed a concentration dependent response (F=61.65, P=0.0087, df=6), which increased with the increase in concentration. On the other hand, the highest percent mortality (71.5 ± 7.4) was recorded after the application of mixture containing Ma-11.1(LC_{40}) + spinosad (LC_{40}) (F=176, P=0.0023, df=6) under field conditions (Table III).

Table II.- Calculated doses of fungi (spores/ml) and insecticides (ppm) for binary treatment on *C. pipiens* (laboratory and field trials).

Isolates (spores/ml)	Laboratory trials					Field trials				
	LC_{50}	LC_{40}	LC_{30}	LC_{20}	LC_{10}	LC_{50}	LC_{40}	LC_{30}	LC_{20}	LC_{10}
Bb-01	4.67×10^{7}	4.39×10^{7}	4.21×10^{7}	4.16×10^{7}	4.01×10^{7}	5.52×10^{7}	5.43×10^{7}	5.37×10^{7}	5.20×10^{7}	5.01×10^{7}
Bb-10	6.57×10^{7}	6.10×10^{7}	6.03×10^{7}	5.97×10^{7}	5.74×10^{7}	7.84×10^{7}	7.65×10^{7}	7.53×10^{7}	7.31×10^{7}	6.99×10^{7}
Ma-2.4	6.33×10^{8}	6.13×10^{8}	6.01×10^{8}	5.99×10^{8}	5.81×10^{8}	8.81×10^{8}	8.54×10^{8}	8.32×10^{8}	8.12×10^{8}	8.00×10^{8}
Ma-11.1	1.62×10^{7}	1.52×10^{7}	1.50×10^{7}	1.49×10^{7}	1.40×10^{7}	2.01×10^{8}	1.91×10^{8}	1.8×10^{8}	1.6×10^{8}	1.53×10^{8}
If-02	7.61×10^{7}	7.12×10^{7}	7.01×10^{7}	6.82×10^{7}	6.19×10^{7}	9.52×10^{7}	9.37×10^{7}	9.15×10^{7}	9.01×10^{7}	8.91×10^{7}
If-2.3	5.48×10^{8}	5.46×10^{8}	5.11×10^{8}	4.92×10^{8}	4.71×10^{8}	7.82×10^{8}	7.53×10^{8}	7.41×10^{8}	7.32×10^{8}	7.11×10^{8}
Insecticides (ppm) × 10^{-5}										
Triazophos	7.9	56	3	1	2.1	9	8.2	6.1	5.2	4
Spinosad	5.2	4	2.1	0.97	0.42	7	6.6	6.1	5	4.9
Imidacloprid	6	3.8	1.3	0.2	0.12	8.1	7.8	7.1	6	5.9
Pyriproxyfen	3.7	2.1	1.1	0.6	0.1	5.8	5.1	4	3.9	3.1
Nitenpyram	4	3	2.8	1.7	0.1	4	3.9	3.1	2.8	2
Lambda cyhalothrin	5	3.7	3.1	1.1	0.21	5.7	5.2	4.8	4.1	3.7
Bifenthrin	9	7.1	5.4	1.2	0.87	9.8	9	8.7	7.6	7.1

Table III.- Larval mortality (%) of *C. pipiens* in laboratory and field trials as a result of binary treatment of fungi and insecticides.

