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Efficacy of some entomopathogens against *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae) in stored date fruits

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Abstract

Background: *Ephestia cautella* (Walker) (Lepidoptera:Pyralidae) is one of the most economically important date fruits insect pests, which causes major losses in quantity and quality of the date yield. The present study aimed to evaluate 3 of the entomopathogens: *Beauveria bassiana* (Basonym 1836), *Metarhizium anisopliae* (Metchnikoff 1879), and *Bacillus thuringiensis* (Berliner 1915) var. *kurstaki* individually and mixed with each other against *E. cautella* in stored date fruits.

Results: Four concentrations from spores' suspension of each entomopathogen were sprayed on 50 eggs of *E. cautella* (24:48 h. old) (for fungal pathogens) and 30 2nd instar larvae of *E. cautella* in glass jars. Pathogenicity was evaluated at different time intervals post treatments 3, 5,7 and 14 days. Obtained results showed that mortality percentages of *E. cautella* stages increased by increasing the tested entomopathogen concentrations and increasing of the exposure time. Results indicated that the reduction percentages of *E. cautella* eggs hatchability after separately treated at the concentrations (3.0×10^5 , 3.0×10^6 , 3.0×10^7 and 3.0×10^8 spores/ml) were (27.50, 39.17, 48.33 and 62.50%) for *B. bassiana*, (35.83, 44.17, 50.83 and 69.17%) for *M. anisopliae*, respectively. After 14 days of treatment, the recorded larval mortalities were (49, 62, 77, and 81%) for *B. bassiana* and (60, 66, 78, and 89%) for *M. anisopliae* at concentrations (3.0×10^5 , 3.0×10^6 , 3.0×10^7 and 3.0×10^8 spores/ml), respectively. In case of treating *E. cautella* larvae with *B. thuringiensis*, the mortality percentage was (44, 52, 63, and 72%) at concentrations (2.0×10^8 , 2.0×10^9 , 2.0×10^{10} and 2.0×10^{11} spores/ml), respectively, after 14 days of exposure. *E. cautella* larvae were more susceptible to *B. bassiana* and *M. anisopliae* than the eggs. *M. anisopliae* was more effective than *B. bassiana*. The combination of the entomopathogens LC50 (*B. bassiana* + *M. anisopliae*, *B. bassiana* + *B. thuringiensis*, *M. anisopliae* + *B. thuringiensis* and *B. bassiana* + *M. anisopliae* + *B. thuringiensis*) caused *E. cautella* larval mortality percentages of 67.00, 73.33, 63.33 and 86.29%, respectively, 14 days after exposure. The combination of the 3 tested entomopathogens (*B. bassiana* + *M. anisopliae* + *B. thuringiensis*) increased their efficacy for controlling *E. cautella*.

Conclusions: The combination of the tested entomopathogens: *B. bassiana*, *M. anisopliae* and *B. thuringiensis* could be recommended for controlling *E. cautella* stages in stored date fruits.

Keywords: Virulence, *Ephestia cautella*, *Metarhizium anisopliae*, *Beauveria bassiana*, *Bacillus thuringiensis*, Stored date fruits

Background

The date palm has great importance as a source of food and heritage symbols, especially in the Arab world, which is characterized by the breadth of its area and variety of its climate, which helped within the spread of date palms cultivation in many areas of it. Egypt occupies the

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primary of date production within the world since 2001, accounting 21.5% of the world's date production. Several pests attack all parts of the date palms, causing great losses affecting the yield quantity, and quality of the date fruits. One of the most important and economic date fruits insect pests is *Ephestia cautella* Walker (Lepidoptera: Pyralidae).

Ephestia cautella was a major worldwide insect of stored products. It founds in tropical and temperate zones and commonly infests date fruit, grains, nuts, dried fruits, and many varieties of other stored products (Horak 1994). Larval stage was the harmful stage and its feeding pollutes stored products with feces and web-bings that caused products spoiling. Also, the almond moth (fig moth) *E. cautella* was recorded in Siwa Oasis and the Governorate of New Valley in Egypt (Kashif et al. 2002) in Iraq (Hameed et al. 2011) and in Tunisia (Ben Jemaa et al. 2012). The larvae of *E. cautella* infest dry and semi-dry fallen date fruits moreover, stored dates during storage.

