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Characterization of Egyptian *Beauveria bassiana* Strain for Biocontrol of *Musca domestica*

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Abstract

Beauveria spp., an entomopathogenic fungus, has recently been widely used as a biocontrol agent of house fly populations to evade the critical effects of chemical insecticides on humans, animals, and the natural ecosystem. In the presented study, different types of fungi were isolated from house flies collected from various locations in different Egyptian governorates between 2021 and 2022. The flies were caught in garbage dumps, dairy and meat production units on cattle farms and poultry farms. The entomopathogenic fungi were identified morphologically and genetically, showing that the isolate was molecularly identified as *Beauveria bassiana*. This efficacy of the isolated fungal strain against the house fly, *M. domestica*, was estimated using laboratory bioassay by the immersion method. The isolated fungal strain successfully induced fatal changes with a high mortality rate in the laboratory-reared house flies, which confirms the scientific world research which has been carried out. The achieved result showed a direct correlation between fungal concentrations and the mortality rate, which reached up to 99.16 %. They also exhibited great destruction and a direct relationship between the increase in spore concentration and the degree of overall DNA genotoxic damage in the exposed fly's DNA to *Beauveria bassiana*. *Beauveria bassiana* MN960454.1 can be used as an efficient way for controlling house fly biologically reached up to 99.16 %. Thus, it is recommended to apply *Beauveria* spp. as a spray in house fly breeding areas.

KEYWORDS
Musca domestica, Entomopathogens, *Beauveria bassiana*, Biological control, Immersion method, Genotoxic.

INTRODUCTION

The most prevalent and widespread species of flies in the world is the house fly, *Musca domestica* (Diptera: *Muscidae*). It is a member of a fly species commonly referred to as "filth flies," and it coexists closely with people (Khamesipour *et al*., 2018). More than 65 potentially fatal diseases, including typhoid, cholera, tuberculosis, bacillary dysentery, infantile diarrhoea, and anthrax, are carried by this fly (Scott *et al*., 2014). According to numerous studies, *M. domestica* is a deadly transmitter of bacteria, viruses, parasites, and fungi, most notably *Aspergillus* spp., which is responsible for serious illnesses such as fungal pneumonia in humans and animals as well as nail infections in humans (Amaike and Keller, 2011; Gauthier and Keller, 2013).

The conventional approach to controlling *M. domestica* is to employ chemical pesticides. Unfortunately, these substances lead to serious issues, such as the comeback of pests due to insecticide resistance. Another significant disadvantage is the poisonous and harmful lingering effects of these compounds in ingested animal corpses. Many common insecticide classes, such as organophosphates, organochlorines, carbamates, and pyrethroids, are ineffective against house flies (Khan *et al*., 2013). These issues and the high cost of insecticides cleared the door for other less expensive and environmentally benign solutions, like biological controls.

Entomopathogenic fungi have recently attracted considerable interest across the globe for the biological management of pests, especially due to their distinctive properties (Kidanu and Hagos, 2020). A vast variety of fungus known as entomopathogenic fungi have the ability to infect, assault, and kill a variety of insect hosts (Singkaravanit *et al*., 2010; Kidanu and Hagos, 2020). These entomopathogens' bio-persistence and eco-friendliness stand out as their most notable traits. They become infected through their cuticle, which presents a special opportunity to kill the insect and can spread from one person to another through simple touch (Kidanu *et al*., 2020). Various entomopathogenic strains, including *Beauveria bassiana*, *Metarhizium anisopliae*, and *Isaria fumosorosea*, have been studied for their possible utility in the management of *Bemisia tabacci*, *Musca domestica*, *Dysdercus koenigii*, and *Oxycarenus hyalinipennis* (Khan *et al*., 2012; Akmal *et al*., 2013; Zafar *et al*., 2016). However, more investigation is required to identify which pathogen isolates can be employed successfully as an alternative to conventional control approaches in specific geographical areas.

Random amplified polymorphic DNA (RAPD) based on the polymerase chain reaction (PCR) has been widely used to distinguish and identify several strains of *Beauveria bassiana*. The genetic diversity of *Beauveria* spp. has also been evaluated using nucleotide variations in the ITS sections of the DNA (Imoulan *et al*., 2017).

In the current study, house flies were gathered from various

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locations in Egypt. The collected flies were then be bred in a lab. environment, and the fungal isolates were isolated and identified using PCR. The efficacy of the identified entomopathogenic strains against houseflies was detected using a bioassay study and DNA damage test via the immersion method and the comet technique, respectively.

MATERIALS AND METHODS

Collection of M. domestica from different localities

Throughout 2021 and 2022, house flies were gathered in Egypt's four governorates north and south of Cairo from waste piles, dairy facilities, meat processing industries, and poultry farms (Fig. 1). The governorates were Kafr El-sheikh, Beheira, Gharbia, and Giza (Nahia, Talbia, and El-harm). With the aid of a sweeping net, the houseflies were captured. The collected flies were recognized as *M. domestica* morphologically by Hafez *et al.* (1971) and Soulsby (1982).

