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Evaluation of binary mixtures of entomogenous fungi and botanicals on biological parameters of *Culex pipiens* (Diptera: Culicidae) under laboratory and field conditions

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Abstract
Present study was conducted to evaluate binary mixtures of *Beauveria bassiana* (isolates Bb-01, Bb-10), *Metarhizium anisopliae* var. *anisopliae* (isolates Ma-11.1, Ma-2.4) and *Isaria fumosorosea* (isolates If-2.3, If-02) and botanicals extracts of *Azadirachta indica, Syzygium cumini, Acacia nilotica, Capsicum annum, Coriandrum sativum* and *Mentha longifolia* against 3rd instar larvae of *Culex pipiens* and the after effects on its progeny under laboratory and field conditions. The results revealed that the mixtures containing Bb-01 (LC_{40}) + *A. indica* (LC_{40}) showed maximum percent mortality, pupal duration, percent emergence and reduced percent pupation, followed by Ma-11.1 (LC_{40}) + *A. indica* (LC_{40}), while sex ratio of all treatments were non-significantly different. Entomopathogenic fungi showed synergistic effect when mixed with botanicals and provide a good management of *C. pipiens* under both field and laboratory conditions. This eco-friendly approach can be used for better management of mosquitoes under field conditions.

Keywords: *Culex pipiens*, mosquito, larvicides, biological parameters, evaluation, entomopathogenic fungi, botanicals, progeny

1. Introduction
Mosquitoes act as vectors of parasites and pathogens of a number of human and animal diseases [1]. About 3200 species of mosquito belonging to 37 genera have been reported [2] and out of these 6 genera and 45 species are present in Pakistan [3]. *Anopheles gambiae* G. and *Culex* species (Say) are widely responsible in spreading parasites of diseases like malaria and filariasis [4]. Management of Mosquito is a main concern now a days for which mechanical and chemical methods including insecticides impregnated nets are used [5, 6]. In the developing countries, management of mosquito is carried by the use of insecticides but due to continuous use of these synthetic poisons, problems like insecticide resistance and health hazards to life arises. There has been a high level of resistance reported in mosquitoes against conventional insecticides i.e., organophosphates, organochlorines, carbamates and pyrethroids [7]. Conversely the extensive use of diethyl-3-methylbenzamide (DEET) can cause skin irritation and erythema in human beings and other animals [8, 9].
Due to resistance against various groups of insecticides, other possible tactics including bio pesticides are being evaluated. Different bio-control agents including entomopathogenic fungi [10], bacteria [11] and plant extracts [12, 13] are most toxic and provide promising management of mosquitoes. Fungi infect mosquitoes by directly attacking on cuticle [14, 15], while, botanical insecticides are used for being less toxic and eco-friendly [16]. Plant based chemicals derived from bark and fruits of different plants and trees are now a day's replacing insecticides for killing larvae and adult mosquitoes [17]. Approximately 1200 species of different plants has been reported for insect control, most of plant extracts showed chronic effects for insects [18], and larvae of medically important mosquitoes including Culex showed greater or less susceptibility towards the botanical insecticides. The research has shown that the larvae of *Cx. pipiens pallens* have shown susceptibility to *Piper nigrum* with least LC_{50} values [19] and leaf litter of various plants [20]. The effect of the botanicals on the growth inhibition of the mosquito is administered by the plant species, plant parts and the method of extraction and more than one thousand plants contain certain chemical which act as insect growth regulators [21] e.g., the metabolites of *Ajuga remota* against mosquitoes.

The objective of the current study was to evaluate six different entomopathogenic fungi and botanicals individually (for calculating sub lethal doses) and in mixtures for their effects on *C. pipiens* mortality and on other biological parameters under laboratory and under field conditions.

2. Materials and Methods

2.1 Collection and rearing of mosquito

Larvae of different instars of *C. pipiens* were collected from Multan, Punjab, Pakistan which were transported in plastic jars containing water to the Laboratory of Insect Microbiology and Biotechnology, Bahauddin Zakariya University, Multan and later on identified on the basis of pictorial keys. Fish food was used as larval diet, and on reaching 80 percent pupation, jars were shifted in plastic cages with dimension (1.5×0.5 ft). The adult mosquitoes on emergence were shifted to another cage disinfected with ethanol and reared up to F_{11}. Adult males were feed on 10% sugar solution while females were provided blood meal by feeding on white albumen mice for egg laying. The rearing conditions were maintained at 25±1 °C, 75±2% relative humidity (RH) and 10L-14D hr photoperiod.