Many chemical insecticides and fumigates have been used as protectants against insect infestation in stored grains and fruits. But their indiscriminate use and residual toxicity affects the non-target animals and human beings. The sustainable alternative to pesticides is biological control which could give a share in reducing the use of synthetic insecticides. The promotion and use of entomopathogens as classical, conservation, biological control agents have achieved successes and a few failures in the past 15 years. The long-run directions of insect-specific viruses, bacteria, fungi, and nematodes as components of integrated pest management strategies for control of crops arthropod pests of forests, urban habitats, and insects of the medical and veterinary field. The entomopathogenic bacteria are commercially developed for controlling the lepidopteran larval pests as *B. thuringiensis* var. *kurstaki*. These pathogens merge the advantages of chemical pesticides and MCAs: they're fast-acting, inexpensive to make, cheap to combine, have a protracted-time period, and could be delivered using traditional application equipment and systems (Lacey et al. 2015).

Fungi are common natural entomopathogens that frequently cause epizootics in their hosts and they have several beneficial characteristics that support their growth as MCAs. Some species such as: *B. bassiana* and *M. anisopliae* var. *anisopliae* that controlled a numerous numbers of pests' species have been effectively utilized on a wide scale as microbial control agents (Srinivasan 2012). Commercialized microbial pesticides based on entomopathogenic fungi (EPF) are now primarily confined to niche markets. EPF control invertebrate pests, according to Lacy et al. (2001). The efficacy of 4 fungal

isolates against Indian meal moths: *B. bassiana*, *Verticillium lecanii* (Balazy), *M. anisopliae* var. *anisopliae*, and *Paecilomyces farinosus* (Brown & Smith) was tested. All of the fungal isolates tested were pathogenic, but to varying degrees (Büda and Pečiulytė 2008).

The aim of the present work was to evaluate the efficacy of 3 entomopathogens, 2 fungi: *M. anisopliae* and *B. bassiana*, and the bacterium, *B. thuringiensis* separately and on combinations against the stored date fruit pest *E. cautella*.

Methods

Rearing of tested insect

The tested insect pest, *E. cautella*, was collected from infested date fruits and was reared at Date Palm Pests and Diseases Department, Central Laboratory of Date Palm, Agricultural Research Center, Giza, Egypt. Adult insects were reared on semi-dry date fruits Siwi cultivar. The date fruits used in rearing culture were conserved at the freezer for 2 weeks before using them to kill potential contamination with other pests. The 2 stages of *E. cautella* eggs and larvae were separately evaluated. All insect's cultures and experiments were conducted at 26 ± 2 °C and $65 \pm 5\%$ (RH), with 16 h of light and 8 h. of darkness (Abd El-Aziz et al. 2012).

Entomopathogens tests

Isolation and identification of fungi

Two isolates of the EPF were isolated at Bio-insecticide Production Unit, Plant Protection Research Institute, Agricultural Research Center (ARC), Giza, Egypt. The first isolate, *Beauveria Bassiana* (AUMC 9808) was isolated from diseased larvae of the cotton leaf worm *Spodoptera littoralis* (Boisduval 1833) collected from a sugar beet field and was identified at the Mycological Center, Faculty of Science, Assiut University (Sahar and Mohararam 2014). The second isolate, *Metarhizium anisopliae* was isolated from red palm weevil, *Rhynchophorus ferrugineus* (Olivier 1790). Eggs were obtained from the Department of Date Palm Pests and Diseases, Central Laboratory for Date Palm, ARC, Giza, Egypt, covered with fungi placed on Czapek-dox agar (CZA) medium and incubated at 27 ± 2 °C for 7–10 days to allow growth of infecting fungus. Pure slant culture of fungus was identified in Plant Pathology Research Institute, ARC, Giza, Egypt.