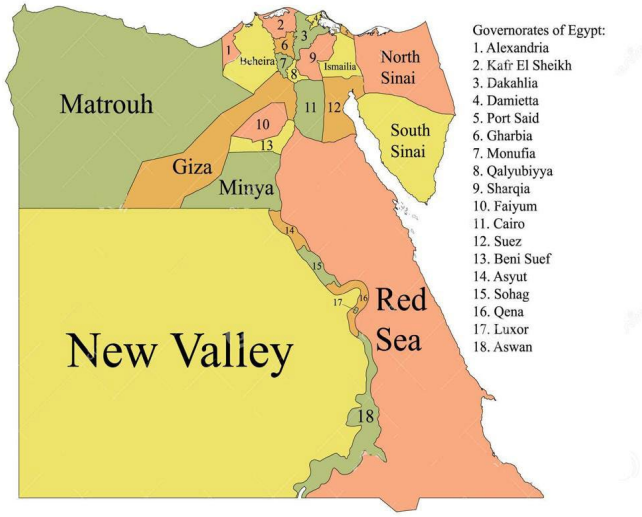

Fig. 1. Map displaying the locations of Egypt's governorates.

Rearing of M. domestica

The house flies were reared in a wooden cage at a temperature of 25–28°C and a relative humidity of 40–65%. Equal amounts (25 g) of powdered milk and granulated sugar combined with water were fed to the adult flies. In the breeding cage, petri dishes in which a piece of cotton had been soaked in the mixture were used. Larvae were transferred from incubators to 250 ml glass beakers with a larval diet of 5 g wheat bran, 2 g milk powder, and 1 ml honey blended with 10 ml of water. The diet was replaced every day up until the pupae started to emerge. The pupae were moved to 250 ml glass beakers with sawdust in the bottom, covered with a piece of paper, secured with an elastic band, and placed in the breeding cage at 25 to 28°C and 40 to 65 % relative humidity.

Fungus isolation from the samples collected

Six groups of collected flies were created from each location. Following divisions were made among the first three Giza groups: The first group was made up of 30 flies that were gathered from trash cans in the Nahia district, while the second and third groups, each of which had 25 flies that were obtained from cans in the Talbia and El-harm districts, respectively. The other three groups, from the poultry farm in Gharbia, the meat processing factory in Kafr El-Sheikh, and the dairy processing plant in Beheira, each had 50 flies. The test tubes containing the preserved flies were put in the freezer for five minutes to induce anesthesia. By adding a saline solution drop by drop using smooth grinding, the flies were moved to a porcelain crucible for maceration. On a fungal growth medium made of 32.5 g of Sabouraud Dextrose Agar in 500 ml of distilled water, along with 0.025 g of chloramphenicol to prevent the development of bacteria and 0.125 g of cycloheximide, a saprophytic fungicide, and other additives, about 0.1 ml of this macerated preparation was applied. Each group of the gathered flies received three duplicates and six plates of the prepared fungal growth media. The plates were kept at 28 to 30°C and ≥ 80% relative humidity. For 15 days, daily observations were made to track the development of a fungus colony. The isolated colony was cleaned, identified macroscopically by its distinctive colour and shape, and studied microscopically in accordance with the colour atlases of pathogenic fungi and entomopathogenic fungi (Doolotkeldieva *et al.*, 2019).

Molecular identification, sequencing, and phylogeny of fungal isolate

The ITS rRNA gene sequence analysis was done using two universal primers: ITS4 5′ (TCCTCCGCTTATTGATATGC) 3′ and ITS4 5′ (TCCTCCGCTTATTGATATGC) 3′. Living fungal cultures were subsequently sent to Macrogen in Korea for DNA extraction using the InstaGene Matrix (Bio-Rad) (Catalog #732-6030). According to Macrogen's instructions, the 20-µl PCR mixture contained 2 µl of DNA template, 0.2 µl of 2.5 U/µ KOMA-Taq, 2 µl of 10X Taq PCR mixture, 1.6 µl of 2.5 mM per deoxynucleoside triphosphate, and 10 pmol of each primer. The following were the PCR conditions: Initial pre-heat at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 2 min, extension at 68°C for 1.5 min, and final extension at 68°C for 10 min. The amplified product was purified using a Montage PCR Clean-up kit from Millipore, and it was sequenced using an automated DNA sequencing equipment at Macrogen, Inc. in Seoul, Korea and a dye terminator cycle sequencing kit from Applied Bio System, USA. The ITS rRNA region sequence similarity was then evaluated using BLASTn, and the sequences were then submitted to the NCBI database after being modified using the Bioedit sequence assembly software. The CLUSTALW multiple sequence alignment tool, version 1.83, was used to compare the sequences (Thompson *et al.*, 1994; Ramadan *et al.*, 2021; Khalifa *et al.*, 2023b). The MEGA6 neighbor-joining method was used to conduct phylogenetic analyses.

Infection of Adult M. domestica

During each experimental infection trial, fungal spore suspension was administered to three groups, each of which contained forty adult *M. domestica* flies, at concentrations of 10^5, 10^8, and 10^{10} spore/ml. By placing a patch of the same quantity of flies only in a solution containing 0.05% Tween 80, a control group of flies was created. Both the infected and control groups were kept in rearing cages and fed according to the prior description. For 14 days, they were watched every day, and fly mortality was noted.

