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Efficacy of entomopathogenic fungi and botanicals on development of *Musca domestica*

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Abstract
The house fly, *Musca domestica* L. (Diptera: Muscidae) is a cosmopolitan insect pest and an important vector of number of diseases. Entomopathogenic fungi i.e., *Beauveria bassiana Metarhizium anisopliae* and *Isaria fumosorosea* and botanicals i.e., *Azadirachta indica*, *Syzygium cumini*, *Acacia nilotica*, *Capsicum annum*, *Coriandrum sativum* and *Mentha piperita* individually and as binary mixture were evaluated against *M. domestica*. The results depicted that the response was concentration dependent. The highest percent mortality (92.5±4.8) was observed in the treatment with LC_{40} of *B. bassiana* and LC_{40} of *A. nilotica* followed by (85.0±6.5) in treatment with LC_{40} of Ma-4.1 and LC_{40} of *A. indica*, respectively. Synergistic action of entomopathogenic fungi with botanicals was determined in the present study which could be exploited for integrated pest management (IPM) programs. The developed "sugar bait" technique is simple and promising enough to encourage further investigations.

Keywords: *Musca domestica*, entomopathogenic fungi, botanicals, binary mixture, biological parameters, progeny

1. Introduction

Musca domestica L. (Diptera: Muscidae) is a general insect-pest of livestock and community health which has afflicted human beings during the ancient times [1, 2]. In the medicinal point of view, *M. domestica* is a medically important arthropod vector for carrying various pathogens that cause avian influenza, cholera, hematic carbuncle, bovine mastitis, typhus fever, dysentery, conjunctivitis and poliomyelitis, that may artifice infrequent pressures to humans and as well as poultry production [3-5]. Poultry farm houses provide best habitat for production of *M. domestica* due to high temperature and humidity. Presence of *M. domestica* raises issues of food-borne disease causing organisms particularly *Escherichia coli*, *Salmonella* spp. and *Shigella* spp. [6, 7]. Continuous use of synthetic insecticides like Diethyl-3-methylbenzamide (DEET) had shown resistance development in many insect pests [8]. In the past synthetic chemicals like organophosphates, carbamates, pyrethroids and new chemical groups provide control on *M. domestica* but the injudicious used of these insecticides has caused the development of resistance in this pest. Diseases like erythema in human beings was also seen in effect these synthetic pesticides [9]. Extensive and broad use of these chemicals has developed resistance in *M. domestica* [10, 11].

Integrated pest management (IPM) plays a vital role in control of *M. domestica* in which traditional botanicals and or biological control agents like entomopathogenic fungi could be used [12]. Entomopathogenic fungi are cosmopolitan in nature and found all over the world [13] which provides promising control of different insect pest. The fungal pathogens, *Beauveria bassiana Metarhizium anisopliae* and *Isaria fumosorosea* are being used for the control of *Bemisia tabacci, Musca domestica, Dysdercus koenigii, Oxycarenus hyalinipennis* and aphid species without development of resistance [14-19]. Use of fungal pathogens for the control of housefly could have a lot of potential due to their low mammalian toxicity and natural occurrence among flie's population [20]. Plant extracts of approximately different plants 1200 species has been reported for insect control [21, 22]. One of the greatest economical methods for the mosquito control lies in personal protection, public awareness, and proper use of larvicides and eradication of larval breeding Natural plant extracts are also providing control for *M. domestica*. Different plant extracts have great potential for repelling or controlling of *M. domestica* [23]. A number of studies have shown the probability to control eggs, larvae, pupae and adults of *M. domestica* via plant compounds [24]. For enhancing effect of botanicals, fungi could be added, to synergise the effect by applying the mixture containing botanicals with entomopathogenic fungi. Rapid larval mortality was recorded followed by low percent pupation, longevity in pupal duration and low percent emergence [25]. Keeping in view the importance of housefly as medical and veterinary pest, insecticide resistance, the current study was planned to check the effectiveness of the entomopathogenic fungi and botanicals against housefly and to evaluate the effects of binary mixture of sub-lethal doses of fungi and botanicals on biological parameters of housefly.

2. Materials and Methods

2.1 Insect Rearing

The *M. domestica* collected from poultry farms were reared in cages (30× 30×30cm^3) under laboratory conditions. Adults were feed on sugar solution, while for egg laying and larval diet containing wheat bran, rice meal, sugar yeast and milk powder (40:10:10:3:1) was provided. Rearing conditions were maintained at 28±2 °C, 60-70% relative humidity and photoperiod of 14L: 10D hrs.

