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Insecticidal activity of toxic crude proteins secreted by entomopathogenic fungi against *Musca domestica* L. (Diptera: Muscidae)

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Abstract

The housefly, *Musca domestica* L. (Diptera: Muscidae), a major insect pest in poultry and dairy farms, has developed resistance against a variety of insecticides worldwide. In order to avoid resistance development and negative impacts of insecticides, the use of entomopathogenic fungi can be an effective option for the management of *M. domestica*. The present study was performed to derive toxic crude proteins from entomopathogenic fungi *Beauveria bassiana, Metarhizium anisopliae* var. *anisopliae* and *Isaria fumosorosea* and test their effect on survival of *M. domestica*. The crude proteins produced in Czapek medium by the six different isolates (two each) of the entomopathogenic fungi were tested against adults of *M. domestica*. A significant effect was observed on the survival of *M. domestica*, ranging from 52.0 to 91.0% mortality. Isolates Bb-01, Ma-4.1, and If-03 showed maximum percent mortalities. These three isolates also exhibited concentration and exposure time based response toward survival of *M. domestica*. The crude protein concentrations i.e., 8 and 10 mg/ml caused the maximum mortality (100.0%) of *M. domestica* population in 2.77 to 3.77 days. In addition, the lowest exposure of duration (96 hrs) of houseflies to *B. bassiana* (Bb-01) crude protein (10 mg/ml) caused 100.0% mortality of tested population as compared to other isolates. In conclusion, crude proteins of entomopathogenic fungi showed good potential for the eco-friendly management of *M. domestica*. However, further purification of the anti-insect proteins and their evaluation under field conditions is required.

Keywords: *Beauveria bassiana*, crude proteins, *Isaria fumosorosea, Metarhizium anisopliae* var. *anisopliae*, *Musca domestica*.

1. Introduction

House fly, *Musca domestica* L. (Diptera: Muscidae) is a notorious insect pest with ubiquitous relationship and mechanical vector of human and animal diseases (Alam & Zurek, 2004; Graczyk *et al*., 1999; Ahmad *et al*., 2007). The causal agents of several important diseases such as *Salmonella pullorum, S. typhimurium, Pasteurella multocida, Erysipelthrix rhusiopaththiae, Staphilococcus* sp., enteric bacteria, protozoan oocysts and several viruses are transmitted by house flies (Malik *et al*., 2007; Omalu *et al*., 2011). In addition to disease transmission and public health concerns, *M. domestica* activity in husbandry brings about lower levels of egg and milk production in livestock (Gullan & Cranston, 2005).

Although, insecticides are considered as the first line of defense against *M. domestica*, this option is very limited due to development of insecticide resistance in flies and deleterious effects of chemicals on the environment (Scott *et al*., 2000; Siriwattanarungsee *et al*., 2008). There are several non-chemical approaches including essential oils, botanicals, MdSGHV virus, bacteria, nematodes and fungi (Geden, 2012; Manzoor *et al*., 2016). The use of naturally occurring entomopathogenic fungi such as *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metschnikof) Sorokin and *Isaria fumosorosea* (Wize) Brown and Smith is one such non-chemical option for the management of flies (Lecouna *et al*., 2005; Sharififard *et al*., 2011; Farooq & Freed, 2016). These fungi target their hosts by producing toxins and anti-insect secondary metabolites resulting in an array of effects ranging from paralysis to immune-suppression (Kershaw *et al*., 1999; Vey *et al*., 2001). The known toxins produced by entomopathogenic fungi include beauvericins, bassianolides, bassiacridin, destruxins, oosporein, oxalic acid and tenellin (Amiri-Besheli *et al*., 2000; Quesada-Moraga & Vey, 2004; Kirkland *et al*., 2005; Xu *et al*., 2008, 2009). Small amounts of bassianolides induce atonical symptoms in insects (Kanaoka *et al*., 1978), while destruxins inhibit the insect defense system by inducing tetanic paralysis and cytopathetic effects on insect's midgut (Boucias & Pendland, 2012). Oosporein and tenellin are known to inhibit ATPS activity of erythrocyte membrane (Flor, 2006). However, the mechanism of action of these compounds is still unclear.

Owing to insecticides being prone to resistance development by insects and deleterious to environment, researchers have been concentrating their efforts on anti-insect natural products derived from microbes as a substitute to insecticides for controlling insects (Quesada-Moraga *et al*., 2006a). In order to search for an active natural compound for controlling insect pests, monitoring approach has been

employed, based on selection of diverse biotypes of different microorganisms (Schulz *et al*., 2002). The secondary metabolites produced by entomopathogenic fungi have good insecticidal properties (Quesada-Moraga *et al*., 2006a; Freed *et al*., 2012) and are considered safe for humans, non-target organisms and environment (Skrobek & Butt, 2005; Skrobek *et al*., 2008). Modern techniques have aided in understanding the biochemistry of fungal metabolites (Kershaw *et al*., 1999). The fungal secondary metabolites have variety of bioactive chemicals including depsipeptides, terpenoids, polyketides and non-ribosomal peptides with wide range of biological activities i.e. antifungal, antiviral, anti-tumoral, antiproliferative, anti-inflammatory, cytotoxic, immuno-suppressant, insecticidal and phytotoxic activities (Ballard *et al*., 2002; Uchida *et al*., 2005; Lozano-Tovar *et al*., 2013).