To promote fungal sporulation, the cadaver was taken every day and put on wet filter sheets in Petri plates (Farooq and Freed 2016). By evaluating the colour and shape of the fungal growth, which each type of entomopathogenic fungi, it was possible to identify mortality caused by fungal infection (Abdel-Gawad *et al.*, 2020). The cadavers were washed with 10 ml of sterile distilled

water for the purpose of collecting fungal conidia, swirled for 30 seconds to extract the spores, and observed under a light microscope (Mwamburi *et al*., 2010).

DNA Damage Analysis (Comet Assay)

Tissue suspension preparation

In 1 mL of cold HBSS containing 20 mM EDTA/10% DMSO, small pieces of *M. domestica* adults were obtained from three groups treated with *Beauveria* strain at 10^5, 10^8, and 10^{10} spore/ml, along with a group of untreated insects. For 10 minutes, everyone was chopped into tiny pieces in the vortex machine. For 10 minutes, the material was thoroughly stirred in the vortex machine. The suspension was put into a clean centrifuge tube, centrifuged at 1000 x g, removed the supernatant, took the pellet, mixed 5–10 µl with 75 µl LMPA, and processed according to Dhawan *et al*. (2003) and Packiam *et al*. (2015).

Comet assay to evaluate DNA damage in *M. domestica* cells

The acquired cell suspension conducted a comet analysis according to Taha *et al*. (2022) and Khalifa *et al*. (2023a). On thoroughly frosted microslides, 75 microliters of 1% normal melting agarose in phosphate buffer saline were gently poured, covered right away with a cover slip, and left to sit for 5 minutes over an ice pack. The coverslip was taken off once the gel had dried. At 37°C, 1% low melting agarose was combined with the cell solution (10,000/mL). The slide was coated with 75 microliters of the cell solution, which was promptly put on top of the gel and given time to set as before. On the gel containing the suspended cell, a third coating of 75 µL of 1% low melting agarose was applied, and the gel was then let to set. There was prepared a duplicate set of slides.

Lysis of cell

The coverslips were taken off once the agarose had solidified, and the slides were then submerged in the ice-cold lysis solution and maintained in the freezer at 4°C for at least one hour. Low lighting was used for all the aforementioned procedures in order to prevent further cell damage. The lysis solution was taken from the slides, and they were then put in an electrophoretic tank. The electrophoresis buffer was poured into the reservoirs until the slides were completely submerged. After around 20 minutes of standing time in the buffer, the slides underwent 10 minutes of electrophoresis at 26 V. The slides were taken out after electrophoresis, cleaned three times in the neutralizing buffer, and then gently tapped to dry (Ramadan *et al*., 2022).

Analysis of Image

Using a ZEISS fluorescence microscope, the stained DNA in the cells was analyzed at 20x magnification with excitation (460-590 nm). The comet score software version five was used to take and evaluate the photos of 50 randomly nuclei on each slide. The percentage of total fluorescence for each nucleus that was made up of damaged DNA that moved in the tail was calculated.

Statistical analysis

Data were showed as DNA damage and a mean percentage of adult mortality. One-way analysis of variance (ANOVA) and a Multiple Comparison Test (MCT) were applied to analyze the data using a statistical application called Minitab 16.0 v. At $P \leq 0.05$, the significance level was evaluated (Ramadan *et al*., 2020; El Akkad *et al*., 2022).

RESULTS

Identification of the Isolated Fungi from Different Governorates

The present study revealed the isolation of different types of fungi from the localities under investigation. Samples from Beheira revealed the existence of the entomopathogenic fungus (*Beauveria* spp.) (Fig. 2 and Table 1). The most prevalent fungal species, other than the entomopathogenic fungus, were *A. flavus* and *Fusarium* spp. (Table 1). From the other samples, the author noticed the existence of the non-entomopathogenic fungus *Fusarium* spp. (Fig. 3). In another different group of non-entomopathogenic fungi, *Aspergillus* spp. was also isolated, namely *Aspergillus niger* (Fig. 4), *Aspergillus flavus* (Fig. 5), *Aspergillus parasiticus* (Fig. 6), and *Aspergillus fumigatus* (Fig. 7). *Aspergillus flavus* predominated in all of the collected samples.

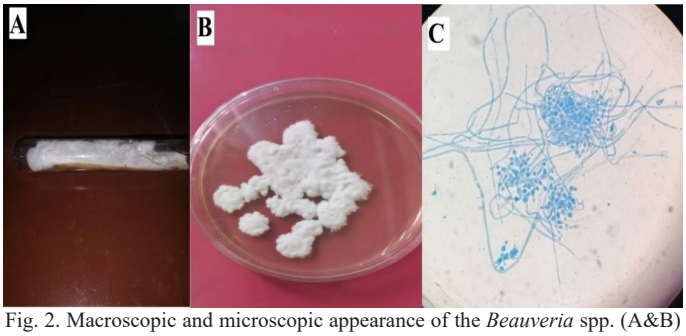

Fig. 2. Macroscopic and microscopic appearance of the *Beauveria* spp. (A&B) White and densely woolly fungus with reverse white color; (C) Microscopic examination of *Beauveria* spp. shows globose in shape conidia (Lactophenol cotton blue stain X400).