2.2 Fungal propagation and concentration

Isolates of *B. bassiana* (Bb01), *M. anisopliae* (Ma4.1) and *I. fumosorosea* (If03) were acquired from Laboratory of Insect Microbiology, Bahauddin Zakariya University, Multan for testing against *M. domestica*. Slants of mono conidial cultures of the isolate already grown on PDA at 25 °C in darkness and then stored at 4 °C were used. For further propagation the spores were spread on to the PDA plates (9 cm diameter) and kept at 25 °C in darkness at 70-75% RH for 14 days. Number of spores were counted with help of haemocytometer, and the desired concentrations (3×10^8, 2×10^8, $1\times10^8$and 1×10^7 spores/ml) were made by serial dilution.

2.3 Botanical extracts preparations and concentration formation.

Botanicals i.e., *Azadirachta indica*, *Syzygium cumini*, *Acacia nilotica*, *Capsicum annum*, *Coriandrum sativum* and *Mentha piperita* leaves and young shoots were taken and sun dried for 15 days. Crushing was done for producing fine powder. For preparation of liquid (w/v) method was used, while serial dilution was done to make the desired concentrations (2000, 1000, 500, 250 and 125ppm).

2.4 Work layout

All above fungal and botanicals concentrations were applied on *M. domestica* separately for determining sub lethal doses. Fungi were mixed with botanicals in the following manner i.e., LC_{20} of fungi + LC_{20} of botanical, LC_{30} of fungi + LC_{30} of botanical and LC_{40} of fungi + LC_{40}. Moreover fungal and botanical mixtures were evaluated against mortality, longevity of both sexes and fecundity of females respectively.

2.5 Bioassay

Preliminary experimentation was done for the measurement of sub lethal doses of selected fungi and botanicals. Later the sub lethal doses were mixed in combination as described above. The experiment was conducted under the Completely Randomized Design (CRD) with four replications in each treatment. Transparent plastic boxes with size of 15×6×6 cm^3 were used for experiment and 40 insects were used per treatment. Cotton swab soaked in water was placed as water source, mixture of fungi and botanicals were applied as bait in sugar crystals.

2.6 Data collection and analysis

Mortality data of mixtures was taken for 7 days, while the longevity of both sexes of *M. domestica* was recorded separately as reported previously by Fletcher [26]. In addition, female fecundity was also noted by dividing total number of eggs laid to total number of females over entire duration of the experiment. The sub-lethal doses of fungi and botanicals were calculated by submitting POLO-PC software (LeOra Software). The means were analyzed by using statistical software (Statistix 8.1) and compared by using LSD test at 0.05 probability levels

3. Results

The preliminary experimentation was done in order to calculate the sub lethal doses of fungi and botanicals separately which are represented in Table 1, 2 respectively. Later on the mixtures of these sub lethal doses were evaluated against *M. domestica* for the effects on different biological parameters.

3.1 Mortality of *M. domestica* as a result of fungi and botanical mixtures

The mortality percentage after the application of mixtures of fungi and botanicals are presented in Table 3. The combination of fungi with botanicals showed impacts on the mortality of *M. domestica*. The response was concentration dependent, higher mortality was recorded at higher sub-lethal doses of fungi and botanical mixtures. The highest percent mortality (92.5±4.8)was observed in the treatment with LC_{40} of Bb-01 and LC_{40} of *A. nilotica* followed by 85.0±6.5 in treatment with LC_{40} of Ma-4.1 and LC_{40} of *A. indica* (F=36.32, P=0.007,df=6). In addition, significant difference was observed in the mortality of *M. domestica* due to mixture of fungi and botanicals except on lower level of sub lethal doses (LC_{10} of fungi and LC_{10} of botanicals).

3.2 Sub-lethal effects of fungi and botanicals mixtures on the longevity of *M. domestica* adults

The results regarding sub-lethal effects of fungi and botanical mixtures on the longevity of male and female *M. domestica* are presented in Table 4, respectively. Overall, the dose dependent response was observed for the longevity of adults. Higher the doses, the less number of days *M. domestcia* survived irrespective of the sex. For the male longevity, a significant difference was observed for all treatments at each level of sub-lethal doses mixtures. The males survival was lowest 8.7±0.2 days when the LC_{40} of Bb-01 was combined with the LC_{40} of *A. indica* followed by 9.7±0.5 days treated with combination of Bb-01 (LC_{40}) and *M. piperita* (LC_{40}) (F=79.8, P=0.0001,df=6)(Table 4).