The potential use of entomopathogenic fungi and their derived compounds have been demonstrated on insect pests in recent studies (Quesada-Moraga *et al*., 2006c, 2008; Ortiz-Urquiza *et al*., 2009, 2010; Freed *et al*., 2012; Yousef *et al*., 2013, 2014). In our previous study, we have evaluated the potential of entomopathogenic fungi against *M. domestica* (Farooq & Freed, 2016). However, fungal derived proteins have not been evaluated against *M. domestica*. Keeping in view the importance of *M. domestica* as a pest and potential of insecticidal proteins from entomopathogenic fungi, the current study was designed to derive toxic proteins from different entomopathogenic fungi i.e., *B. bassiana, M. anisopliae* var. *anisopliae* and *I. fumosorosea* and investigate their effects on *M. domestica* under laboratory conditions.

2. Materials and methods

2.1 Rearing of *M. domestica*

Adult *M. domestica* were collected from poultry farms of Multan, Punjab, Pakistan and brought to Laboratory of Insect Microbiology and Biotechnology. They were reared in the transparent cages (30×30×30 cm) having mesh screen on the opposite sides and cloth sleeve opening at front for handling. Temperature was maintained at 26±2°C, RH (relative humidity) of 50±5% and 12:12 light and dark period. The adults were provided with sugar and powdered milk (3:1) in Petri dish as diet and water as ad libitum. The plastic cups containing oviposition medium, (water based paste of wheat bran, rice meal, yeast, sugar and dry milk powder (40:10:3:3:1) were placed in the cages as reported by Bell *et al*. (2010) with slight modifications (using rice-meal instead of grass-meal and replacing sugar with malt due to its unavailability). The larval food was changed after 2-4 days depending on the number of larvae per cup.

2.2 Source of entomopathogenic fungi

Different isolates of *B. bassiana* (Bb-01, Bb-08), *M. anisopliae* var. *anisopliae* (Ma-2.3, Ma-4.1) and *I. fumosorosea* (If-2.3, If-03) were obtained from the entomopathogenic fungal culture collection of the Laboratory of Insect Microbiology and Biotechnology, Department of Entomology, Bahauddin Zakariya University, Multan, Pakistan and used in different experiments of the current study. Potato dextrose agar (PDA) medium was used to culture fungi (Freed *et al*., 2011a, b). PDA plates were inoculated with fungi and cultured for 14 days at 25°C. The conidia were harvested from the plates with aid of sterilized scalpel and mixed with sterile solution of Tween-80 (0.05%).

2.3 Production of fungal toxic proteins in Czapek's medium

The crude proteins from fungi were extracted as described by Quesada-Moraga *et al*. (2006a). The fungus grown on PDA plates at 25°C for 14 days, was scrapped off and conidial concentration was determined with the help of haemocytometer. In order to derive toxic proteins from fungal culture, conidia of different isolates of *B. bassiana M. anisopliae* var. *anisopliae* and *I. fumosorosea* (1×10^8 spore/mL) were introduced in 250 mL of Czapek's medium (cane sugar = 30g, bacteriological peptone = 5g, $NaNO_3$=3g, K_2HPO_4 = 1g, KCl = 0.5g, $MgSO_4.7H_2O$=0.5g, $FeSO_4.7H_2O$ = 0.01g, , and water 1000 mL) in 500 mL conical flasks and were incubated at 25°C on a rotary shaker at 110 rpm for a period of 10 days. Mycelia were removed by filtration through Whatman No. 3 filter paper. The filtrate (pellet) was saturated with 90% ammonium sulphate and centrifuged at 10,000 g (revolutions per minute) for 40 minutes. For desalting purpose, fungal filtrate was separated with pure water at ratio of 1:2 for 24 h at 4°C. For this purpose, sample was introduced to porous membrane with 68- kDa cut off. The resultant fraction (desalted) was concentrated by implantation of similar membrane in polyethylene glycol 20000 at 4°C.

2.4 Determination of crude protein concentration

Bovine serum albumin (BSA) was used as a standard to determine soluble proteins content. The quantity of proteins was estimated by recording absorbance at 595nm (Bradford, 1976).

2.5 Insecticidal activity of fungal toxic proteins

The fungal protein extracts were tested for their toxicity by bait method. For this purpose, newly emerged 2-3 day old adult flies (sex ratio 1:1) were provided with baits containing 1 mL protein extract with a concentration of 5 mg protein/mL, while in control, insects were provided with baits containing only desalted media. Water was provided as ad libitum. In all treatments, baits were changed after every 24hr for seven

consecutive days. Each treatment had four replications with total number of 100 adults per treatment. The entire experiment was performed under similar conditions as described previously. The mortality data was recorded on daily basis and percentage mortality was calculated.