Fungi culture

The isolates were cultured on (CZA) medium with 1% yeast extract plates in several Petri dishes (9 cm in diameter), and were grown for 15 days at 27 ± 2 °C. The conidia were harvested by scraping the surface of 14–15 days old culture gently with inoculation needle. The conidia were

suspended in distilled water containing 0.1% Tween-80. The mixture was stirred by a magnetic shaker for 10 min and then 4 concentrations of spore's suspension were prepared from each of the fungal isolates (3×10^5 , 3×10^6 , 3×10^7 and 3×10^8 spores/ml).

Culture of entomopathogenic bacterium

One strain of *Bacillus thuringiensis* subsp. *Kurstaki* (Btk) was obtained from the Bio-insecticide Production Unit, Plant Protection Research Institute ARC, Giza, Egypt. T3 medium which was composed of (tryptone 3.0 g, tryptose 2.0 g, yeast extract 1.5 g, MnCl₂ 0.005 g, and NaH₂PO₄·H₂O 8.9 g, adjusted PH at 6.8), was prepared and the final volume was made up to 1 L with distilled water. The medium was sterilized at 121 °C for 20 min, and inoculation was occurred with the standard inoculums. The inoculated flask was incubated on a shaker (142 rpm) at 28 °C for 72 h. (Attathom et al. 1995). Four concentrations (2×10^8 , 2×10^9 , 2×10^{10} , and 2×10^{11} spores/ml) were prepared by plate count method.

Bioassay tests

Efficacy of entomopathogenic fungi on eggs of *E. cautella*

Fifty eggs of *E. cautella* (24:48 h. old) were placed each into a small glass jar (200 ml volume), every jar was treated with the 4 concentrations of each fungal concentration (3×10^5 , 3×10^6 , 3×10^7 and 3×10^8 spores/ml) then filled with about 50 g Siwi date fruits and covered with cloths fixed with rubber bands. The reduction percentage of eggs hatchability was calculated after treatments. Then the calculated LC₅₀ values of each fungus were combined to study their effects on *E. cautella* eggs. Then the percentage reduction in egg hatching was calculated.

Efficacy of entomopathogenic fungi and bacterium on larvae of *E. cautella*

B. bassiana, *M. anisopliae* and *B. thuringiensis* were sprayed against 30 individuals of 2nd *E. cautella* larvae that placed into a glass container (200 ml volume). The larval instar was chosen before penetrating the date fruit and feeding on it from inside. Four concentrations of each fungus (3.0×10^5 , 3.0×10^6 , 3.0×10^7 and 3.0×10^8 spores/ml) and 4 as well of the bacterium *B. thuringiensis* (2.0×10^8 , 2.0×10^9 , 2.0×10^{10} and 2.0×10^{11} spores/ml) were tested. Each pathogen was applied by 2 methods (single and mixed) against the date fruit pest *E. cautella* in stored date fruit under laboratory conditions of 26 ± 2 °C and $65 \pm 5\%$ R.H. The experiment was replicated 4 times. The number of dead larvae in each jar was counted on specific dates after 3, 5, 7, and 14 days from the treatments and the percentages of mortality were recorded.

Combination of the three entomopathogens

The LC₅₀ value of the tested fungus and bacterium was calculated, to study their mixing effects. The different combinations *B. bassiana* + *M. anisopliae*, *B. bassiana* + *B. thuringiensis*, *M. anisopliae* + *B. thuringiensis* and *B. bassiana* + *M. anisopliae* + *B. thuringiensis* were evaluated to study the pathogens combination on *E. cautella* mortality.

Statistical analysis

Mortality rates of insects were corrected using the Abbott formula (Abbott 1925) compared to the control (untreated). LC₅₀ and LC₉₀ were calculated through the probit analysis as described by Finney (1971). Comparison between the mortality percentages using the LC₅₀ values of the three tested pathogens combinations were analyzed by Proc., ANOVA in SAS (SAS Institute 2006).