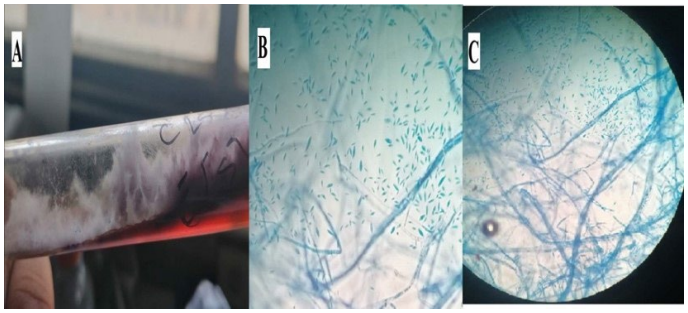

Fig. 3. Macroscopic and microscopic images of *Fusarium* spp. (A) Macroscopic images show atypical woolly texture with an entirely delicate purple color; (B&C) Microscopic images showing macro and microconidia (lactophenol cotton blue stain X400).

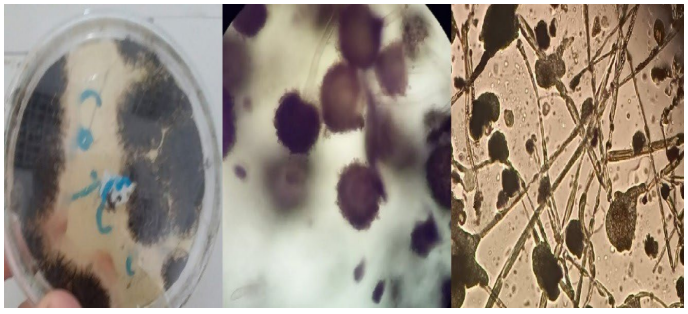

Fig. 4. Macroscopic and microscopic images of *A. niger*. (A) Macroscopic images showing dark brown to black colonies with rough-walled colonies; (B&C) Microscopic images showing dark brown to black conidial head and conidia radiating all over the vesicle.

A. flavus, *A. niger*, and *Fusarium* species were isolated from three localities in Giza. While *A. flavus*, *A. parasiticus*, *A. fumigatus*, and *Beauveria* spp. were recorded from Beheira. *A. flavus*, *A. niger*, *A. parasiticus*, and *Fusarium* spp. were collected from Gharbia. Furthermore, *A. flavus*, *A. parasiticus*, *A. fumigatus*, and *Fusarium* spp. were recorded in Kafr El-sheik.

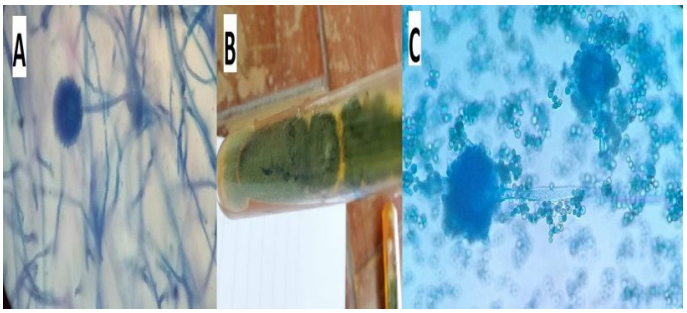

Fig. 5. Macroscopic and microscopic images of *A. flavus*. (A, C) Microscopic images showing the entire vesicle surrounded with conidia of various sizes (lactophenol cotton blue stain X400); (B) Olive green in color colonies with a granular texture.

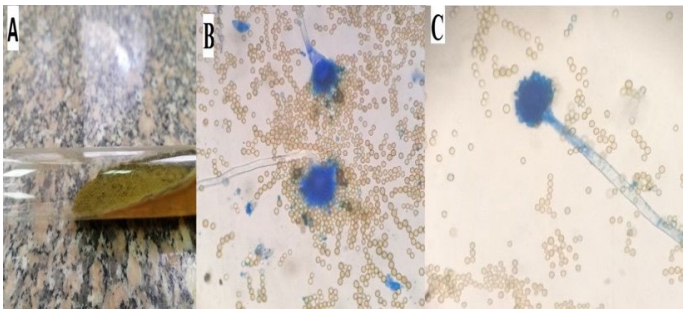

Fig. 6. Macroscopic and microscopic images of *A. parasiticus*. (A) Dark green colonies with a granular appearance; (B&C) Microscopic images showing swollen conidiophore forming the vesicle and the conidia covering the entire vesicle (Lactophenol cotton blue stain X400).

Molecular Identification and Phylogenetic Analysis of the Isolated Fungus

The fungus B1 was identified using 16s rRNA gene sequencing. The amplified product of the ITS region was 569 bp long using the former 2 universal primers. On comparing the sequence of the amplified product with the NCBI database using BLASTn, the isolate was identified as *Beauveria bassiana* with a 99.48% similarity to *Beauveria bassiana* MN960454.1 (Table.2). The accession number of the sequence was ON693245.1. Then, the phylogenetic analysis was conducted on our strain ON693245.1 among 14 other *Beauveria* strains returned from the NCBI database. The Maximum Likelihood method and Tamura 3-parameter model were used to infer the evolutionary history. Furthermore, the bootstrap consensus tree concluded from 100 replicates was possessed to state the evolutionary history of the taxa analyzed. Comparative analyses indicated that the ON693245.1 strain exhibited close identities to *Beauveria* strains from Costa Rica, India, and Italy, respectively (Fig. 8).