2.6 Effect of different concentrations of crude fungal proteins against *M. domestica*

Based on insecticidal bioassay, isolates showing better results from *B. bassiana* (Bb-01) *M. anisopliae* var. anisopliae (Ma-4.1) and *I. fumosorosea* (If-03) were cultivated and protein toxicity was assessed as described earlier. Each isolate was tested at concentrations of 2, 4, 6, 8 and 10 mg protein/ mL, while in control, baits were provided with desalted media. The treated insects were provided with same conditions as explained earlier. The data were recorded for seven consecutive days in order to calculate percent mortality.

2.7 Effect of exposure time on the toxicity of crude protein extracts

The effect of exposure time on toxicity of protein extracts of Bb-01, Ma-4.1 and If-03 were studied at concentrations of 5 and 10 mg/mL of protein using six exposure times and control as an experimental protocol. Newly emerged adults were placed in plastic boxes and fed on bait. Diet was incorporated with 1mL of protein extracts of desired concentration and insects were fed on this diet for 24, 48, 72, 96, 120 and 144 hrs, respectively. Later, baits were replaced with untreated diet. On the other hand, in control, insects were fed on bait with desalted media only. Each treatment was replicated four times with 100 insects per treatment. Similar conditions were provided for experiment as explained earlier and mortality data were taken on daily basis.

2.8 Data analysis

Mortality data were corrected with the help of Abbot's formula (Abbott, 1925) (as mortality was recorded in control). One-way analysis of variance (ANOVA) was used for analyzing the data and means were compared with Tukey (HSD) test in Statistix version 8.1 (Statistix, 2005). With the aid of Probit analysis time required to kill 50% of population (LT50) was determined using POLO PC software (LeOra Software, 2003). Percentage corrected mortality was calculated for each day and Duncan's Multiple Range Test (DMRT) was used to separate means. Data was subjected to Statistix 8.1 software for statistical analysis (Statistix, 2005).

3. Results

3.1 Toxicity of crude protein of different isolates of entomopathogenic fungi against *M. domestica*

A significant effect was observed on *M. domestica* mortality ranging from 52.0 to 91.0% as compared to control (Figure 1). *B. bassiana* (Bb-01), *M. anisopliae* var. *anisopliae* (Ma-4.1) *I. fumosorosea* (If-03) showed maximum mortality (Means ± SE) 91.0 ± 3.42 %, 84.0 ± 5.16 % and 69.0 ± 5.32 % respectively. In addition, Bb-08 caused 60.0 ± 6.32 % followed by Ma-2.3 and If-2.3 with 58.0 ± 5.42 % and 52.0 ± 4.32 % mortality in 7 days after treatment.

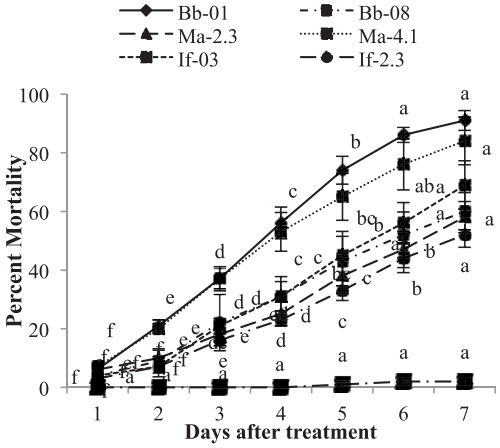

Fig. 1. Percent mortality (mean ± SE) of *Musca domestica* adults after seven days of exposure of diet containing 5 mg / ml of protein extract of strains (Bb-01, Bb-08, Ma-2.3, Ma-4.1, If-03 and If-2.3) of the entomopathogenic fungi. Similar letters on each day are statistically not different by Duncan's Multiple Range Test (DMRT) ($P<0.05$). The isolates were compared on different days, respectively.

3.2 Effect of concentrations on the toxicity of crude proteins

Based on insecticidal activity bioassay, *B. bassiana* (Bb-01), *M. anisopliae* var. *anisopliae* (Ma-4.1) *I. fumosorosea* (If-03) were chosen and effect of different concentrations of their crude proteins was assessed. The data showed a significant effect and a dose dependent trend.

In case of Bb-01, a significant effect was recorded with mortality ranging from 42.0-100.0%. The concentrations 10 and 8 mg/mL of protein showed maximum mortality with LT_{50} of 2.12 and 2.70 days, respectively. Similarly, Ma-4.1 caused mortality ranging from 37.0-100.0% and LT_{50} of 2.48 and 2.86 days for similar concentrations. While in case of If-03, mortality ranged from 31.0-100.0% with least LT_{50} value of 3.54 and 3.77 days (Table 1, $F_{2, 36}$ =112.08, $P<0.05$).