Results

Virulence of entomopathogens

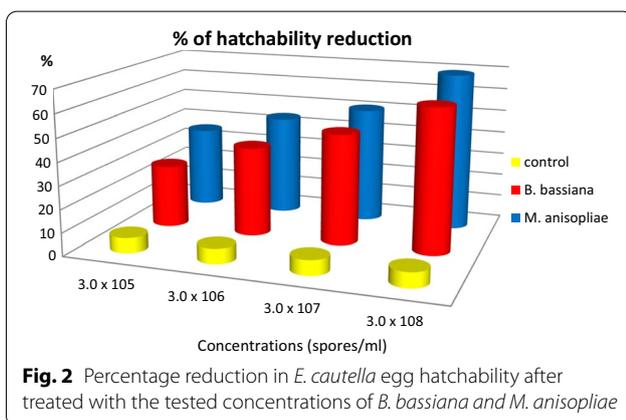
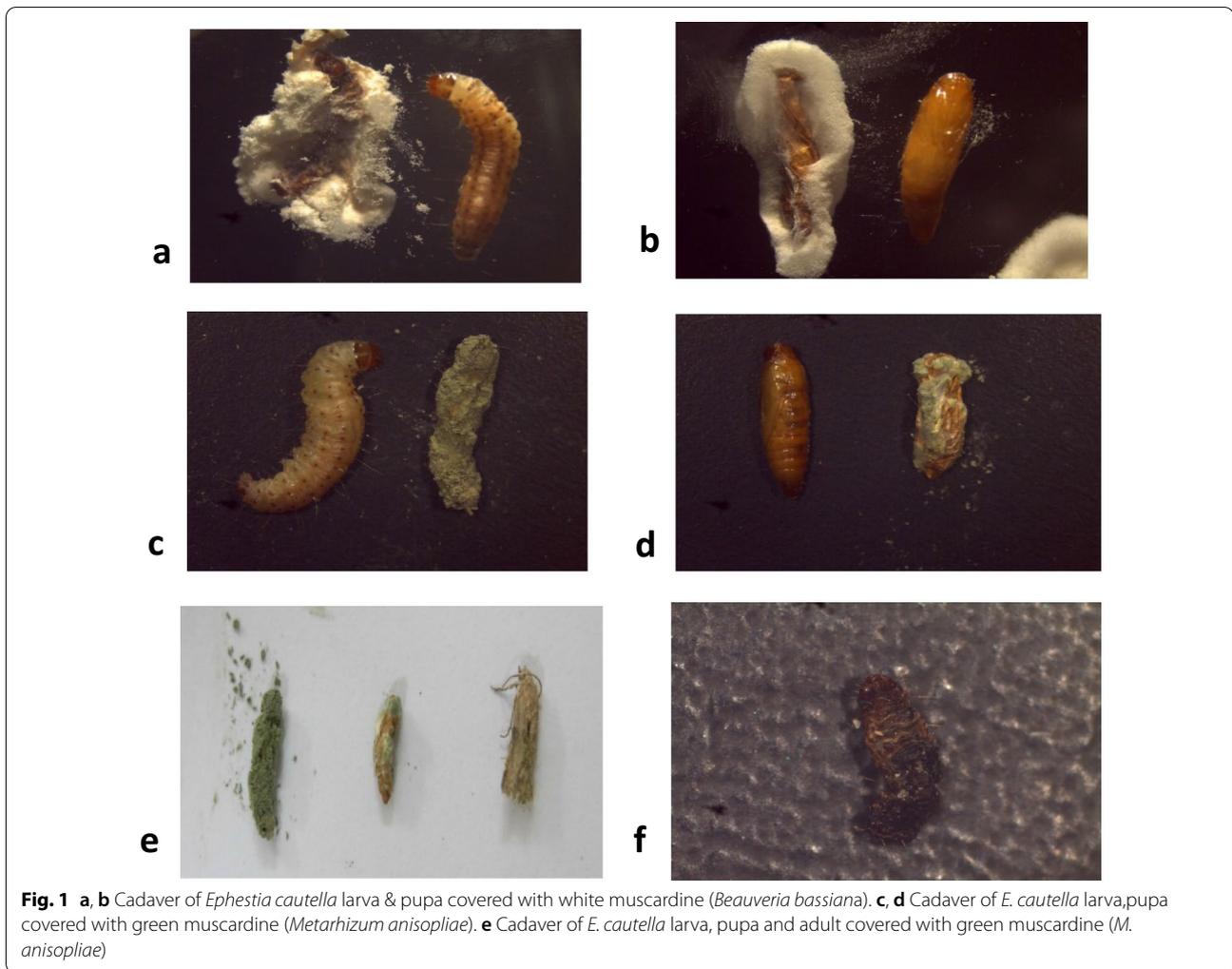
Infections' symptoms of *E. cautella* stages with the tested entomopathogens were illustrated in (Fig. 1).