	$LC_{10}+LC_{10}$			$LC_{20}+LC_{20}$			$LC_{30}+LC_{30}$			$LC_{40}+LC_{40}$		
	Bb-01	**Ma-11.1**	**If-02**	**Bb-01**	**Ma-11.1**	**If-02**	**Bb-01**	**Ma-11.1**	**If-02**	**Bb-01**	**Ma-11.1**	**If-02**
Laboratory trials												
Triazophos	36.7±5.7c	31.7±3.1cd	20.0±2.7ef	48.3±7.8ab	43.3±6.3bc	25.0±3.1e	56.7±1.9b	51.7±3.1bc	55.0±6.8b	65.0±3.1ab	60.0±2.7b	45.0±5.0d
Spinosad	38.3±7.8bc	41.7±6.1b	16.6±1.9fg	46.7±4.7b	51.7±3.1ab	23.3±1.9ef	51.7±3.1bc	61.7±4.1a	31.7±5.6e	58.3±7.8bc	73.3±4.7a	41.7±5.6de
Imidacloprid	31.7±1.6cd	26.7±2.7de	26.7±2.7de	40.0±4.1c	30.0±1.9de	30.0±5.7de	50.0±6.9bc	38.3±4.1d	38.3±8.3d	61.7±1.6b	48.3±1.6cd	51.7±3.1cd
Pyriproxyfen	33.3±5.4cd	25.0±3.1e	15.0±3.1g	40.0±7.2c	30.0±3.3de	20.0±2.7f	50.0±4.3bc	40.0±2.7d	23.3±1.9f	61.7±4.1b	50.0±5.7cd	33.3±2.7ef
Nitenpyram	43.3±1.9ab	48.3±1.6a	28.3±3.1de	50.0±1.9ab	55.0±1.6a	33.3±2.7d	63.3±5.7a	65.0±4.1a	50.0±5.7bc	70.0±3.3a	71.7±4.1a	60.0±3.8bc
Lambda cyhalothrin	23.3±1.9ef	18.3±3.1f	18.3±1.6f	30.0±3.3de	23.3±3.3ef	23.3±5.7ef	40.0±2.7d	33.3±4.1de	28.3±1.6e	50.0±1.9cd	43.3±1.9d	33.3±2.7ef
Bifenthrin	35.0±4.1c	23.3±3.3ef	20.0±2.7f	46.7±4.7b	31.7±3.1de	26.7±2.7e	56.7±4.3b	40.0±2.7d	31.7±5.6e	63.3±1.9b	55.0±1.6c	38.3±1.6e
Control	1.3±0.2h	2.1±0.0h	1.8±0.8h	1.7±1.6g	1.0±1.4g	3.7±1.6g	1.4±1.6g	1.7±0.6g	1.4±0.1g	1.9±1.6g	1.4±0.3g	1.4±0.2g
F-values		43.22			43.67			53.76			61.65	
P-values		0.0002			0.0019			0.0013			0.0087	
LSD-values		5.64			6.87			8.11			8.75	
Field trials												
Triazophos	30.9±5.8bc	27.5±3.1bc	16.5±2.6f	44.9±6.1a	38.1±6.4b	20.5±2.8f	49.5±1.2bc	47.3±2.8cd	30.0±4.5fg	61.1±3.9bc	53.9±3.0c	38.6±4.1ef
Spinosad	37.0±7.0a	33.2±8.1b	13.0±2.1g	41.0±5.9ab	45.5±3.2a	20.8±1.6f	46.5±3.2cd	54.8±4.6bc	28.1±5.7fg	52.6±7.2d	71.5±7.4a	38.3±4.7ef
Imidacloprid	26.3±2.2cd	21.2±1.3e	21.9±2.6de	35.4±4.6bc	25.5±1.7e	25.4±5.4e	46.2±6.8cd	34.6±4.4ef	33.1±7.6ef	54.0±2.5cd	42.6±1.8ef	42.2±1.3ef
Pyriproxyfen	31.2±4.9bc	20.8±2.7e	12.7±2.8g	35.4±6.8bc	25.7±4.0e	17.7±2.3fg	47.4±4.0c	36.6±2.6ef	19.3±1.2h	57.4±4.0cd	46.3±5.7e	30.3±3.1gh
Nitenpyram	37.4±1.7a	33.7±0.4ab	21.7±1.9de	45.6±1.9a	43.5±2.6a	27.5±2.3de	58.8±5.9ab	54.5±4.1bc	44.6±5.9d	62.4±2.1c	61.8±4.4bc	53.5±5.3cd
Lambda cyhalothrin	18.3±2.1ef	14.5±2.6fg	15.1±2.0fg	23.1±2.3ef	20.7±3.0f	17.8±5.0fg	34.8±2.4ef	29.6±3.6fg	23.3±1.7gh	45.1±2.2e	37.8±2.7fg	28.3±2.2gh
Bifenthrin	29.6±4.1bc	18.0±3.0ef	16.5±2.5f	38.0±3.1b	25.4±2.6e	22.0±2.7ef	49.7±3.9bc	35.0±2.8ef	27.3±5.4fg	58.9±1.0c	50.3±1.1d	32.1±1.9g
Control	1.8±0.1i	2.4±0.9i	1.9±0.4i	1.5±0.6i	2.0±0.3i	1.6±0.1i	2.0±0.3i	1.8±0.2i	1.7±0.4i	1.8±0.5i	2.0±0.1i	1.2±0.6i
F-values		131			165			154			176	
P-values		0.0049			0.0039			0.0001			0.0023	
LSD-values		4.03			4.76			5.11			6.98	