2.2 Formulation of entomopathogenic fungi

Isolates of entomopathogenic fungi were obtained from laboratory pre-culture. Rice based media was used for the inoculation of different isolates of *Beauveria bassiana* (isolates, Bb-01, Bb-10), *Metarhizium anisopliae* var. *anisopliae* (isolates, Ma-11.1, Ma-2.4) and *Isaria fumosorosea* (isolates, If-2.3, If-02) (Table 1). Flasks (500 mL) containing 100 gm of water soaked rice were inoculated with different isolates of insect pathogenic fungi and kept at 25 °C in darkness at 70-75% RH for 14 days. Subsequently spores were harvested in 0.05% Tween solution. The concentrations of stock suspensions were estimated by hemocytometer and the desired concentrations (1×10^7, 1×10^8, 2×10^8, 3×10^8 and 4×10^8 spores/ml) of each isolate were prepared by serial dilution from the stock suspension [22].

2.3 Botanical extracts preparation

Leaves and new shoots of *Azadirachta indica*, *Syzygium cumini*, *Acacia nilotica*, *Capsicum annum*, *Coriandrum sativum* and *Mentha longifolia* were taken and shade dried for 15 days and later on crushed to fine powder. For preparation of liquid form solid (powder), weight/volume method was used. Serial dilution was done for the preparation of required concentrations.

2.4 Preparation of binary mixtures of fungi and botanicals

Preliminary experimentation was done in order to calculate different concentrations (LC_{10}, LC_{20}, LC_{30}, LC_{40} and LC_{50}) of fungi and botanicals individually. Later on, for binary mixtures applications following sequence was followed i.e., fungus LC_{10} + botanical LC_{10}, fungus LC_{20} + botanical LC_{20}, fungus LC_{30} + botanical LC_{30}, and fungus LC_{40} + botanical LC_{40}.

2.5 Bioassay

The experiment was conducted under the Completely Randomized Design (CRD) with four replications in each treatment for both field and laboratory experiments. 250 ml serially diluted solution (botanicals and fungi) was poured into small transparent plastic trays (capacity of 450 ml) and 15 larvae of same age belonging to 3rd instar were released in each tray with sufficient fish food. In case of laboratory studies experimental trays were labeled and placed under laboratory conditions. While for the field studies all experimental trays were placed in shady place (humidity and temperature varied with day timings). Mortality data was taken for seven consecutive days for fungi [23], five for botanicals and seven days for binary mixtures of sub lethal doses (fungi and botanicals). Data regarding percent pupation, pupal duration, percent emergence and sex ratio were recorded till the end of experiment [24].

2.6 Data analysis

Mortality data was corrected where necessary with the help of Abbott's formula [25]. POLO-PC software [26] was used for determining lethal and sub lethal doses of fungi and botanicals. The means regarding percent pupation, pupal duration, percent emergence and sex ratio were analyzed by using analytical software (Statistix version 8.1) and compared by LSD test at 0.05 probability levels.

3. Results

Pre-experimentation was done for estimating sub-lethal doses of all isolates of insect pathogenic fungi and botanicals for laboratory and field populations (Table 2). On basis of least LC_{50} values three fungi were selected for further use in binary combination with botanicals.

Table 1: The isolates of entomopathogenic fungi isolated from different soils

S. No	Fungi	Name of isolate	Source
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

Table 2: Calculated doses of fungi (spores/ml) and botanicals (ppm) for binary treatment on *C. pipiens* (laboratory and field trail)

Isolates	Laboratory population					Field population				
	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

Botanicals										
Azadirachta indica	81.21	78.67	69.78	63.44	58.97	98.76	92.33	87.52	76.91	71.22
Capsicum annum	79.11	75.23	69.99	65.43	62.11	86.52	79.98	72.97	67.55	61.98
Acacia nilotica	169.09	153.42	149.80	141.23	138.22	178.97	165.44	151.90	143.22	138.91
Mentha longifolia	301.11	298.71	276.43	267.89	261.32	320.11	308.23	296.53	289.72	275.65
Coriandrum sativum	489.76	483.43	476.52	461.23	441.97	501.31	492.18	481.21	473.67	468.51
Syzygium cumini	398.77	386.41	379.49	373.22	364.12	411.98	401.31	392.31	383.12	378.31