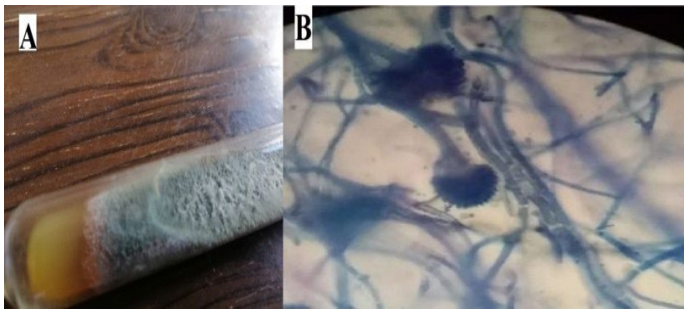

Fig. 7. Macroscopic and microscopic images of *A. fumigatus*. (A) Macroscopic image showing bluish-green in color with a powdery texture; (B) Microscopic picture showing conidial heads of *A. fumigatus* with conidia on the upper two third of the vesicle (Lactophenol cotton blue stain X400).

Bioassay on Adult Musca domestica Adult Flies Using Beauveria bassiana

The hazard of the isolated entomopathogenic fungus, identified as *Beauveria bassiana* ON693245.1, was evaluated through a topical application (immersion method) on the adult housefly. Three serial dilutions were used; there were three replicates per treatment. The adult fly mortality was inspected after application by immersion method. It was revealed that the isolated fungus showed a lethal effect on adult house flies.

The result of different concentrations of *Beauveria* spp. on the mortality percentage of adult flies is shown in Table 3. Data revealed a direct relationship between increasing concentration of the fungal spores and increasing the mortality percentage in

Table 1. Different types of fungal species isolated from different regions.

Governorates	No. of collected flies	Fungus species					
		A. flavus	*A. fumigatus*	*A. niger*	*A. parasiticus*	*Beauveria* spp.	*Fusarium* spp.
Giza (Nahia)	30	+	_	_	_	_	+
Giza (Talbia)	25	+	_	+	_	_	+
Giza (El-harm)	25	_	_	+	_	_	+
Beheira	50	+	+	_	+	+	_
Gharbia	50	+	_	+	+	_	+
Kafr El-Sheikh	50	+	+	_	+	_	+

Table 2. BLASTn results of *Beauveria bassiana* isolate in the NCBI database.

Query					Score		Identities
Accession	Description	Length	Start	End	Coverage	E- Value	Percentage %
ON693245.1	*Beauveria bassiana*	225	11(+)	201(+)	84%	2.00E-91	99.48%
Subject							
Accession	Description	Length	Start	End			
MN960454.1	*Beauveria bassiana*	521	142(-)	333(-)			

adult *M. domestica* flies (p ≤0.05); mortality increased by increasing fungal concentration. The mortality records were compared to control mortality. The minimally recorded morality in adult stages was (19.16%), using a concentration of $1x10^5$ spore/ml (Fig. 9). Increasing fungal concentration to $1x10^8$ spore/ml significantly affected the mortality and caused a 4.6-fold increase in adult mortality (89.16%). The maximum mortality level was recorded by increasing the fungal concentration to $1x10^{10}$, and the adult mortality reached (99.16%). In contrast to the infected *M. domestica* adult flies, in the control group there was no record of mortality.

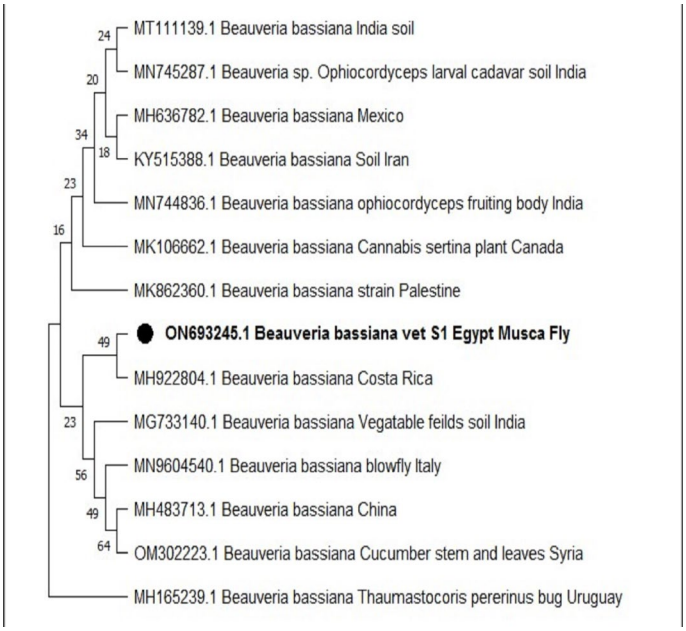

Fig. 8. Phylogenetic tree of Egyptian *Beauveria bassiana* strain constructed by Maximum Likelihood method in Mega 1.