Means followed by same letters in row and columns are non-significantly different (LSD=0.05). ± indicates upper and lower limit of standard error.

Table IV.- Pupation (%) of *C. pipiens* under laboratory and field trials as a result of binary treatment of fungi and insecticides.

	$LC_{10}+LC_{10}$			$LC_{20}+LC_{20}$			$LC_{30}+LC_{30}$			$LC_{40}+LC_{40}$		
	Bb-01	**Ma-11.1**	**If-02**	**Bb-01**	**Ma-11.1**	**If-02**	**Bb-01**	**Ma-11.1**	**If-02**	**Bb-01**	**Ma-11.1**	**If-02**
Laboratory trials												
Triazophos	63.0±0.3ef	68.3±0.5de	81.7±0.9c	51.0±0.3hi	56.7±0.5gh	77.2±0.3d	43.0±0.5h	48.3±0.8j	66.3±0.3g	26.7±0.4o	31.7±0.7n	49.6±0.9jk
Spinosad	61.7±0.5ef	58.3±0.2f	83.3±0.4bc	51.3±0.6hi	48.3±0.2i	76.7±0.7de	48.3±0.3j	38.3±0.4f	68.3±0.8fg	23.3±0.4p	16.3±0.8q	46.7±0.7k
Imidacloprid	68.3±0.4de	73.3±0.5d	73.3±0.3d	60.0±0.9g	70.0±0.3ef	70.0±0.7ef	50.0±0.6j	61.7±0.7gh	67.7±0.3fg	31.7±0.5mn	43.3±0.8l	38.3±0.4m
Pyriproxyfen	66.7±0.9de	75.0±01cd	85.0±0.5bc	60.0±0.8g	70.0±0.3ef	80.0±0.3d	50.0±0.3j	60.2±0.6h	76.0±0.4e	26.7±0.9o	35.0±0.4mn	50.7±0.3j
Nitenpyram	56.7±0.4f	51.7±0.3fg	71.3±0.6b	50.0±0.6i	45.7±0.8ij	65.0±0.4fg	36.7±0.4f	35.0±0.8k	60.9±0.3h	20.0±0.4pq	18.3±0.4q	33.8±0.6n
Lambda cyhalothrin	76.7±0.1cd	81.7±0.4c	83.2±0.1bc	70.0±0.5ef	76.7±0.8de	74.2±0.4e	60.0±0.3h	66.7±0.3g	71.3±0.6f	38.3±0.9m	45.0±0.7kl	57.5±0.7i
Bifenthrin	65.0±0.4e	76.7±0.3cd	81.7±0.7c	53.3±0.4hi	68.3±0.3f	71.7±0.8ef	43.3±0.1h	60.0±0.6h	65.4±0.8g	23.3±0.8p	33.3±0.9n	53.3±±0.7j
Control	96.3±0.9a	98.0±0.5a	97.0±0.2a	98.1±0.06a	98.7±0.8a	98.0±0.3a	98.7±0.09a	99.0±0.4a	99.3±0.9a	97.4±0.2a	98.0±0.6a	99.0±0.9a
F-values		45			49			52			65	