3.1 Percent larval mortality of *C. pipiens* after application of binary mixtures of fungi and botanicals

The binary mixtures application of insect pathogenic fungi and botanicals on the laboratory population of *C. pipiens* showed concentration dependent response and highest percent mortality (68.3 ± 8.3) was recorded in treatment with Bb-01 (LC_{40}) +*A. indica* (LC_{40}) as compared to other treatments (F=91.0, df=6, P=0.0007) (Figure 1). Similar trend was observed in case of field trail of *C. pipiens* in which binary treatment of Bb-01 (LC_{40}) +*A. indica* (LC_{40}) caused percent larval mortality of 65.5 ± 7.3 (F=63.0, df=6, P=0.0021) (Figure 2).

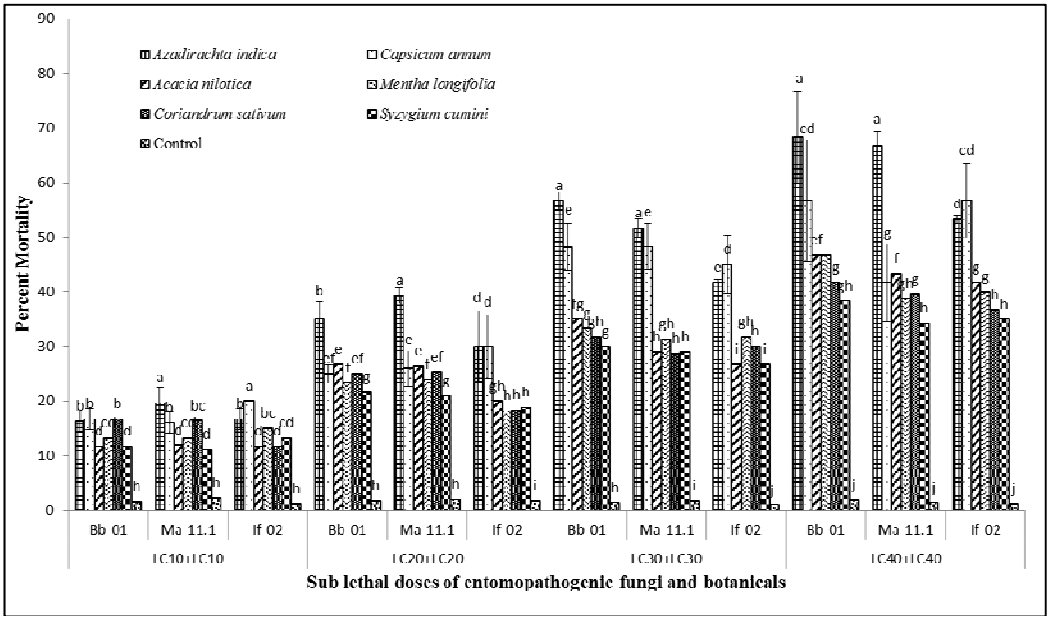

Fig 1: Percent larval mortality of *C. pipiens* (laboratory trail) after application of binary mixtures of fungi and botanicals. Means with different letters in each day are statistically different among treatments and control at $P<0.05$.