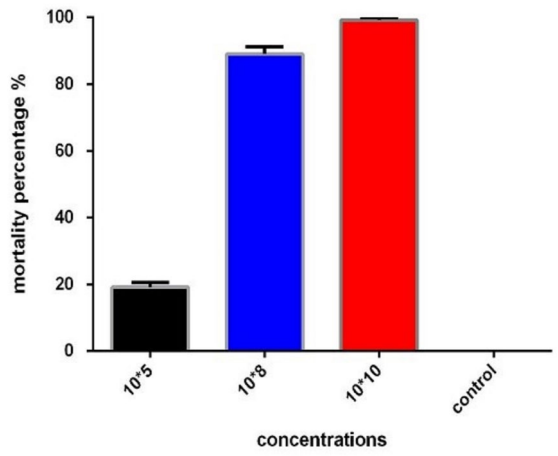

Fig. 9. Effect of naturally isolated *Beauveria* spp. fungal suspension on the mortality of *M. domestica* adult.

However, the abundance of setae on adult flies promotes and spreads entomopathogenic fungus infection, which leads to an abnormally high mortality rate for adult house flies (19.16–99.16). In the present study, the daily recorded mortality in adult *M. domestica*, treated with a fungal suspension of *B. bassiana*, revealed a gradual effect expressing a maximum lethal effect after 7-9 days post-infection (Fig. 10).

Considering the lethal effect of *B. bassiana* ON693245.1 on adult mortality decreased the adult house fly numbers significantly within a week after application, thus reducing the bothering effect of house flies on both humans and animals.

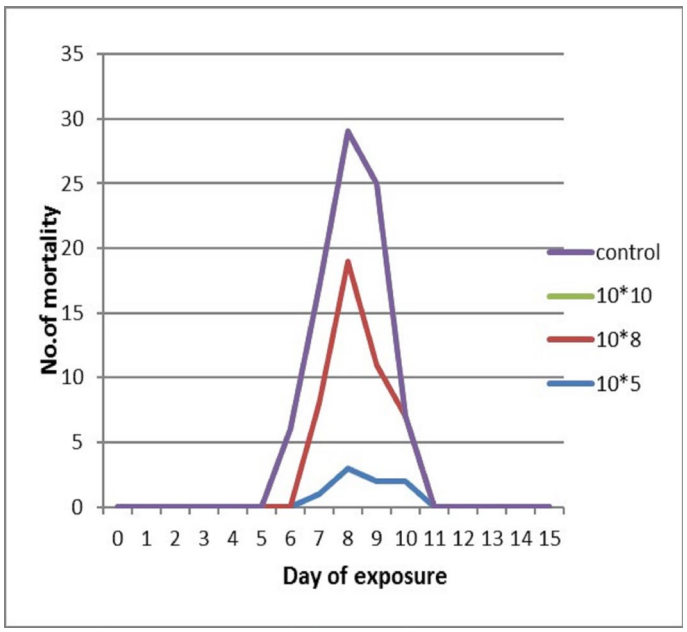

Fig. 10. Daily mortality of *M. domestica* adults upon exposure to *Beauveria bassiana*.

DNA Damage in the Exposed Adult Fly (Comet Assay)

In the exposed and control *Musca domestica* adult flies' DNA damage was examined using the comet test. DNA damage was observed in adult flies exposed to the three concentrations of *Beauveria bassiana* strain, $1x10^5$ spore/ml, $1x10^8$ spore/ml, and $1x10^{10}$ spore/ml, as well as in control flies (Table 4 and Fig. 11).

The results revealed appositive correlation between the elevation in spore concentration and the level of total DNA genotoxic damage. They were also elucidated by variance in the tail length, % of DNA in the tail segment, and tail moment. The migration of the fragments of DNA by agarose gel electrophoresis showed, a significant rise (P ≤ 0.05) in the % of DNA damage was detected from13.6±1.9 to 18.3±0.4, the mean tail length elevated from 7.6±0.7 to 11.5±0.5, the % of DNA in the tail raised from 24.4±1.1 to 28.1±0.7, and the tail moment increased with

Table 3. Effect of naturally isolated *Beauveria* spp. fungal suspension on the mortality of *M. domestica* adult.

Concentration (sp/ml) *Beauveria bassiana*	Mean Corrected Mortality (%)±S.E. (Range or Number)	
	Total mortality	Mortality (%)
10^5	7.7±1.2	19.2±2.5
10^8	35.8±1.5	89.1±3.7
10^{10}	39.6±0.6	99.2±0.8
Control	0	0
Correlation coefficient (r)	0.9	0.9

increasing the concentration from 1.7±0.6 to 2.9±0.1. The values elevated gradually with the examed concentrations from 1x105 spore/ml to 1x10^{10} spore/ml (Table 4 and Figure 12). Simultaneously, in the control group a very low damage (4.8±0.3%) in DNA and the other parameters were observed (Table 4 and Figure 13).