P-values		0.0003			0.0021			0.0001			0.008	
LSD-values		7.91			6.03			5.29			5.02	
Field trials												
Triazophos	67.3±0.6e	72.4±0.6de	86.6±0.3c	56.7±0.3fg	67.8±0.9fg	81.2±0.7c	50.0±0.1fg	62.3±0.9e	69.3±0.4d	38.0±0.3h	53.8±0.8f	63.4±0.3e
Spinosad	64.9±0.1ef	62.3±0.7f	86.5±0.8c	54.3±0.4g	56.4±0.9fg	80.3±0.3c	51.7±0.4f	47.9±0.6g	76.5±0.6cd	45.6±0.8g	42.3±0.9gh	64.6±0.3de
Imidacloprid	71.7±0.6de	79.8±0.4cd	81.2±0.5c	68.4±0.3e	72.1±0.6de	77.9±0.9d	59.4±0.7ef	65.4±0.8de	73.6±0.9d	47.2±0.8fg	54.6±0.6f	52.3±0.7f
Pyriproxyfen	68.4±0.7e	76.9±0.4d	87.7±0.8bc	64.9±0.6ef	73.3±0.8de	84.3±0.8c	59.1±0.7ef	61.2±0.7e	75.8±0.8cd	43.7±0.3gh	53.5±0.6f	63.5±0.8e
Nitenpyram	57.6±0.5fg	58.6±0.8fg	73.5±0.6de	54.3±0.6g	52.3±0.6g	66.6±0.3ef	41.2±0.8gh	41.7±0.8gh	54.8±0.8f	39.6±0.8gh	34.2±0.9h	47.9±0.9fa
Lambda cyhalothrin	81.5±0.7c	81.7±0.6c	87.7±0.9e	78.3±0.7cd	78.6±0.3cd	81.32±0.9c	67.5±0.3de	72.0±0.8de	78.6±0.5c	56.5±0.7ef	63.5±0.7e	69.8±0.3d
Bifenthrin	67.4±0.3e	81.3±0.4c	86.3±0.4e	56.6±0.8fg	72.3±0.6de	80.0±0.5c	49.9±0.6fg	65.4±0.6de	72.5±0.7d	41.3±0.3gh	58.7±0.3ef	67.82±0.6de
Control	97.9±0.7a	98.8±0.5a	98.8±0.7a	99.8±0.3a	97.9±0.7a	99.0±0.8a	98.7±0.1a	99.0±0.3a	97.8±0.6a	98.3±0.3a	98.6±0.4a	97.0±0.5a
F-values		89			96			101			109	
P-values		0.0042			0.0076			0.0031			0.0001	
LSD-values		7.13			7.43			8.75			9.03	

Means followed by same letters in row and columns are non-significantly different (LSD=0.05). ± indicates upper and lower limit of standard error.

Table V.- Pupal duration of *C. pipiens* under laboratory and field trials as a result of combined application of fungi and insecticides.