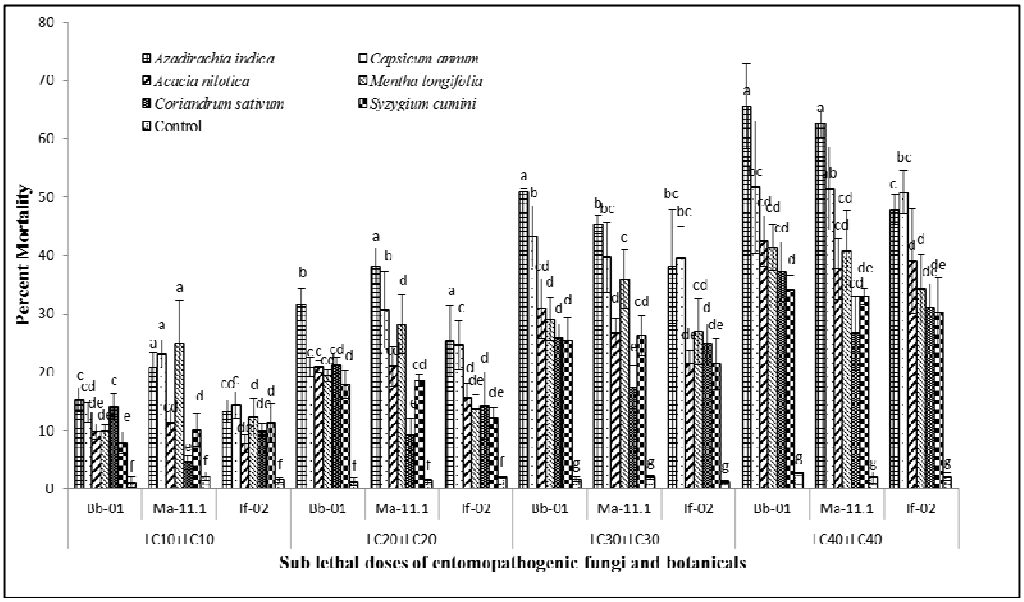

Fig 2: Percent larval mortality of *C. pipiens* (Field trail) after application of binary mixtures of fungi and botanicals. Means with different letters in each day are statistically different among treatments and control at $P<0.05$.

3.2 Percent pupation of *C. pipiens* as a result of binary mixtures application of fungi and botanicals

Data regarding percent pupation of laboratory population of *C. pipiens* after application of binary mixtures of fungi and botanicals showed significant different responses for all treatments. Lowest percent pupation (37.7 ± 0.5) was observed in case of Bb-01(LC_{40}) + *A. indica* (LC_{40}) followed by 33.3±0.9 in case of Ma-11.1 (LC_{40}) and *A. indica* (LC_{40}) (F=126.0, df=6, P=0.0001)(Table 3). Similar trend was recorded in case of the field experiment, in which binary combination of LC_{40} of Bb-01 and LC_{40} of *A. indica* showed least percent pupation (37.3±0.6), followed by (47.6±0.7) in combination of LC_{40} of Ma-11.1 with LC_{40} of *A. indica* (F=109.0,df=6,P=0.004)(Table 4).

Table 3: Percent pupation of *C. pipiens* (laboratory trail) as a result of binary treatment of fungi and botanicals

	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**
Azadirachta indica	83.3±1.7bc	73.3±0.7cd	83.3±0.3bc	65.0±0.3de	56.7±0.3e	70.0±0.3d	43.3±0.5f	48.3±0.2f	58.3±0.4e	31.7±0.5de	33.3±0.9de	46.7±0.4c
Capsicum annum	81.3±0.2c	70.0±0.5d	80.0±0.8c	75.0±0.3cd	61.7±0.5e	70.0±0.9d	68.3±0.8d	51.7±0.4ef	55.0±0.3e	58.3±0.4b	38.3±0.3d	43.3±0.8c
Acacia nilotica	84.8±0.8bc	85.0±0.5b	83.3±0.4bc	73.3±0.3cd	76.7±0.8cd	80.0±0.4bc	65.0±0.6d	66.7±0.4d	73.3±0.3cd	53.0±0.4bc	56.7±0.5b	58.3±0.8b
Mentha longifolia	86.7±0.8b	71.7±0.5b	85.0±0.3bc	76.7±0.7d	66.7±0.8e	81.0±0.6bc	66.7±0.9d	60.0±0.5de	68.3±0.9d	53.3±0.5bc	51.7±0.3bc	60.0±0.5cd
Coriandrum sativum	83.3±0.4bc	95.0±0.3a	83.3±0.5bc	75.0±0.5cd	88.3±0.4b	81.7±0.1bc	68.3±0.9d	80.0±0.4bc	70.0±0.9cd	58.3±0.4b	68.3±0.5ab	63.3±0.6ab
Syzygium cumini	88.3±0.3b	86.3±0.4bc	86.7±0.6b	78.3±0.7c	78.3±0.9c	81.7±0.1bc	70.0±0.3cd	73.7±0.9cd	73.3±0.3bc	61.7±0.4b	58.3±0.3b	65.0±0.3ab
Control	97.7±0.5a	98.7±0.1a	98.0±0.3a	98.3±0.9a	98.7±0.9a	98.3±0.5a	99.1±0.6a	99.1±0.3a	98.6±0.7a	99.0±0.3a	9876±0.4a	98.0±0.4a
F-value		151			148			139			126	
P-values		0.0003			0.0006			0.0009			0.0001	
LSD-value		8.65			9.31			10.02			11.76	