On the other hand, for female longevity, a significant difference was observed for all treatments at each level of sub-lethal doses mixtures as reported in male longevity. The females survival was lowest 10.7±0.3 days when the LC_{40} of Bb-01 was combined with the LC_{40} of *A. indica* followed by 10.8±0.5 days treated with combination of Bb-01(LC_{40}) and *C. annum* (LC_{40}) (F=17.65, P=0.0002,df=6)(Table 4).

3.3 Sub-lethal effects of fungi and botanicals mixtures on the fecundity and hatching percentage of *M. domestica*

A significant difference was observed for all the treatments at each level of sub-lethal doses mixtures for fecundity of *M. domestica*. The lowest number of eggs 189.0±3.2 were recorded, when the LC_{40} of Bb-01 was combined with the LC_{40} of *A. indica* followed by 198.0±4.7 eggs in treatment with combination of Ma-4.1 (LC_{40}) and *A. indica* (LC_{40}) (F=9.65, *P*<0.0001,df=6) (Table 5). Lowest percent hatching (63.1±0.10) was observed when If-03 (LC_{40}) and *A. indica* (LC_{40}) was applied (F=98.32, P=0.005,df=6), while maximum hatching was recorded in treatment with Bb-01 (LC_{10}) and *C. sativum* (LC_{10}) (F=93.64, P=0.003, df=6)(Table 5).

3.4 Effects of sub lethal doses of fungi and botanicals mixtures on larval duration and percent pupation of *M. domestica*

Rate of larval duration after the application of binary mixtures of fungi and botanical are presented in Table 6. The results depicted longest larval duration (9.3±1.3) after application of If03 (LC_{40}) + *A. nilotica* (LC_{40}) followed by 9.0±1.2 when binary mixtures of Bb01 (LC_{40}) + *A. nilotica* (LC_{40}) was applied (F=99.86, P=0.001, df=6) (Table 6). Rate of percent pupation was concentration dependent. Least percent pupation (60.2±1.9) was recorded after application binary mixture If03 (LC_{40}) + *A. indica* (LC_{40}) followed by (61.3±2.2) after treatment of Bb01 (LC_{40})+ *A. indica* (LC_{40}) (F=145,P=0.0089,df=6) (Table 6).

3.5 Effects of sub-lethal doses of fungi and botanicals on pupal weight and pupal duration of *M. domestica*

Lowest pupal weight (7.3±2.9) was recorded after application of If03 (LC_{40}) + *A. indica* (LC_{40}) followed by (7.6±2.3) post treatment of binary mixtures containing Ma4.1 (LC_{40}) + *A. indica* (LC_{40}) (F=109,P=0.0001,df=6). Pupal duration was significantly affected by the combined applications of fungi and botanicals. Longest pupal duration (7.5±1.9)(days) was recorded when If03 (LC_{40})+ *A. indica* (LC_{40}) were applied (F=132, P=0.0025.df=6) (Table 7).

3.6 Effects of sub-lethal doses of fungi and botanicals on percent emergence of *M. domestica*

The data regarding Percent emergence after application binary (fungus + botanical) is shown in Table 8. Lowest percent emergence (41.7±0.2) was recorded when If03 (LC_{40})+ *A. indica* (LC_{40}) was applied followed by (42.5±0.3) post treatment of Ma-4.1 (LC_{40}) + *A. indica* (LC_{40}) (F=132,P=0.008,df=6).

Data regarding sex ratio after treatment of binary mixtures of fungi and botanicals on *M. domestica* was non-significantly different at all levels of treatment.

Table 1: Sub-lethal (Spores/ml) doses of entomopathogenic fungi used against *M. domestica*

S. No.	Common Name	LC_{40}	LC_{30}	LC_{20}
1.	*B. bassiana* (Bb01)	1.02×10^{6}	9.25×10^{5}	7.34×10^{5}
2.	*M. anisopliae* (Ma4.1)	2.10×10^{7}	1.64×10^{7}	9.43×10^{6}
3.	*I. fumosorosea* (If03)	1.3×10^{8}	1.20×10^{8}	0.75×10^{8}

Table 2: Sub-lethal doses (ppm) of botanicals used for treatment of *M. domestica*

S. No.	Common Name	Scientific Name	LC_{40}	LC_{30}	LC_{20}
1.	Neem	*Azadirachta indica*	107.26128	75.61591	50.22534
2.	Jamun	*Syzygium cumini*	283.95463	162.59750	84.67143
3.	Kiker	*Acacia nilotica*	724.05555	289.57985	99.07739
4.	Red pepper	*Capsicum annum*	750.30416	500.67423	311.85301
5.	Mentha	*Mentha piperita*	1040.47209	688.29526	424.37833
6.	Dhania	*Coriandrum sativum*	2325.17806	1272.41554	628.35603