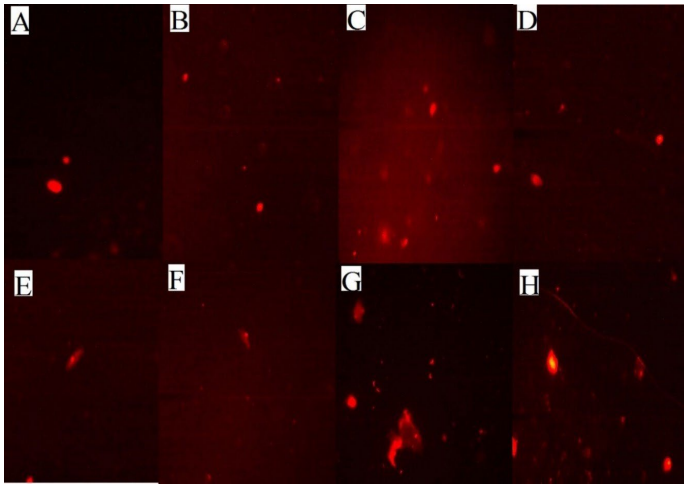

Fig. 11. Comet assay demonstrating the damage in DNA of *Musca domestica* adult flies exposed to *Beauveria bassiana* :(A&B) controlled non-exposed fly, (C&D) low level of damage recorded in that exposed to 10^5 sp/ml, (E&F) showed damage in that exposed to 10^8 sp/ml, and (G&H) showed a elevation in DNA damage to that exposed to 10^{10} sp/ml.

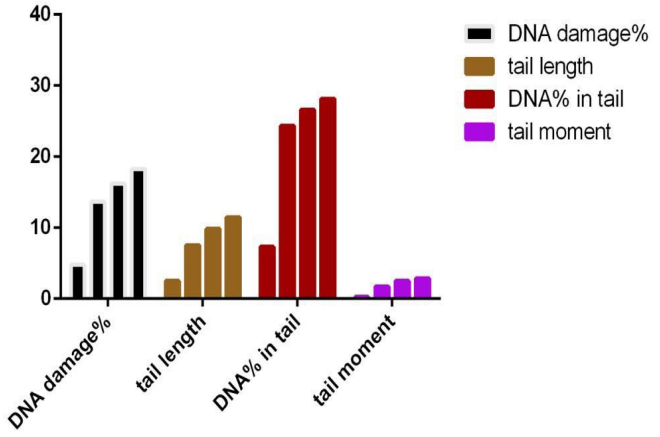

Fig. 12. Different comet parameters and DNA damage levels in adult flies exposed to *Beauveria bassiana* in different concentrations.

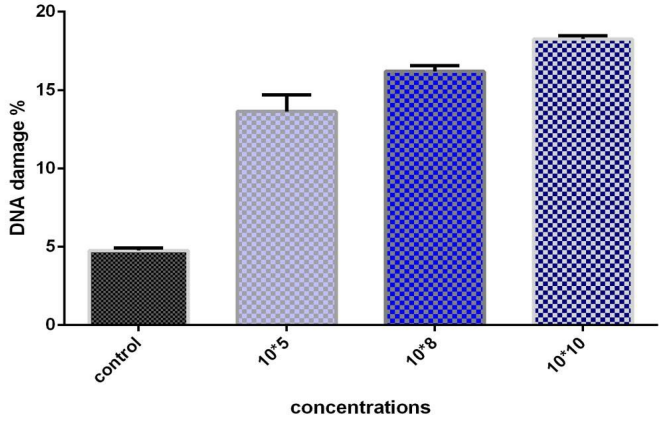

Fig. 13. DNA damage level in adult flies exposed to *Beauveria bassiana* in different concentrations.

DISCUSSION

From the previous results, *Beauveria bassiana* is the most entomopathogenic fungus isolated from localities under investigation. This result agreed with Steinkraus *et al.* (1990), who illustrated the first record of natural infection and isolation of *B. bassiana* from *M. domestica* in New York. Siri *et al.* (2005) also stated the occurrence of *Beauveria bassiana* on *M. domestica* in La Plata, Buenos Aires province, Argentina. Skovgård and Steenberg (2002) elucidated the isolation of *B. bassiana* from house flies in Denmark.

Our study showed that the most prevalent non-entomopathogenic fungal species were *A. flavus* and *Fusarium* spp. This result agreed with Phoku *et al.* (2014), who isolated *Aspergillus* and *Fusarium* from *M. domestica*. Abid *et al.* (2018) reported that the predominat fungi in houseflies, rice, and wheat grains were *Aspergillus* and *Fusarium* followed by *Alternaria* and *Penicillium*. Additionally, Srivoramas *et al.* (2012) stated that On *M. domestica* and the blow fly, *Chrysomya megacephala*, *A. niger* and *A. fumigatus* were isolated. The flies were gathered in September 2010 from Muang and Warinchamrap districts in Ubon Ratchathani.

Davari *et al.* (2012); Srivoramas *et al.* (2012) and Phoku *et al.* (2016) isolated different types of fungi from *M. domestica*, including *Aspergillus* spp. Kaaya and Okech (1990) identified several fungal species isolated from pupae and adults of *Glossina pallidipes*, including *A. flavus*, *A. niger*, *Penicillium* spp., and *Fusarium*. These findings were part of another study on the isolation and identification of fungi in *Muscidae dipterous*, which agreed with the present study.