Means followed by same letters in row and columns are non-significantly different (LSD=0.05)

Table 4: Percent pupation of *C. pipiens* (field trail) as a result of binary treatment of fungi and botanicals

	LC_{10}+LC_{10}			LC_{20}+LC_{20}			LC_{30}+LC_{30}			1. 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**
Azadirachta indica	82.7±0.3c	77.0±0.7c	85.6±0.7b	67.0±0.9cd	67.9±0.8cd	78.6±0.6bc	48.9±0.6g	58.9±0.4e	64.4±0.7e	37.3±0.6de	47.6±0.7d	53.5±0.6cd
Capsicum annum	84.0±0.8c	74.6±0.3cd	82.3±0.8b	79.7±0.3c	63.8±0.8cd	75.4±0.4bc	72.4±0.3cd	59.9±0.3e	62.4±0.7e	64.8±0.4c	49.6±0.4d	53.4±0.4cd
Acacia nilotica	89.7±0.8ab	90.1±0.7ab	85.3±0.6b	74.9±0.3bc	68.9±0.6cd	82.5±0.2b	67.5±0.9cd	62.3±0.7de	78.7±0.3bc	57.4±06cd	53.7±0.8c	75.6±0.7ab
Mentha longifolia	89.3±0.3ab	79.0±0.4cd	87.5±0.4b	78.6±0.1bc	73.6±0.7cd	83.2±0.5b	68.9±0.3cd	65.7±0.7de	73.6±0.4cd	55.3±0.3cd	59.8±0.3cd	69.9±0.3bc
Coriandrum sativum	87.7±0.1b	98.5±0.3a	87.5±0.5b	77.8±0.3bc	88.9±0.3b	83.9±0.3b	70.0±0.5d	80.0±0.4c	77.0±0.3c	62.0±0.4c	69.3±0.8bc	69.8±0.3bc
Syzygium cumini	92.5±0.9a	91.7±0.3ab	93.4±0.8ab	81.6±0.3b	87.7±0.4b	84.5±0.6b	74.6±0.4c	78.5±0.3bc	79.6±0.8c	67.2±0.9bc	70.0±0.5b	72.8±0.5b
Control	98.9±0.4a	97.9±0.5a	99.0±0.3a	99.4±0.3a	99.8±0.1a	98.7±0.5a	97.7±0.8a	99.0±0.3a	98.6±0.3a	99.0±0.1a	98.7±0.4a	99.3±0.3a
F-value		121			115			112			109	
P-values		0.0071			0.0031			0.008			0.004	
LSD-value		11.23			10.12			9.87			8.71	

Means followed by same letters in row and columns are non-significantly different (LSD=0.05)

3.3 Pupal duration of *C. pipiens* as a result of binary mixture application of fungi and botanicals

The results showed that the pupal duration in laboratory population differed significantly for all treatments of binary mixtures of fungi and botanicals. Longest pupal duration (10.0±0.6) days was observed with the combination of Bb-01 (LC_{40}) and *A. indica* (LC_{40}) followed by (9.9±0.6) after application of Ma-11.1 (LC_{40}) +*A. indica* (LC_{40}) (F=65.0, df =6, P=0.003) (Table 5). In case of the field experiment, parallel results were observed, where pupal duration showed concentration dependent response and Bb-01(LC_{40}) + (LC_{40}) *A. indica* showed longest pupal duration(10.91±0.81) days (F=66.0, df=6, P=0.0002) (Table 6) as compared to the control.