In addition, the mortality rates of *M. domestica* adults after exposure to toxic protein at different concentration levels i.e., 2, 4, 6, 8, 10 mg/mL of protein (Figures 2a, b, c) showed that mortality of *M. domestica* population was directly proportional to concentration i.e., as concentration increased mortality rate of *M. domestica* increased.

Table 1. Percent mortality and LT_{50} values after exposure of *Musca domestica* to different concentrations of crude protein extracts of *Beauveria bassiana*, *Metarhizium anisopliae* var. *anisopliae* and *Isaria fumosorosea*.

Fungi	Protein concentration (mg/ml)	Percent mortality (±SE)	LT_{50} (days)	FD[a]	Slope	$\chi 2$
Beauveria bassiana (Bb-01)	0	2.0±0.13f	-	-	-	-
	2	42.0±4.76de	-	-	-	-
	4	65.0±8.70c	5.73	5.13-6.40	2.60±0.26	4.56
	6	97.0±3.00a	3.06	2.51-3.72	3.87±0.44	14.08
	8	100.0±1.40a	2.70	1.88-3.89	4.57±0.76	21.95
	10	100.0±0.00a	2.12	1.85-3.87	5.19±1.27	17.01
Metarhizium anisopliae var. *anisopliae* (Ma-4.1)	0	2.0±0.13f	-	-	-	-
	2	37.0±1.00de	-	-	-	-
	4	57.0±7.19cd	6.73	5.84-7.76	2.38±0.26	3.98
	6	88.0±5.89ab	3.11	2.86-3.39	2.92±0.22	6.86
	8	100.0±2.31a	2.86	2.51-4.48	3.63±0.69	26.17
	10	100.0±0.00a	2.48	2.20-3.01	4.37±0.76	15.67
Isaria fumosorosea (If-03)	0	2.0±0.13f	-	-	-	-
	2	31.0±3.00e	-	-	-	-
	4	52.0±8.64c-e	7.27	6.17-8.57	2.25±0.26	2.58
	6	73.0±6.40bc	4.38	3.81-5.02	2.67±0.23	2.95
	8	91.0±3.42ab	3.77	3.89-4.20	3.47±0.43	14.18
	10	100.0±0.0a	3.54	2.66-4.73	4.11±0.90	52.46

Means followed by same letter in columns are not statistically different at $P < 0.05$; Tukey (HSD) test
a=fudicial limit, X^2=Chi square

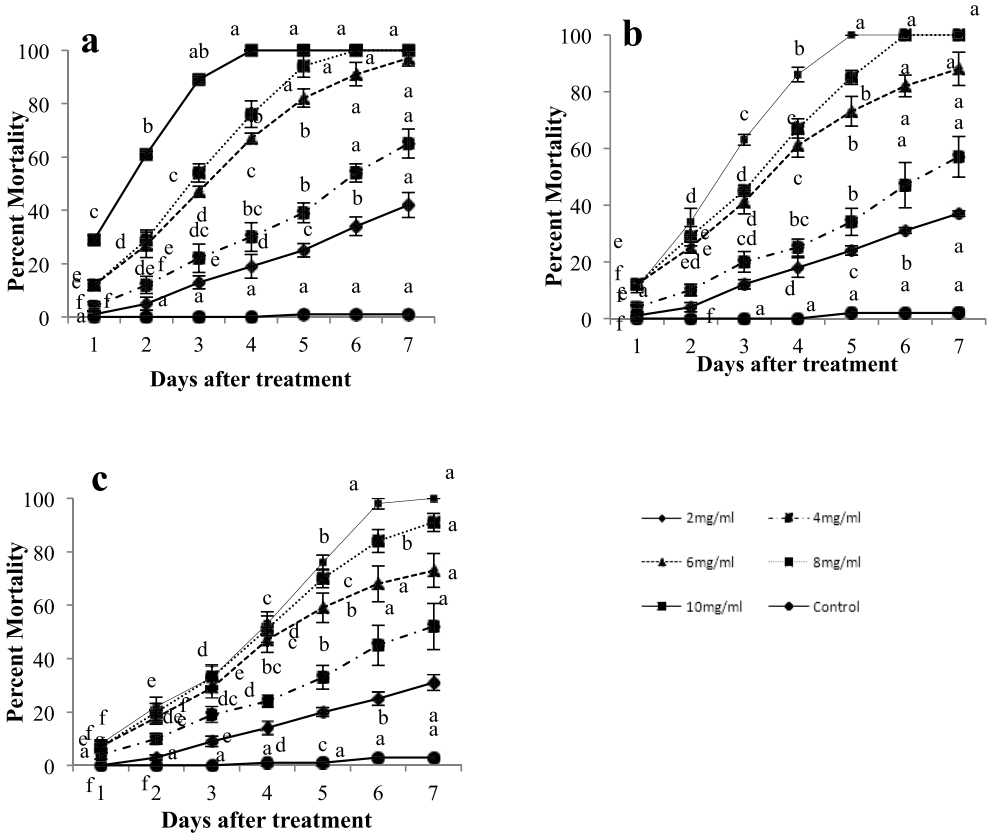

Fig. 2. Cumulative mortality (±SE) of *Musca domestica* adults after exposure to different concentrations of protein extracts of different entomopathogenic fungi (a) *B. bassiana* (Bb-01), (b) *M. anisopliae* var. *anisopliae* (Ma-4.1) and (c) *I. fumosorosea* (If-03). Similar letters on each concentration are statistically not different by Duncan's Multiple Range Test (DMRT) ($P<0.05$). The concentrations were compared on different days, respectively.