Table 3: Mortality of *M. domestica* after application of fungi and botanicals in mixture

	$LC_{20}+LC_{20}$			$LC_{30}+LC_{30}$			$LC_{40}+LC_{40}$		
	Bb01	**Ma4.1**	**If03**	**Bb01**	**Ma4.1**	**If03**	**Bb01**	**Ma4.1**	**If03**
A. indica	55.0±11.9ab	50.0±4.0bc	70.0±4.1a	67.5±4.8ab	70.0±5.1ab	80.0±4.1ab	92.5±4.8a	85.0±6.5 ab	80.0±6.1ab
S. cumini	30.0±4.0cd	32.5±2.50cd	40.0±4.0c	37.0±4.8d	42.5±4.7cd	45.0±2.8cd	52.5±2.5cd	57.5±6.3c	55.0±8.6cd
C. annum	52.5±8.5bc	32.5±4.70cd	50.0±5.7bc	70.0±6.5ab	45.0±6.4cd	62.5±4.8b	84.0±6.5ab	62.5±4.1bc	75.0±9.5ab
M. piperita	45.0±6.4bc	35.0±2.88d	35.0±2.8d	65.0±8.7ab	55.0±2.1b	50.0±7.1c	80.0±4.1ab	72.5±2.5ab	65.0±4.1bc
A. nilotica	50.0±6.0bc	37.5±4.7cd	37.5±2.5cd	70.0±7.1ab	50.0±0.2cd	52.5±6.3cd	82.5±6.5ab	70.0±2.1ab	72.5±1.1ab
C. sativum	37.5±4.7cd	32.5±4.7cd	27.5±2.8cd	42.5±4.8cd	45.0±6.5cd	37.5±6.2d	55.0±1.9cd	62.5±4.4bc	50.0±4.0cd
Control	3.0±1.1d	2.8±0.53d	2.3±1.8d	3.1±1.12d	4.11±0.3d	2.5±0.30d	3.2±1.1d	3.0±0.5d	2.11±0.3d
F-value	7.00			50.97			36.23		
P-value	0.02			0.02			0.007		
LSD-value	15.21			18.91			23.95		

Table 4: Effect of sub-lethal doses of fungi and botanicals on male and female longevity

	$LC_{20}+LC_{20}$			$LC_{30}+LC_{30}$			$LC_{40}+LC_{40}$		
Male longevity	**Bb01**	**Ma4.1**	**If03**	**Bb01**	**Ma4.1**	**If03**	**Bb01**	**Ma4.1**	**If03**
A. indica	14.6±1.2c	15.6±0.3bc	15.3±0.1bc	12.4±0.3d	13.4±0.3cd	14.2±0.8bc	8.7±0.2d	10.3±0.5d	10.5±0.5cd
S. cumini	15.3±1.2bc	15.5±0.4bc	15.2±0.9bc	13.5±0.4cd	13.5±0.4cd	14.4±0.7bc	10.3±0.3cd	11.3±1.2c	10.4±0.5cd
C. annum	16.2±0.2b	16.5±0.6b	15.9±1.2bc	13.4±0.6cd	13.5±0.4cd	12.4±0.5d	9.8±0.5d	10.5±1.3cd	10.7±0.3cd
M. piperita	15.7±1.1bc	16.2±0.5b	16.1±0.7b	12.5±0.3d	12.8±0.4d	13.4±0.5cd	9.7±0.5d	10.8±1.5cd	10.9±0.1cd
A. nilotica	16.3±0.6b	15.3±0.4bc	16.1±0.3b	13.6±0.4cd	13.7±0.1cd	14.6±0.6	12.4±0.7b	12.9±1.2b	11.8±0.1c
C. sativum	15.7±0.6bc	16.3±0.4b	16.0±0.5b	14.7±0.3bc	15.2±0.9b	14.9±0.4b	12.4±0.8b	11.6±0.9bc	11.2±0.5bc
Control	21.5±0.4a	19.7±0.5ab	20.1±0.3ab	20.1±0.4a	20.3±1.3a	20.2±0.4a	20.5±0.8ab	21.1±0.8a	19.8±1.1ab
F-value	67.3			35.7			79.8		
P-value	0.001			0.03			0.001		
LSD-value	2.37			1.56			1.43		
Female longevity									
A. indica	15.6±1.7d	16.6±0.2cd	16.3±0.1d	13.4±0.1d	14.4±0.2cd	15.2±0.6b	10.7±0.3d	12.3±0.5c	12.6±0.5c
S. cumini	16.4±1.1d	17.1±0.4b	17.2±0.2bc	13.6±0.3d	13.1±0.3d	14.3±0.1cd	11.4±0.6d	12.3±0.2cd	12.3±0.5cd