Table 5: Pupal duration of *C. pipiens* (laboratory trail) as a result of mixture of fungi and botanicals

	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**
Azadirachta indica	4.4±0.7ab	5.3±0.8a	4.6±0.3bc	6.7±0.6a	7.0±0.3a	6.8±0.4a	8.0±0.3	7.3±0.4	7.4±0.7	10.0±0.6a	9.9±0.6a	9.5±0.4a
Capsicum annum	4.7±0.8ab	5.1±0.4a	4.8±0.4b	6.7±0.8a	6.8±0.4a	6.5±0.5a	7.8±0.5	7.2±0.7	7.3±0.4	9.9±0.7a	9.7±0.8a	9.3±0.6a
Acacia nilotica	4.0±0.6bc	4.3±0.5ab	3.7±0.6b	6.6±0.6a	6.0±0.3ab	6.4±0.4a	7.8±0.6	7.1±0.7	6.5±0.7	9.7±0.4a	9.4±0.3ab	9.0±0.6ab
Mentha longifolia	4.0±0.6b	4.7±0.6ab	4.4±0.2ab	6.0±0.4ab	6.0±0.8ab	6.0±0.2ab	7.6±0.6	7.0±0.6	7.2±0.5	9.6±0.8a	9.2±0.5ab	8.8±0.3ab
Coriandrum sativum	4.4±0.7ab	5.0±0.4a	3.5±0.3bc	6.5±0.7a	6.6±0.3a	5.4±0.6ab	6.8±0.5	6.9±0.6	6.9±0.4	8.8±0.7ab	9.0±0.4ab	8.5±0.5ab
Syzygium cumini	3.5±0.8bc	4.4±0.6b	4.0±0.5b	5.3±0.6b	5.3±0.4ab	6.0±0.7ab	6.7±0.6	6.7±0.5	6.2±0.5	8.4±0.6ab	7.7±0.9b	8.3±0.5ab
Control	2.3±0.1d	3.3±0.4c	2.5±0.5cd	2.3±.6de	3.0±0.6d	2.9±0.9d	3.0±0.9	2.6±0.8	3.1±0.8	3.2±0.7cd	3.6±0.6cd	3.9±0.1cd
F-value	39			41			53			65		
P-values	0.0001			0.0003			ns			0.003		
LSD-value	1.00			1.32						2.42		

Means followed by same letters in row and columns are non-significantly different (LSD=0.05)

Table 6: Pupal duration of *C. pipiens* (field trail) as a result of mixture of fungi and botanicals

	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**
Azadirachta indica	4.2±0.7a	4.0±0.7a	3.8±0.5ab	6.5±0.7a	6.4±0.6a	6.3±0.3a	7.9±0.3a	7.6±0.4a	7.5±0.3a	10.9±0.8a	10.0±0.3a	9.8±0.3a
Capsicum annum	4.6±0.8a	4.2±0.6a	4.0±0.5a	6.5±0.1a	6.3±0.3a	6.0±0.1a	7.6±0.4a	7.4±0.7a	7.2±0.2a	9.8±0.3a	9.1±0.4a	9.1±0.6a
Acacia nilotica	3.8±0.2ab	3.7±0.2ab	3.3±0.6b	6.1±0.7a	6.0±0.2b	5.4±0.2ab	7.0±0.6a	6.8±0.3a	6.7±0.3a	8.9±0.4ab	9.0±0.3a	8.8±0.6ab

Mentha longifolia	4.0±0.2a	3.5±0.1a	3.1±0.8b	5.8±0.4ab	5.5±0.6ab	5.2±0.3a	7.0±0.3a	6.5±0.9ab	6.3±0.2a	8.6±0.2ab	8.5±0.2ab	8.3±0.8ab
Coriandrum sativum	4.0±0.2a	3.7±0.2ab	3.6±0.2a	5.3±0.2ab	5.0±0.4ab	4.8±0.9ab	6.7±0.1ab	6.4±0.9ab	6.4±0.3a	8.5±0.6ab	8.3±0.3ab	8.0±0.5b
Syzygium cumini	3.1±0.2bc	3.7±0.7ab	3.8±0.1ab	5.0±0.1ab	4.8±0.3ab	4.5±0.3ab	6.5±0.3ab	6.3±0.6ab	6.0±0.7a	8.0±0.3b	7.4±0.8bc	7.4±0.6b
Control	3.2±0.1b	3.0±0.6bc	3.1±0.8b	3.9±0.1bc	3.0±0.3cd	3.1±0.6bc	3.7±0.1bc	3.0±0.8b	3.4±0.3bc	2.9±0.3de	3.0±0.7d	3.1±0.6cd
F-value		51			56			59.11			66	
P-values		0.0004			0.0006			0.0008			0.0002	
LSD-value		1.17			2.41			2.59			2.63	