3.3 Effect of exposure time on the toxicity of crude proteins

In order to study effect of exposure time on toxicity of crude proteins extracts of Bb-01, Ma-4.1 and If-03, adults of *M. domestica* were fed on bait treated with 5 and 10 mg/mL of proteins for 24, 48, 72, 96, 120 and 144 hrs, respectively and then fed on untreated diet. A significant influence was observed on the toxicity of crude extract at both levels.

In case of 5mg/mL of protein, adults exposed to 144 and 120 hrs to treated baits showed maximum mortalities and lowest LT_{50} values in Bb-01, Ma-4.1 and If-03 (Table 2, $F_{2,45}$= 14.7, P<0.05). Additionally, the mortality rate of *M. domestica* adults after exposure to three different entomopathogenic fungi isolates at different exposure time i.e., 24, 48, 72, 96, 120 and 144 hrs (Figures 3a, b, c) showed that mortality rate of *M. domestica* population was directly proportional to exposure time.

In case of exposure of adults to 10mg/mL protein, for 96 and 120 hrs to treated baits showed complete mortality of *M. domestica* population and lowest LT_{50} values for Bb-01 and Ma-4.1. While in case of If-03, exposure of flies to 144 hrs showed complete mortality of insects. (Table 3, $F_{2, 45}$ =19.3, P<0.05).

Table 2. Percent mortality and LT_{50} values after exposure of *Musca domestica* to crude protein extracts (5mg/mL) of *Beauveria bassiana*, *Metarhizium anisopliae* var. *anisopliae* and *Isaria fumosorosea*.

5 mg/ml						
Fungi	**Exposure time (Hrs)**	**Percent mortality**	**LT_{50} (days)**	**FD[a]**	**Slope**	**$\chi 2$**
Beauveria bassiana (Bb-01)	0	2.0±0.13i	-	-	-	-
	24	20.0±3.65gh	-	-	-	-
	48	40.0±1.63d-f	-	-	-	-
	72	59.0±5.74b-e	4.83	4.27-5.45	2.09±0.2	1.3
	96	71.0±1.91a-c	3.97	3.63-4.33	2.65±0.2	1.4
	120	82.0±4.76ab	3.46	3.23-3.73	3.19±0.2	3.8
	144	88.0±4.32a	3.36	3.11-3.62	3.29±0.2	4.7
Metarhizium anisopliae var. *anisopliae* (Ma-4.1)	0	2.0±0.13i	-	-	-	-
	24	17.0±1.91gh	-	-	-	-
	48	38.0±2.00e-g	-	-	-	-
	72	55.0±8.23c-f	5.49	4.86-6.21	2.24±0.2	2.8
	96	63.0±5.97b-d	4.79	4.23-5.39	2.42±0.2	3.3
	120	71.0±6.40a-c	4.18	3.58-4.58	2.90±0.2	1.4
	144	80.0±4.32ab	3.96	3.68-4.28	3.18±0.2	2.9
Isaria fumosorosea (If-03)	0	2.0±0.13i	-	-	-	-
	24	11.0±3.00h	-	-	-	-
	48	21.0±3.79gh	-	-	-	-
	72	34.0±6.22f-h	-	-	-	-
	96	47.0±4.73d-f	-	-	-	-
	120	53.0±2.52c-f	6.11	5.32-7.01	2.22±0.2	0.5
	144	60.0±4.32b-e	5.36	4.83-5.94	2.61±0.2	1.6

Means followed by same letter in columns are not statistically different at P < 0.05; Tukey (HSD) test
a=fudicial limit, X^2=Chi square