	$LC_{10}+LC_{10}$			$LC_{20}+LC_{20}$			$LC_{30}+LC_{30}$			$LC_{40}+LC_{40}$		
	Bb-01	**Ma-11.1**	**If-02**	**Bb-01**	**Ma-11.1**	**If-02**	**Bb-01**	**Ma-11.1**	**If-02**	**Bb-01**	**Ma-11.1**	**If-02**
Laboratory trials												
Triazophos	4.5±0.2a	4.3±0.3ab	3.9±0.7b	6.0±0.9ab	6.3±0.7ab	6.0±0.1ab	8.5±0.4a	8.1±0.4a	8.6±0.3a	8.8±0.2b	9.5±0.6ab	9.7±0.4a
Spinosad	4.6±0.3ab	4.6±0.9a	3.7±0.5bc	5.9±0.3b	6.6±0.4a	6.1±0.7ab	7.8±0.0ab	8.2±0.2a	7.3±0.4ab	9.9±0.2ab	9.9±0.4a	9.6±0.7ab
Imadacloprid	4.3±0.1ab	4.0±0.6b	4.2±0.2ab	5.9±0.2b	5.9±0.3b	5.3±0.6bc	7.6±0.5ab	7.8±0.6ab	7.8±0.5ab	9.5±0.6ab	9.7±0.3ab	9.9±0.5ab
Pyriproxyfen	3.8±0.6b	3.9±0.3b	3.8±0.2b	5.8±0.3b	6.0±0.4ab	5.0±0.0bc	7.5±0.3ab	7.6±0.5ab	7.6±0.8ab	9.0±0.1b	9.5±0.6ab	9.3±0.3ab
Nitenpyram	4.8±0.5a	5.0±0.4a	4.7±0.7a	6.5±0.2ab	6.9±0.3a	6.5±0.4ab	8.8±0.8a	8.9±0.4a	8.0±0.2a	11.0±0.3a	10.1±0.7a	9.9±0.5ab
Lambda cyhalothrin	4.1±0.2ab	3.7±0.7b	3.5±0.2bc	5.3±0.2bc	5.3±0.6bc	5.4±0.2b	7.9±0.3ab	7.3±0.2ab	8.0±06a	9.8±0.3ab	9.2±0.5ab	9.0±0.3b
Bifenthrin	4.6±0.3a	3.3±0.4bc	3.1±0.5bc	5.7±0.8b	5.8±0.5b	5.9±0.3b	8.6±0.9a	7.2±0.3ab	7.9±0.3ab	9.6±0.4ab	9.8±0.4ab	8.9±0.6b
Control	3.1±0.6bc	2.9±0.2c	3.5±0.6bc	3.0±0.4de	3.0±0.9de	3.6±0.3de	2.9±0.6d	3.1±0.3de	2.8±0.5de	3.2±0.4e	3.1±0.7e	3.7±0.8de
F-values		39			41			49			56	
P-values		0.003			0.0065			0.0091			0.002	
LSD-values		0.98			1.02			1.76			1.81	
Field trials												
Triazophos	4.2±0.5ab	4.1±0.4ab	3.6±0.5b	5.9±0.5ab	6.1±0.5ab	5.9±0.33ab	7.9±0.4a	7.6±0.6ab	8.1±0.5a	8.7±0.3ab	8.5±0.3a	8.5±0.6a
Spinosad	4.6±0.6a	4.5±0.3a	3.6±0.5b	5.2±0.2b	6.4±0.3a	5.8±0.52b	7.1±0.2b	8.0±0.6a	7.0±0.3b	8.8±0.2a	8.5±0.1a	8.1±0.1ab
Imidacloprid	4.2±0.5ab	4.1±0.2ab	4.2±0.3a	5.6±0.4b	5.2±0.3b	5.2±0.56bc	7.5±0.4b	7.9±0.5ab	7.8±0.5ab	8.8±0.5ab	8.3±0.6ab	8.1±0.9ab
Pyriproxyfen	3.7±0.6b	3.6±0.2b	3.7±0.6b	5.6±0.2b	6.0±0.3a	5.1±0.05bc	7.2±0.2b	7.3±0.4bc	7.5±0.3b	8.8±0.1a	7.2±0.1ab	7.1±0.1b
Nitenpyram	4.8±0.6a	4.2±0.3a	4.6±0.8a	6.1±0.5a	6.9±±0.3ab	6.5±0.35a	8.6±0.7a	8.9±0.2a	7.9±0.2ab	10.0±0.3a	8.9±0.3a	8.7±0.2ab
Lambda cyhalothrin	4.2±0.2ab	3.5±0.3bc	3.5±0.3b	5.3±0.2bc	5.1±0.5b	5.3±0.23b	7.5±0.3b	7.1±0.1b	7.9±0.3ab	9.6±0.3a	8.9±0.2a	8.7±0.1ab
Bifenthrin	4.6±0.3a	3.5±0.4b	3.0±0.5bc	5.4±0.6bc	5.8±0.3b	5.8±0.44b	8.6±0.8a	7.0±0.8bc	7.6±0.1ab	9.6±0.3a	8.2±0.3a	8.3±0.9ab
Control	2.9±0.5bc	2.8±0.5bc	3.6±0.1b	2.9±0.4dc	2.6±0.9c	3.5±0.35d	2.7±0.3ef	3.2±0.7de	3.0±0.3de	2.7±0.4de	3.2±0.9d	2.8±0.2de
F-values		111			129			138			151	
P-values		0.0003			0.001			0.006			0.008	
LSD-values		0.91			1.04			1.26			2.11	