Means followed by same letters in row and columns are non-significantly different (LSD=0.05)

3.4 Percent emergence of *C. pipiens* as a result of binary mixtures application of fungi and botanicals

Percent emergence in the laboratory and field population after the treatment of binary mixtures fungi + botanicals is shown in Figure 3 and 4, respectively. Binary treatment comprising of Bb-01 (LC_{40}) + *A. indica* (LC_{40}) showed least percent emergence (19.2±0.3) (F=83.0, df=6, P=0.0001) (Figure 3). Whereas in case for the field trial, least percent emergence (27.67±0.18) was observed in case of combined application of If-02 and *A. indica* (LC_{40}) (F=61.0,df=6, P=0.0001)(Figure 4). Conversely the data regarding sex ratio after binary mixtures application of fungi and botanicals was non-significantly different on all treatment levels.

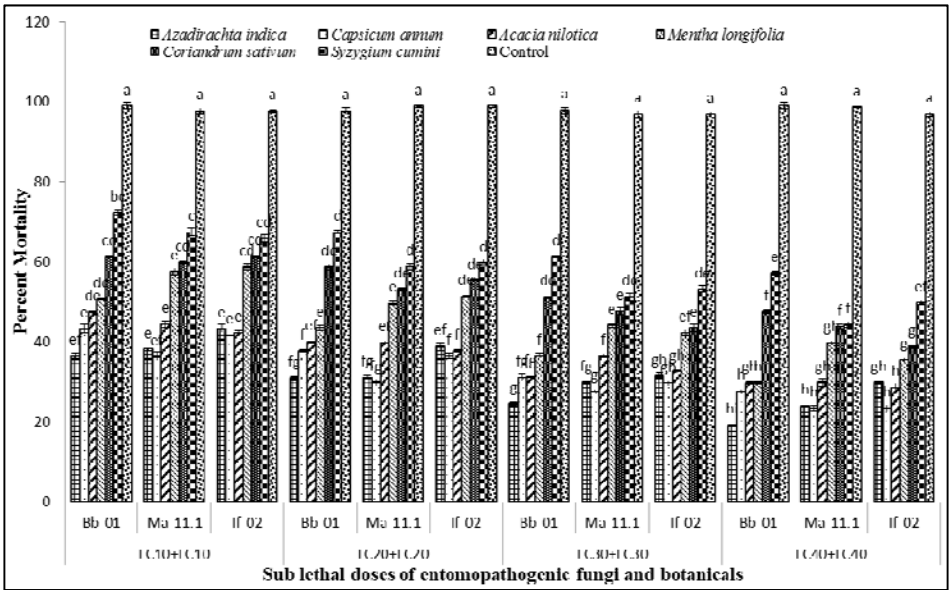

Fig 3: Percent emergence of *C. pipiens* (laboratory trail) after application of binary mixtures of fungi and botanicals. Means with different letters in each day are statistically different among treatments and control at *P*<0.05