Concerning the precise identification tool of entomopathogenic fungi is the DNA-based molecular characterization. Thus, using rDNA-ITS regions, isolates were genetically characterized consequently, the ITS region of rDNA were amplified result in a single product approximately 545bp fragment size for the isolate. It was the same to the 500bp fragment size demonstrated from ITS-rDNA regions of isolates amplified with ITS1-ITS4 primers for 8 *Beauveria bassiana* isolates (Richter *et al.*, 2016) and 560bp fragment size for 8 isolates of *Beauveria bassiana* (Belay *et al.*, 2017). Earlier studies showed amplicons of *B. bassiana* at 320bp12. In contrast, other more relevant studies showed that the DNA band of *B. bassiana*, separated using agarose gel electrophoresis was about 750bp14. Surprisingly, our strain showed more similarity to an isolate from Costa Rica than those isolated from neighboring countries, such as Italy, India, and Syria. Fernandes *et al.* (2009) and Wang *et al.* (2003) also agreed with this stating that the higher the genetic distances were associated with greater geographical distances.

The popular way to control different flies biologically is using entomopathogenic fungi. *B. bassiana* is the most popular insecticidal fungus that destroys house flies (Mwamburi *et al.*, 2009).

Entomopathogenic fungi have been revealed to penetrate the host cuticle after short time from germination. The spores germinate and penetrate the insect skin and enter the host. Once the fungus penetrates the host, it produces toxins that overcome the insect's immune system (Hasaballah *et al.*, 2017).

The result of different concentrations of *Beauveria* spp. on the mortality percentage of adult house flies is shown in (Table 3). As a result of the high density of setae on adult flies, entomopathogenic fungi are supported and spread, leading to a relatively significant mortality of adult house flies (16.6-99.2). These setae are well known for supplying the ideal moisture level to facilitate conidial germination (Mishra 2016). In the present study, the daily recorded mortality in adult *M. domestica*, treated with a fungal suspension of *B. bassiana*, revealed a gradual effect expressing a maximum lethal effect after 7-9 days post-infection. The same duration was recorded by Sharififard *et al.* (2011) using *M. anisopliae* against *M. domestica*. A concentration-dependent response was evaluated by Farooq and Freed (2016), who used immersion and bait method infection on the adult flies and larvae of *M. domestica*, recording mortality ranging from 53 to 96% using various isolates of entomopathogenic fungi, *B. bassiana*,

Table 4. Different comet parameters and DNA damage levels in adult flies exposed to *Beauveria bassiana* in different concentrations.

Concentrations of *Beauveria bassiana*	DNA damage (%)	Tail length (PX)	DNA in tail (%)	Tail moment	Olive tail moment
Control	4.80±0.30	2.50±0.20	7.30±0.70	0.20±0.05	0.60±0.06
10^5	13.60±1.90	7.60±0.70	24.40±1.10	1.70±0.60	1.50±0.50
10^8	16.20±0.60	9.80±0.60	26.60±0.30	2.50±0.10	2.50±0.05
10^{10}	18.30±0.40	11.50±0.50	28.10±0.70	2.90±0.10	2.80±0.20
p-value ≤ 0.05					

M. anisopliae, and *Isaria fumosorosea*. The role of entomopathogenic fungi, such as *M. anisopliae*, was also assessed against *M. domestica* by Sharififard *et al*. (2011), they measured the pathogenicity of *M. anisopliae* against adult house flies, causing 48% and 72% mortality using 10^5 and 10^7 sp/g. larval mortality was 36% and 69% using 10^6 and 10^8 spore/g.

Entomopathogenic fungi are a natural approach for controlling pests that expresses a promising tactic in integrated pest management programs. The presented study aimed to collect and identify fungal isolates from the same habitat as *M. domestica* and explain the virulence of the Egyptian isolate of *B. bassiana* against *M. domestica* adult stages. It is worth mentioning that the isolated strain differs in pathogenicity from other studied strains. The reasons for this difference may be attributed to fungi-related characteristics, including species origin, application strategy, as well as environmental and mass culturing factors (Farooq and Freed, 2016).

The toxic effect of *Beauveria bassiana* on *Musca domestica* adult fly was recognized. *Beauveria bassiana* induced an evident damage in DNA of *Musca domestica* adult flies that increased by raising the concentration of the spores. Dua *et al*. (2013) explained that the Psoralea corylifolia Linn essential oil resulted in a genotoxic effect on Culex quinquefasciatus, inducing DNA damage of 6.713% and 8.864% and with mean comet tail lengths of 6.2548 µm and 8.47 µm, respectively. Kumar *et al*. (2015) additionally observed the genotoxic effect of *Acorus calamus* on *Drosophila melanogaster* adult flies. Comet tail length revealed equivalent DNA damage concerning controls. Zahoor *et al*. (2020) showed that *Penganum harmala, Datura stramonium* caused percentage of DNA damage, which show positive correlation between increasing the concentration and DNA damage. Additionally, it has been noted that alkaloids destroy a target organism by attaching to their DNA and interfering with DNA synthesis and replication.

CONCLUSION

Considering the lethal effect of *B. bassiana* on adult mortality, it is a significant lethal agent decreasing the adult house fly numbers within a week after application. Thus, reducing the bothering effect of house flies on both human and animals, rendering the fecundity of the whole population of house flies and decreasing the oviposition individuals for building up further insect generations, hence, it is recommended to apply *Beauveria* spp. as a spray in house fly breeding areas.

CONFLICT OF INTEREST

The authors declare no competing interests.

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