C. annum	16.1±0.2cd	17.4±0.2b	16.7±0.2d	13.2±0.6d	14.5±0.1cd	13.4±0.7d	10.8±0.5d	11.6±0.3d	11.7±0.6d
M. piperita	16.0±0.1cd	17.3±0.5bc	17.1±0.6bc	13.6±0.4d	14.8±0.2cd	14.3±0.5cd	12.4±0.7cd	12.4±0.5cd	12.7±0.5c
A. nilotica	17.3±0.4bc	16.3±0.2d	17.1±0.7bc	14.6±0.3cd	14.7±0.1cd	15.8±0.6b	12.7±0.5c	12.7±0.6c	12.5±0.7cd
C. sativum	16.6±0.3cd	17.2±0.3bc	17.0±0.4b	15.7±0.8b	15.6±0.4b	15.9±0.4b	13.4±0.6b	12.7±0.9c	12.7±0.6c
Control	22.5±0.2a	21.7±0.5a	22.1±0.3a	22.1±0.4a	21.3±1.1a	21.3±0.4a	21.6±0.6a	22.2±0.8a	21.8±0.8a
F-value	79.4			145.8			17.65		
P-value	0.002			0.0001			0.002		
LSD-value	2.73			2.87			1.3		

Table 5: Effects of sub-lethal doses of fungi and botanicals on fecundity and hatching percentage of *M. domestica*

Fecundity	**LC_{20}+LC_{20}**			**LC_{30}+LC_{30}**			**LC_{40}+LC_{40}**		
	Bb01	**Ma4.1**	**If03**	**Bb01**	**Ma4.1**	**If03**	**Bb01**	**Ma4.1**	**If03**
A. indica	289.0±3.4c	298.0±3.1c	304.0±6.4b	256.0±4.3cd	276.0±3.2b	267.0±3.4cd	189.0±3.2cd	198.0±4.7cd	211.0±5.3c
S. cumini	304.0±3.6b	305.0±3.5b	314.0±5.3ab	287.0±4.1ab	289.0±2.5bc	302.0±3.2ab	217.0±5.3bc	243.0±3.6cd	214.0±3.5c
C. annum	306.0±4.9ab	301.0±3.4bc	299.0±1.5bc	267.0±4.7cd	277.0±4.3b	271.0±3.6c	215.0±2.5bc	223.0±2.2bc	210.0±3.5c
M. piperita	301.0±3.1b	306.0±2.1ab	312.0±3.1ab	276.0±3.2b	281.0±4.7bc	271.0±4.2c	216.0±2.6bc	225.0±2.7bc	243.0±2.8b
A. nilotica	298.0±2.6bc	296.0±5.7c	299.0±3.6bc	276.0±3.2b	267.0±4.2c	278.0±3.2b	232.0±2.1b	245.0±2.6b	234.0±3.6b
C. sativum	312.0±3.4ab	309.0±1.6c	305.0±3.1ab	278.0±2.1b	265.0±4.2cd	276.0±3.5b	203.0±3.8c	214.0±1.2c	215.0±2.7c
Control	398.0±3.5a	379.0±2.5a	379.0±1.3a	403.0±2.0a	392.0±3.4a	406.0±3.6a	397.0±3.7a	393.0±1.4a	385.0±2.7a
F-value	6.43			5.76			9.65		
P-value	<0.0001			<0.0001			<0.0001		
LSD-value	3.45			3.21			5.7		
Hatching Percentage									
A. indica	78.17±0.11cd	77.38±0.12cd	76.18±0.18cd	70.76±0.85cd	69.80±0.16e	69.46±0.56e	64.68±0.12g	65.17±.1.9g	63.10±0.1g
S. cumini	85.27±0.61bc	84.61±2.1bc	84.20±3.32bc	81.33±3.11bc	80.61±2.13bc	80.10±1.51bc	78.19±4.08de	77.14±2.7de	77.13±3.3de
C. annum	87.73±1.99b	86.32±0.55bc	85.13±2.83bc	83.10±2.4bc	82.10±0.89bc	83.17±0.76bc	82.59±2.90cd	81.13±3.5d	82.88±0.83cd
M. piperita	86.37±0.50b	85.26±1.8bc	86.35±2.5bc	84.44±0.43b	83.73±0.65bc	84.20±2.7b	82.13±1.97d	82.14±2.3d	80.08±1.42d
A. nilotica	80.33±.16c	79.21±0.19cd	81.28±0.30c	77.43±0.19c	76.37±0.19c	76.16±0.25c	70.14±0.27f	68.31±0.30fg	69.50±0.14f
C. sativum	88.42±0.35b	87.22±0.45b	86.13±1.13bc	85.07±2.10b	85.18±2.55b	84.65±0.43b	83.67±0.28cd	82.87±0.90cd	82.18±1.5d
Control	97.51±0.15a	97.32±0.12a	96.88±0.31a	96.21±0.34a	96.38±0.47a	96.32±0.29a	96.21±0.39a	98.31±0.33a	97.30±0.19a
F-value	95.32			110.15			98.32		
P-value	0.002			0.003			0.005		
LSD-value	6.87			8.67			5.31		