Means followed by same letters in row and columns are non-significantly different (LSD=0.05). ± indicates upper and lower limit of standard error.

Table VI.- Emergence (%) of *C. pipiens* under laboratory and field trials as a result of binary treatment of fungi and insecticides.

	$LC_{10}+LC_{10}$			$LC_{20}+LC_{20}$			$LC_{30}+LC_{30}$			$LC_{40}+LC_{40}$		
	Bb-01	**Ma-11.1**	**If-02**	**Bb-01**	**Ma-11.1**	**If-02**	**Bb-01**	**Ma-11.1**	**If-02**	**Bb-01**	**Ma-11.1**	**If-02**
Laboratory trials												
Triazophos	47.3±0.3ef	51.2±0.9d	54.3±0.6c	41.2±0.7de	47.7±0.8cd	49.9±0.3c	37.7±0.1ef	41.2±0.9cd	41.9±0.4cd	30.0±0.8e	34.5±0.9d	37.8±0.2c
Spinosad	30.9±0.3j	29.9±0.7j	44.2±0.2g	26.3±0.6gh	20.8±0.6ij	41.2±0.8de	19.9±1.0kl	17.8±0.5kl	34.6±0.7g	16.0±0.7hi	13.2±0.9j	29.9±0.2ef
Imidacloprid	49.8±0.2de	54.3±0.9c	61.2±0.3b	41.5±0.4de	47.7±0.9cd	56.7±0.3b	37.6±0.1ef	39.9±0.4e	49.8±0.9b	29.9±0.2ef	29.9±0.6ef	41.8±0.8b
Pyriproxyfen	32.2±0.3i	37.7±0.5hi	45.4±0.2fg	28.2±0.8g	31.6±0.9fg	38.7±0.4e	26.3±0.6ij	27.7±0.7ij	32.3±0.7gh	19.9±0.7h	23.6±0.8g	29.8±0.3ef
Nitenpyram	26.5±0.3k	26.5±0.8k	30.9±0.4j	21.4±0.7i	19.9±0.6k	26.7±0.7k	18.5±0.8kl	14.3±0.6m	21.6±0.8k	14.5±0.1j	10.8±0.6k	17.7±0.7hi
Lambda cyhalothrin	47.5±0.7ef	49.9±0.4de	58.8±0.9b	42.3±0.9de	43.2±0.7d	52.3±0.9bc	36.6±0.1ef	36.6±0.4ef	43.9±0.9c	29.9±0.2ef	29.9±0.5ef	37.7±0.7c
Bifenthrin	38.7±0.9hi	39.8±0.5h	51.1±0.7d	31.3±0.7fg	33.8±0.8f	43.7±0.6d	27.2±0.2ij	29.9±0.5i	38.7±0.5ef	22.4±0.4g	22.8±0.9g	29.8±0.9ef
Control	99.0±0.9a	98.9±0.5a	98.3±0.5a	96.9±0.5a	99.0±0.8a	99.0±0.9a	98.7±0.3a	97.7±0.4a	97.5±0.6a	97.7±0.3a	98.7±0.5a	96.9±0.3a
F-values		195			188			179			191	
P-values		0.000			0.000			0.000			0.000	
LSD-values		0.66			0.71			0.70			0.65	
Field trials												
Triazophos	55.7±0.1de	59.8±0.1d	61.2±0.9bc	51.8±0.4d	55.9±0.6bc	57.9±0.6b	47.7±0.3d	51.9±0.7bc	55.9±0.3b	41.3±0.8de	49.7±0.6bc	51.9±0.9b
Spinosad	34.6±0.8h	39.2±0.1fg	46.7±0.9f	31.2±0.7h	31.2±0.7h	40.1±0.9fg	26.8±0.3h	29.9±0.2h	34.8±0.2fg	21.3±0.3i	26.5±0.1gh	27.6±0.1gh
Imidacloprid	58.8±0.1d	62.4±0.7bc	67.9±0.2b	51.3±0.6d	57.9±0.6b	59.9±0.2b	48.9±0.3d	52.3±0.1bc	52..8±0.4bc	41.6±0.7de	46.6±0.7d	49.8±0.3bc
Pyriproxyfen	38.7±0.1fg	45.3±0.5f	51.2±0.7de	32.1±0.7h	40.9±08fg	48.7±0.4de	27.9±0.3h	37.7±0.3fg	44.8±0.9de	25.5±0.6gh	29.8±0.1gh	38.8±0.9f
Nitenpyram	32.1±0.9hi	34.9±0.5h	37.7±0.9fg	27.7±0.5hi	29.8±0.1hi	30.9±0.8h	22.3±0.4hi	25.5±0.8hi	27.7±0.4h	19.1±0.4ij	21.9±0.1i	23.7±0.8gh
Lambda cyhalothrin	52.3±0.1de	56.7±0.7d	64.8±0.1bc	47.9±0.9de	51.4±0.4d	57.5±0.9b	41.2±0.7de	46.8±0.1d	51.7±0.3bc	39.8±0.3f	41.8±0.5de	45.6±0.9d
Bifenthrin	42.3±0.7fg	48.7±0.2f	57.9±0.3d	38.8±0.6fg	43.9±0.2f	51.9±0.7d	32.7±.9fg	39.9±0.1f	43.9±0.2de	29.9±0.3gh	31.8±0.2g	37.6±0.2f
Control	98.8±0.2a	98.8±0.3a	98.7±0.3a	97.9±0.5a	97.9±0.3a	99.0±0.7a	99.1±0.4a	99.0±0.3a	97.9±0.2a	97.9±0.3a	97.9±0.2a	97.7±0.9a
F-values		174			197			201			182	
P-values		0.000			0.000			0.000			0.000	
LSD-values		0.86			0.89			0.97			0.81	