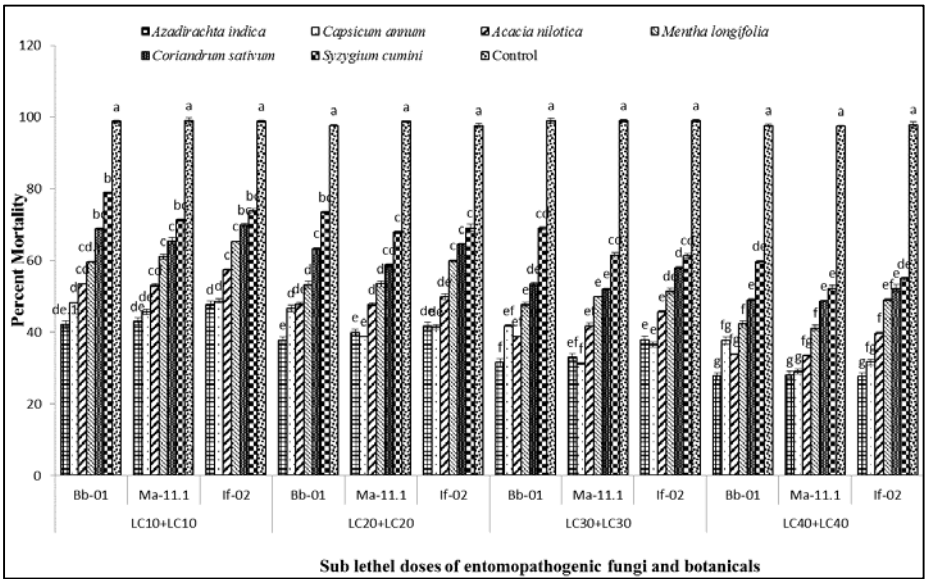

Fig 4: Percent emergence of *C. pipiens* (Field trial) after application of binary mixtures of fungi and botanicals. Means with different letters in each day are statistically different among treatments and control at *P*<0.05.

4. Discussion

Bio control agents like entomopathogenic fungi are distributed throughout the world [27] having potential for management of mosquitoes [28]. Different entomopathogenic fungi i.e., *M. anisopliae* and *I. fumosorosea* have been used in the past for insect pest management [29, 30, 31, 32]. Plant extracts are another eco-friendly approach used for management of mosquitoes [33]. Large number of plants extracts like *Allium sativum* [34], *Curcuma aromatic* [35], *A. indica* [36] *Eucalyptus oblique*, *Citronella*, *M. piperita*, *Asteraceae*, *Carvacryl* [37] have been used against different species of mosquito which revealed efficient control. For refining the efficiency of entomopathogenic fungi sub-lethal doses of botanicals can be added as synergists [38]. In the present study binary mixtures of entomopathogenic fungi and botanicals were applied for the control of *C. pipiens*, which showed significant larvicidal action. The *C. pipiens* mortality (68.33%) in laboratory and (65.52%) field trail in the current study lies in accordance with Roberts [39], Raveen *et al.* [40], Ghosh *et al.* [41], Kovendan and Murugan [42], Liu *et al.* [43] and Wright *et al.* [44] who reported *C. pipiens* susceptibility to entomopathogenic fungi and different plant extracts leading to rapid mortality (50.00%) after the combined treatment of fungi and botanicals. Similar trend was observed when entomopathogenic fungi and botanicals were used against mosquito in the paddy field, which showed rapid mortality and reduction in late instar of larvae and pupae [45]. The present study is in accordance to the previous research which showed the synergistic action of temephos and *Aspergillus flavus* against *Anopheles stephensi* [46] and combined activity *A. flavus* and *Cuscuta reflexa* extract against *An. stephensi* and *Cx. Quinquefasciatus* [47].

The application of binary mixtures of fungi and botanicals significantly affected the percent pupation of *C. pipiens*. The reduction in percent pupation at higher concentrations of binary mixtures confirms the findings of Schmutterer [47] in which binary mixtures significantly affected different life parameters i.e., growth retardation, reproductive inhibition and longevity of *C. pipiens* progeny. The combined treatments of fungi and botanicals considerably prolonged the pupal duration of *C. pipiens* which lies in accordance with Malarvannan [48] who reported enhanced pupal duration of *Spodoptera litura,* Fabricius (Lepidoptera: Noctuidae) after treatment with entomopathogenic fungi. The data regarding the sex ratio showed non-significant results after the application of binary mixtures of fungi and botanicals, which as well corroborates the findings of Shaalan *et al.* [49] who reported non-significant sex ratio in *A. aegypti* after the treatment of mixtures containing insecticides and *Callitris glaucophylla.*

Combination of entomopathogenic fungi and botanicals not only enhanced the mortality, it also affected the progeny of *C. pipiens* by altering the percent pupation, pupal duration and percent emergence. The present study reports the enhanced effectiveness of entomopathogenic fungi and botanicals for the better management of mosquitoes under field conditions and their incorporation in the integrated management program of mosquitoes.

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