Table 6: Effects of sub-lethal doses of fungi and botanicals on larval duration (days) and percent pupation of *M. domestica*

Larval Duration	**LC_{20}+LC_{20}**			**LC_{30}+LC_{30}**			**LC_{40}+LC_{40}**		
	Bb01	**Ma4.1**	**If03**	**Bb01**	**Ma4.1**	**If03**	**Bb01**	**Ma4.1**	**If03**
A. indica	5.9±0.8bc	6.1±1.2b	6.5±0.6bc	6.9±1.4b	7.2±0.8b	7.1±1.2b	7.6±1.7b	7.7±1.3ab	7.6±1.5ab
S. cumini	8.2±0.3a	8.2±1.0a	8.8±0.8a	8.5±1.3a	8.4±1.5ab	8.4±1.6ab	8.6±1.9a	8.6±1.0ab	8.9±1.7a
C. annum	6.1±1.5b	6.8±1.3b	6.7±1.1b	7.0±0.7b	7.1±1.0b	7.6±1.7b	7.2±1.2b	7.3±1.2ab	7.3±0.4ab
M. piperita	7.0±1.6ab	7.5±0.7b	7.1±0.2ab	7.7±1.5ab	7.0±1.7b	7.6±0.6b	7.8±0.8ab	7.3±1.4a	7.2±1.0ab
A. nilotica	8.7±1.3a	8.2±0.5a	8.9±1.9a	9.5±1.9a	9.3±1.9a	9.8±1.4a	9.0±1.2a	9.8±1.6a	9.3±1.3a
C. sativum	5.5±1.0bc	6.1±1.4bc	6.0±1.2bc	6.7±1.8bc	6.9±1.5bc	6.3±1.8bc	6.1±1.3b	6.6±0.8b	6.1±1.1b
Control	6.2±0.3bc	6.4±0.1bc	6.1±0.1bc	6.9±0.2bc	6.3±0.1bc	6.0±0.5bc	6.1±0.4b	6.2±0.5b	6.9±0.2b
F-value		96.67			101.72			99.86	
P-value		0.003			0.02			0.001	
LSD		1.92			2.08			2.59	
Percent Pupation									
A. indica	71.3±2.1c	68.2±1.8d	68.3±2.7d	62.2±2.5c	62.6±2.1e	63.1±1.2c	61.3±2.2c	62.7±2.1d	60.2±1.9d
S. cumini	79.6±1.3b	79.2±2.2b	79.8±3.9b	70.8±1.6b	71.9±1.1ab	71.2±2.8ab	68.7±2.8b	69.6±1.1b	69.1±1.7b
C. annum	76.8±2.0bc	75.9±2.1bc	75.6±2.3bc	66.5±2.0bc	66.4±2.1bc	65.9±1.9d	64.9±1.1bc	63.9±1.2bc	64.4±1.2bc
M. piperita	78.6±1.9b	79.3±2.1b	77.0±2.1b	69.9±0.9b	68.3±2.4b	67.1±2.5d	67.9±3.0c	66.2±2.1c	65.2±1.2cd
A. nilotica	82.6±1.1ab	81.8±2.ab	81.0±1.1ab	72.9±1.1ab	73.4±2.9ab	71.1±1.4ab	72.8±2.9ab	73.2±2.7ab	74.8±1.1ab
C. sativum	73.3±1.8c	74.9±1.4c	73.7±2.0c	64.3±2.8c	65.5±1.7d	65.9±1.6bc	62.3±2.5c	63.3±2.1bc	62.0±2.8c
Control	98.6±2.2a	98.4±1.1a	99.9±2.0a	99.1±1.1a	98.8±2.0a	99.9±2.3a	98.3±2.3a	99.4±2.4a	99.0.±1.7a
F-value	112			132			145		
P-value	0.003			0.042			0.0089		
LSD	9.78			10.28			13.85		