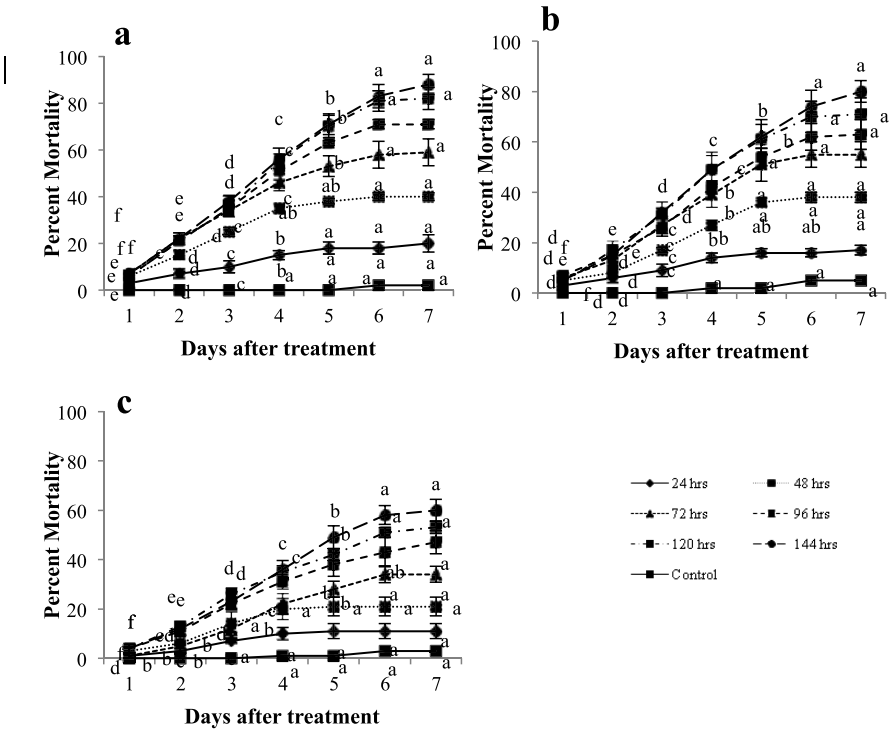

Fig. 3. Cumulative mortality (±SE) of *Musca domestica* after exposure to 5 mg/ml protein extracts of different entomopathogenic fungi (a) *B. bassiana* (Bb-01), (b) *M. anisopliae* var. *anisopliae* (Ma-4.1) and (c) *I. fumosorosea* (If-03) for different time duration. Similar letters on each exposure duration are statistically not different by Duncan's Multiple Range Test (DMRT) ($P<0.05$). The exposure durations were compared on different days, respectively.

4. Discussion

Entomopathogenic fungi i.e., *B. bassiana* and *M. anisopliae* have been used worldwide to overcome defenses of important insect pests and successful parasitism. Different mechanisms are responsible for the fungal virulence against insects. Cuticle-degrading enzymes produced by these fungi have been reported to be associated with their virulence (Freimoser *et al*., 2003; Fang *et al*., 2005; Shah *et al*., 2005).

Table 3. Percent mortality and LT_{50} values after exposure of *Musca domestica* to crude protein extracts (10mg/mL) of *Beauveria bassiana*, *Metarhizium anisopliae* var. *anisopliae* and *Isaria fumosorosea*

10 mg/ml						
Fungus	**Exposure time (Hrs)**	**Percent mortality (±SE)**	**LT_{50} (days)**	**FD[a]**	**Slope**	**$\chi 2$**
Beauveria bassiana (Bb-01)	0	2.0±0.00h	-	-	-	-
	24	23.0±1.00ef	-	-	-	-
	48	48.0±4.32c-e	-	-	-	-
	72	72.0±1.63bc	3.40	3.00-3.87	2.60±0.22	4.00
	96	100.0±0.00a	2.10	1.45-2.99	4.73±1.39	23.42
Metarhizium anisopliae var. *anisopliae* (Ma-4.1)	0	2.0±0.00h	-	-	-	-
	24	20.0±3.65ef	-	-	-	-
	48	45.0±5.26d	-	-	-	-
	72	67.0±1.91b-d	4.15	3.67-4.69	2.60±0.23	4.75
	96	83.0±5.74ab	3.60	2.76-4.70	3.24±0.24	10.94
	120	100.0±0.00a	2..32	2.11-4.26	4.40±1.20	33.83
Isaria fumosorosea (If-03)	0	2.0±0.00h	-	-	-	-
	24	23.0±3.42ef	-	-	-	-
	48	41.0±4.12de	-	-	-	-
	72	64.0±6.73b-d	4.00	3.58-4.49	2.17±0.21	4.63
	96	79.0±3.00bc	3.27	2.98-3.57	2.97±0.23	3.42
	120	96.0±4.00a	2.93	2.39-3.59	4.21±0.48	14.99
	144	100.0±0a	2.82	2.01-3.95	4.51±0.67	25.47

Means followed by same letter in columns are not statistically different at $P < 0.05$; Tukey (HSD) test
a=fudicial limit, X^2=Chi square