Means followed by same letters in row and columns are non-significantly different (LSD=0.05). ± indicates upper and lower limit of standard error.

Pupation after fungi + insecticides treatment

Least pupation (16.3 ± 0.8) was recorded after the application of Ma-11.1+ spinosad (LC_{40}) followed by Ma-11.1 + nitenpyram (LC_{40}) which caused (18.3 ± 0.4) percent pupation (F=65, P=0.008, df = 6) (Table IV). On the other hand, least percent pupation (34.2 ± 0.9) after treatment of mixture (fungi + insecticides) was recorded after treatment with Ma-11.1+ nitenpyram (LC_{40}) mixture, followed by Bb-01 + triazophos (LC_{40}) causing (38.0 ± 0.3) precent mortality (F=109, P=0.0001, df=6) (Table IV).

The results depicted longest pupal duration after the application of higher concentrations of fungi + insecticide on laboratory population (Table V). Longest pupal duration (11.0 ± 0.3) days was recorded when mixture of Bb-01 + nitenpyram (LC_{40}) followed Ma-11.1+ nitenpyram (LC_{40}) (10.1 ± 0.7) days (F=56, P=0.002, df = 6). In contrast to this the binary mixtures application of fungi and insecticides under field condition showed longest pupal duration (10.0±0.3) (days) after treatment with Bb-01 + nitenpyram (LC_{40}) (F=151, P=0.008, df = 6) as compared to the control.