Table 7: Effects of sub-lethal doses of fungi and botanicals on pupal weight (mg) and pupal duration of *M. domestica*

Pupal Weight	**LC_{20}+LC_{20}**			**LC_{30}+LC_{30}**			**LC_{40}+LC_{40}**		
	Bb01	**Ma4.1**	**If03**	**Bb01**	**Ma4.1**	**If03**	**Bb01**	**Ma4.1**	**If03**
A. indica	11.8±1.9c	9.9±2.9c	10.5±3.6c	9.8±1.6c	8.9±1.5c	8.9±2.6c	8.6±1.7c	7.6±2.3c	7.3±2.9c
S. cumini	16.0±3.3ab	16.5±2.9ab	16.6±1.7b	15.8±2.1b	15.7±2.3b	15.7±1.9ab	14.6±1.6b	14.5±1.8b	14.6±2.5b
C. annum	14.2±2.1b	14.1±2.0b	13.8±1.7bc	13.2±1.6b	13.2±1.7b	12.9±2.0bc	10.6±1.3c	9.9±2.6c	10.1±2.2c
M. piperita	15.8±2.1b	15.1±2.1b	15.8±2.3b	14.9±1.2b	14.7±2.1b	14.8±1.7b	12.6±1.3bc	12.5±1.1bc	12.8±1.8bc
A. nilotica	17.0±2.2ab	16.9±1.3ab	16.8±1.5b	16.2±1.6b	15.8±2.6b	15.7±2.1b	15.2±2.2ab	15.1±1.7ab	15.2±1.7ab
C. sativum	12.1±2.1bc	11.9±2.1bc	10.1±1.9c	10.0±3.5c	9.8±1.9c	8.1±1.5c	7.8±1.1c	7.8±2.1c	8.8±2.6c
Control	19.7±0.9a	18.9±1.1a	19.5±2.0a	19.6±1.3a	19.8±1.1a	19.6±1.1a	18.3±1.9a	19.8±1.1a	18.5±0.3a
F-values		135			117			109	

P-value		0.043			0.004			0.0001	
LSD value		4.53			5.09			5.38	
A. indica	6.1±2.0a	6.1±1.7a	6.1±1.2a	6.7±1.9a	6.7±2.0a	6.7±2.0a	7.0±1.0a	7.3±1.6a	7.5±1.9a
S. cumini	4.8±2.2b	4.8±1.6b	4.9±1.9b	4.8±2.3b	4.8±1.8b	4.3±2.1b	4.7±2.5bc	4.7±2.9bc	4.7±2.3bc
C. annum	5.7±1.7a	5.8±1.9a	5.8±2.2a	5.8±19ab	6.1±1.1a	6.2±1.9a	6.4±1.7ab	6.3±2.1ab	6.4±1.2ab
M. piperita	5.1±1.6ab	5.2±1.5ab	5.1±2.0ab	5.2±1.1ab	5.3±2.2ab	5.3±2.8ab	5.4±1.9ab	5.4±2.5b	5.4±2.2b
A. nilotica	4.8±1.7b	4.8±2.3b	4.8±2.3b	4.7±2.1b	4.7±1.3b	4.7±2.1b	4.6±2.3bc	4.0±1.6a	4.7±1.9b
C. sativum	5.8±2.1a	5.8±1.6a	5.7±1.9a	6.0±1.5ab	6.0±2.3ab	6.0±1.5ab	6.2±1.1ab	6.1±2.1ab	6.2±2.1ab
Control	4.4±2.1bc	4.5±2.2b	4.5±2.3b	4.4±2.2b	4.5±2.0b	4.5±1.8b	4.5±2.2bc	4.5±2.8bc	4.5±2.0bc
F-value	156			122			132		
P-value	0.001			0.007			0.0025		
LSD	1.34			1.98			2.01		

Table 8: Effects of sub-lethal doses of fungi and botanicals on percent emergence of *M. domestica*