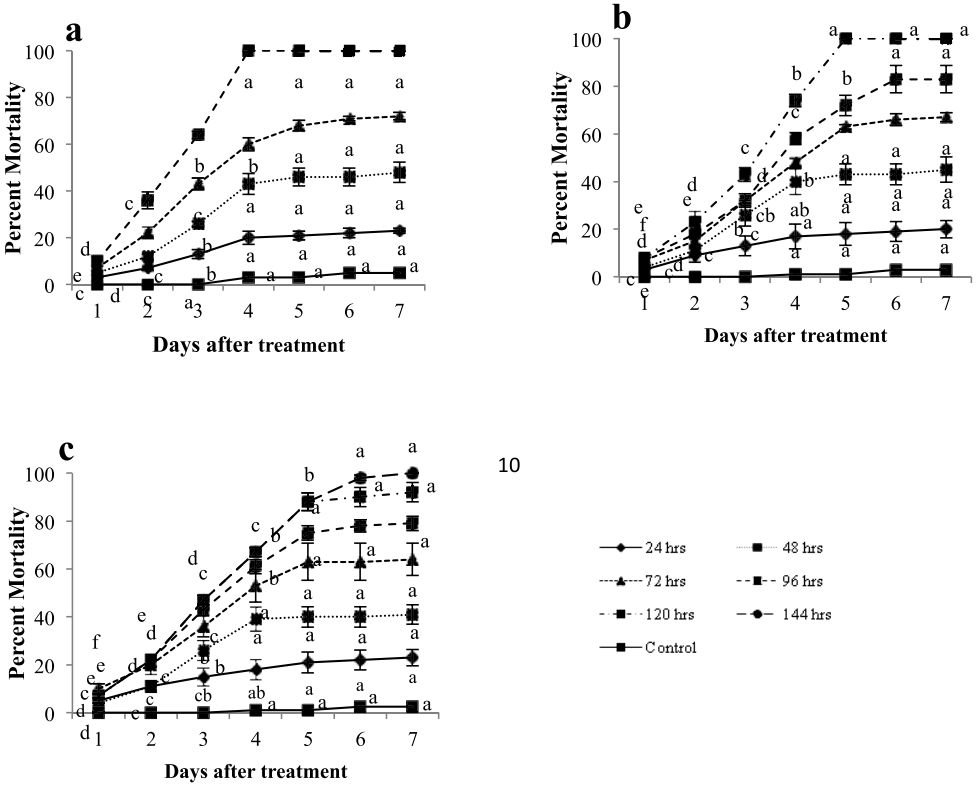

Fig. 4. Cumulative mortality (±SE) of *Musca domestica* after exposure to 10 mg/ml protein extracts of different entomopathogenic fungi (a) *B. bassiana* (Bb-01), (b) *M. anisopliae* var. *anisopliae* (Ma-4.1) and (c) *I. fumosorosea* (If-03) for different time duration. Similar letters on each exposure duration are statistically not different by Duncan's Multiple Range Test (DMRT) (P<0.05). The exposure durations were compared on different days, respectively.

Fungal virulence against insects has also been associated with the production of various toxic compounds secreted during the infection process. Such compounds include small molecular organic acids i.e., oxalates (Kirkland *et al*., 2005), secondary metabolites (Mazet *et al*., 1994; Vey *et al*., 2001; Quesada-Moraga & Vey, 2003; Fuguet *et al*., 2004; Fuguet & Vey, 2004; Quesada-Moraga *et al*., 2006b; Zimmermann, 2007a, b).

Most of described fungal toxins are secondary metabolites with low molecular weight exhibiting insecticidal or antibiotic properties (Strasser *et al*., 2000; Vey *et al*., 2001). A major obstacle in the development of insect pathogenic fungi as myco-pesticides is the length of time needed to kill an insect pest (St Leger & Screen, 2001).

The current study was designed to endow information regarding the insecticidal activity of crude proteins secreted by entomopathogenic fungi. The extracts from *B. bassiana* (Bb-01), *M. anisopliae* var. *anisopliae* (Ma-4.1) and *I. fumosorosea* (If-03) were more toxic and showed promising results against *M. domestica*. The insecticidal activity of extracted proteins exhibited a dose-dependent and exposure time related response against *M. domestica* adults. In previous studies secondary metabolites secreted by fungus exhibited insecticidal activity against *Phaedon cochleariae* F. (Coleoptera: Chrysomelidae) and *Plutella xylostella* L. (Lepidoptera: Plutellidae) with dose-dependent response in both topical and leaf dip bioassay. Moreover, P. *xylostella* was more susceptible than *P. cochleariae* against insecticidal activity of efrapeptins (Bandani & Butt, 1999). Further, a strong dose-related toxicity was observed in *Spodoptera littoralis* F. (Lepidoptera: Noctuidae) by *M. anisopliae* extract. At 40μg protein/insect of extract 100.0% mortality of *S. littoralis* was observed (Quesada-Moraga *et al*., 2006a). In addition, *I. fumosorosea* crude proteins were evaluated against *P. xylostella* for insecticidal and anti-feedant properties. *I. fumosorosea* isolate CNZH was proved to be most toxic and dose-dependent response with maximum 91.6% mortality was observed at 9mg/ml of protein (Freed *et al*., 2012). In the current study, *B. bassiana* (Bb-01), M. *anisopliae* var. *anisopliae* (Ma-4.1) and *I. fumosorosea* (If-03) exhibited maximum mortalities (Means ± SE) 91.0 ± 3.42 %, 84.0 ± 5.16 % and 69.0 ± 5.32 % respectively and later selected for further concentration and exposure bioassay.