Emergence after fungi + insecticides treatment

Lowest adult emergence (10.8 ± 0.6) was recorded after treatment with Ma-11.1+ nitenpyram (LC_{40}) followed by (13.2 ± 0.9) treated with Ma-11.1+ spinosad (LC_{40}) (F=158, P=0.001, df=6) (Table VI). Conversely lowest precent emergence (19.1 ± 0.4) was observed after application of Bb-01 + nitenpyram (LC_{40}) (F=136, P=0.0076, df=6) under field conditions, while maximum precent emergence (97.9 ± 0.3) was recorded in the control treatment. The data regarding sex ratio after application of fungi and insecticides mixtures showed non-significant difference on all levels of treatments.

DISCUSSION

The current study was conducted to test the effectiveness of different insect pathogenic fungi and insecticides individually and in combinations against larval stages of *C. pipiens*. Our previous research has depicted the compatibility of these fungi with the insecticides (Akbar *et al.,* 2012). The previous research has shown the effectiveness of entomopathogenic fungi against dengue mosquitoes (Scholte *et al*., 2007; Paula *et al*., 2008), while *M. anisopliae* and *I. fumosorosea* have been used as virulent fungi for the control of different insect pests of various crops (Kaufman *et al*., 2005; Sharififard *et al*., 2011; Mishra *et al*., 2011). In addition to this various entomopathogenic fungi in combination with insecticides have been used for the management of a number of insect pests in past (Sharififard *et al*., 2011; Archana and Ramaswamy, 2012; Kassab *et al*., 2014).

Present study shows the combined effect of entomopathogenic fungi and synthetic insecticides on mortality and different biological parameters of *C. pipiens*. Highest larval mortality *i.e.,* 73.3 and 71.5% in laboratory and field trials was recorded after the combined treatment of Ma-11.1 with spinosad (LC_{40}), respectively which lies in favour of Anderson *et al*. (1989) who reported rapid mortality of Colorado potato beetle in laboratory and field trials due to combined application of *B. bassiana* with different insecticides. Similar results have been reported by Flores *et al*. (2004) and Pelizza *et al*. (2013) which depict decreased survival rate of *Aedes aegypti* after application of binary mixtures of entomopathogenic fungi and insecticides.

Percent pupation of *C. pipiens* was concentration dependent, higher concentrations of binary mixtures of entomopathogenic fungi and insecticides resulted in low percent pupation which lies in favor of Prasad and Veerwal (2012) who reported same results after application of *B. bassiana* on *Anopheles stephensi*. Pupal duration seemed to be prolonged after application of entomopathogenic fungi and insecticides in laboratory and field trails after application of binary mixtures (Bb-01 + nitenpyram LC_{40}). The results are in accordance to findings of Malarvannan *et al*. (2010) who stated increased pupal duration of *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) after treatment of entomopathogenic fungi.

Percent emergence of *C. pipiens* varied with the concentration of mixtures in field and laboratory trails, which relates with the findings of El-Razik *et al*. (2013) who observed the decrease in the adult development after treatment of binary mixtures (fungi + insecticides) on *Callosobruchus maculatus* (F). On the other hand, the data regarding sex ratio of *C. pipiens* showed non-significant differences after treatment of mixture of fungi and insecticides both in laboratory and field trails. Similar results were observed by Shaalan *et al*. (2005) who reported abnormal sex ratio in *A. aegypti* after application of mixtures of fungi and insecticides. Combination of entomopathogenic fungi and insecticides showed potential to be used in mixtures to counter insecticide resistance (Farenhorsta *et al*., 2009). Binary application of insecticide and fungi cause stress and disease for better management of *C. pipiens*. In conclusion mixture of entomopathogenic fungi and insecticides could be used as an effective tool for integrated management of *C. pipiens*.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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