	LC_{20}+LC_{20}			LC_{30}+LC_{30}			LC_{40}+LC_{40}		
	Bb01	**Ma4.1**	**If03**	**Bb01**	**Ma4.1**	**If03**	**Bb01**	**Ma4.1**	**If03**
A. indica	57.6±0.9bc	55.7±0.1bc	53.6±0.2c	50.6±1.8bc	48.6±0.3c	46.5±0.1c	42.5±0.3cd	43.6±0.2c	41.7±0.2cd
S. cumini	65.4±0.8ab	66.9±0.8ab	64.7±0.3ab	60.5±0.5ab	58.6±0.1ab	57.3±0.7b	56.6±0.7b	55.4±0.1b	52.7±8bc
C. annum	62.5±0.8b	60.1±0.3b	58.4±0.8b	57.7±0.9b	55.5±0.5bc	54.5±0.1bc	52.4±0.3bc	50.6±0.4bc	49.8±0.8bc
M. piperita	63.6±0.9b	64.5±0.4ab	60.9±0.3b	59.0±0.8ab	60.9±0.4sb	57.8±0.4b	58.7±0.3b	56.5±0.8b	54.7±0.2b
A. nilotica	68.8±0.3ab	67.5±0.7ab	66.7±0.2ab	64.7±0.4ab	63.6±0.4ab	62.8±0.3ab	60.7±0.2ab	61.5±0.9ab	59.5±0.8b
C. sativum	60.5±0.6b	58.1±0.2b	57.8±0.5b	55.6±0.9bc	53.8±0.7bc	51.7±0.4bc	48.7±0.1bc	46.6±0.8c	45.3±0.9c
Control	99.9±0.1a	99.8±0.1a	100.0±0.9a	99.0±1.9a	100.0±0.9a	100.0±1.6a	100.0±1.8a	100.0±1.7a	98.8±0.2a
F-values	118			121				132	
P-values	0.0001			0.0087				0.008	
LSD values	8.29			9.12				10.81	

4. Discussion

The co-evolution of plants with insects has equipped them with a plethora of chemical defenses, which can be used against insects. Since botanicals are less likely to cause ecological damage, a large number of plants have been screened for their insecticidal activities against different insect pests and some of these have been found to be promising, specifically, on related Dipterans [27, 28]. Botanical products have become more prominent in assessing current and future pest control alternatives [29]. Over the past two decades, surveys of plant families [30-32] have discovered sources of new botanical insecticides, which could possibly meet some of the desired demands. Identification of novel effective muscacidal compounds is essential to combat increasing resistance rates, concern for the environment and food safety, the unacceptability of many organophosphates and organ chlorines and the high cost of synthetic pyrethroids. To be highly competitive and effective, the ideal phytochemicals should possess a combination of toxic effects and residual capacity. Acute toxicity is required at doses comparable to some commercial synthetic insecticides, while chronic or sub-chronic toxicity is required to produce growth inhibition, developmental toxicity and generational effects [33]. The effectiveness of an insecticidal treatment is influenced not only by the toxicity of the insecticide but also by the primary response of the insect to its mode of application. Repellent or attractant effects are the principal factors affecting insecticidal efficiency and many common insecticides exhibit one or both of these properties depending on concentration. Odour of most insecticides is repellent to certain insects at higher concentrations but act as attractants at lower concentrations [34]. The selected botanicals in the present study (Table 2), are easily obtainable locally. The toxicity of the tested plant extracts was evaluated sugar bait method. The insects fed on the sugar lure are mainly exposed to stomach poisoning action.

In current study, mortality caused by the different botanicals and fungus mixture to the adult of *M. domestica* might be due to the differential toxicity of the active ingredients. The varying results were probably due to the differences in levels of toxicity among the insecticidal ingredients of each plant and fungi. In addition to direct effect on adult mortality, binary treatment of fungus and botanicals significantly reduced the adult longevity as compared to the average survival time of adult. Due to the reduced life of the female, the number of eggs was also affected by the treatment. Similar results have been reported for other dipterans where pathogenic fungi resulted in the decreased survival and fecundity with the increasing dose [35- 37].

Reduction in the egg hatching percent by plant materials was similar to findings reported by many authors using different plant oils and extracts *Matricaria chamomilla* and *Clerodendron inerme* [38] *Melia azedarach* extract, extracts from leaves and flowers of *Datura innoxia* [39] and *Atriplex inflate* [40, 41] against *M. domestica*, in which the decrease in egg production accompanied with increasing sterility has been reported.

The results of the present investigation reveal the broad-spectrum toxic properties of the tested botanical extracts against the adult stage of *M. domestica*. The interesting result is the toxicant efficacy of the extracts against adults. Synergistic action with entomopathogenic fungi was determined in the present study which could be exploited for integrated pest management (IPM) programs. The developed "sugar bait" technique is simple and promising enough to encourage for further investigations.

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