The results of current study showed that insecticidal activity of *B. bassiana* (Bb-01), *M. anisopliae* var. *anisopliae* (Ma-4.1) and *I. fumosorosea* (If-03) crude proteins increased

with the increase in concentrations. The maximum mortality rates after treatment with 8 and 10 mg/mL of protein showed lethal time (LT_{50}) ranging from 2.12 -3.54 days. Similar dose-dependent insecticidal effects of fungal secondary metabolites have been reported in previous studies, where dose related toxicity was exhibited by fungal proteins in S. littoralis with 40μg protein/insect of extract showed 100.0% mortality (Quesada-Moraga *et al*., 2006a). In addition, maximum mortality of *P. xylostella* by *I. fumosorosea* (isolate CNZH) at 9mg/ml of protein (Freed *et al*., 2012) supports the findings of current study.

Exposure duration of 96, 120 and 144 hrs showed complete *M. domestica* mortality by *B. bassiana* (Bb-01), *M. anisopliae* var. *anisopliae* (Ma-4.1) and *I. fumosorosea* (If-03), respectively. Methanol extracts of *Cordyceps militaris* Link (Ascomycotina: Clavicipitaceae) have shown insecticidal activates, which varied as per dose and exposure time (Kim *et al*., 2002) lies in favor of current study with similar dose and exposure dependent response.

Synergism effect of destruxins B (mycotoxin from M. anisopliae) with *Bacillus thuringiensis kurstaki*, tea saponins and entomopathogenic fungi have been reported in earlier studies (Rizwan-ul-Haq *et al*., 2009). Further, interactions of fungal toxic proteins with other tactics i.e. insect bait (Ortiz-Urquiza *et al*., 2009), botanicals insecticides i.e. rotenone, azadirachtin and aeonolum (Yi *et al*., 2012) and their application in field conditions could enhance knowledge of their insecticidal effects.

5. Conclusion

In conclusion, crude proteins exhibited a great potential for the control of *M. domestica*. However, purification of active proteins, persistence of proteins, effect of temperature and their evaluation under the field conditions are needed to be investigated.

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نشاطاً مبيداً للحشرات من البروتينات الخام السامة التي تفرزها فطريات مسببة للأمراض الحشرية ضد الذبابة المنزلية Musca domestica L. (Diptera: Muscidae)

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الملخص

الذبابة المنزلية، Musca domestica L. (Diptera: Muscidae)، هي آفة حشرية رئيسية في مزارع الدواجن والألبان، قد طورت مقاومة ضد مجموعة متنوعة من المبيدات الحشرية في جميع أنحاء العالم. ومن أجل تجنب تطوير المقاومة والآثار السلبية للمبيدات الحشرية، فإن استخدام الفطريات المسببة للأمراض الحشرية يمكن أن يكون خياراً فعالاً للقضاء على M. domestica. أُجريت هذه الدراسة لاستخراج البروتينات الخام السامة من الفطريات المسببة للأمراض الحشرية Beauveria bassiana وMetarhizium anisopliae var. anisopliae و Isaria fumosorosea واختبار تأثيرها على مدى بقاء M. domestica على قيد الحياة. تم اختبار البروتينات الخام المُنتجة في وسط تشابيك Czapek بواسطة ست عزلات مختلفة (اثنان لكل واحد) من الفطريات الممرضة للحشرات ضد M. domestica البالغة. وقد لوحظ تأثير كبير على مدى بقاء M. domestica، حيث تتراوح نسبة الوفيات بين 52.0 إلى 91.0٪. وقد أظهرت عزلات Ma-4.1، Bb-01، و If-03 أعلى نسبة من الوفيات. أظهرت هذه العزلات الثلاث كذلك الاستجابة نحو بقاء M. domestica اعتماداً على التركيز ومدة التعرض. تركيزات البروتين الخام مثل 8 و 10 ملغ / مل تسببت في حدوث أقصى معدل للوفيات (بنسبة 100٪) من عدد M. domestica خلال المدة من 2.77 إلى 3.77 أيام. وبالإضافة إلى ذلك، فإن تعرض الذباب المنزلي لـ (10 ملغ / مل) من البروتين الخام B. bassiana (Bb-01) لمدة أقل (96 ساعة) تسبب في وفيات 100٪ من عدد الذباب قيد الاختبار بالمقارنة مع العزلات الأخرى. وفي الختام، فإن البروتينات الخام المستخرجة من الفطريات المسببة للأمراض الحشرية أظهرت إمكانات جيدة للقضاء على M. domestica بطريقة صديقة للبيئة. ومع ذلك، هناك الحاجة إلى مزيد من التنقية للبروتينات المضادة للحشرات وتقييمها في الظروف الميدانية.