B. bassiana and *M. anisopliae* on *E. cautella* eggs

The reduction percentages of *E. cautella* egg hatchability after separately treated at the concentrations (3.0×10^5 , 3.0×10^6 , 3.0×10^7 and 3.0×10^8 spores/ml) were (27.50, 39.17, 48.33 and 62.50%) for *B. bassiana* and (35.83, 44.17, 50.83 and 69.17%) for *M. anisopliae*, respectively (Fig. 2). The results showed that *M. anisopliae* was more effective than *B. bassiana*, and the percentage reduction in egg hatchability increased by increasing the concentrations. The LC₅₀ values were 6.0×10^7 spores/ml for *B. bassiana* and 2.0×10^7 spores/ml for *M. anisopliae* at 14 days of treatment (Table 3). As compared to control, the percentage reduction in the egg hatching for mixed fungal treatment was 66.6%.

B. bassiana and *M. anisopliae* on *E. cautella* larvae

Efficacy of 4 concentrations of the 2 fungi against *E. cautella* larvae at 26 ± 2 °C, $65 \pm 5\%$ RH, and different exposure periods were presented in Table 1. The results showed that the percentage of corrected mortality of the tested larvae increased by increasing the concentrations and or exposure time. Larval mortality was recorded (49, 62, 77, and 81%) for *B. bassiana* and (60, 66, 78, and 89%) for *M. anisopliae* after 14 days of exposure to the concentrations (3.0×10^5 , 3.0×10^6 , 3.0×10^7 and 3.0×10^8 spores/ml), respectively.



***B. thuringiensis* on *E. cautella* larvae**

The virulence of 4 concentrations of, *B. thuringiensis* against *E. cautella* larvae at 26 ± 2 °C, $65 \pm 5\%$ RH,

and at different exposure periods are listed in Table 2. The results showed that the corrected mortality % of *E. cautella* larvae increased by increasing the concentrations and or the exposure time as it was recorded (44, 52, 63, and 72%) at concentrations (2.0×10^8 , 2.0×10^9 , 2.0×10^{10} and 2.0×10^{11} spores/ml), respectively, after 14 days of exposure.

The LC_{50} for *B. bassiana* and *M. anisopliae* were 0.5×10^6 spores/ml and 1.2×10^5 spores/ml, respectively, after 14 days of treatment and was 1.7×10^9 for *B. thuringiensis* after 5 days of treatments (Table 3). The results showed clearly that *E. cautella* larvae were more susceptible than the eggs. The same trend was recorded at the LC_{90} level for the 2 tested insect stages.

Combined effect of the 3 entomopathogens on *E. cautella* larvae

The LC_{50} of the 3 entomopathogens were mixed in different combinations (*B. bassiana* + *M. anisopliae*, *B.*

Table 1 Corrected mortality percentage of *Ephestia cautella* larvae treated with *Beauveria bassiana* and *Metarhizium anisopliae* at different exposure periods

Concentrations	Mortality % of <i>E. cautella</i> larvae after exposure periods							
	3 days		5 days		7 days		14 days	
	<i>B. bassiana</i>	<i>M. anisopliae</i>	<i>B. bassiana</i>	<i>M. anisopliae</i>	<i>B. bassiana</i>	<i>M. anisopliae</i>	<i>B. bassiana</i>	<i>M. anisopliae</i>
3.0 × 10 ⁵	14.67 d	17.50 d	26.88 d	44.17 b	38.17 c	50.00d	49.17 d	60.50 d
3.0 × 10 ⁶	22.17 c	28.17 c	32.59 c	48.00 b	43.50 c	57.88 c	62.00 c	66.33 b
3.0 × 10 ⁷	28.33 b	35.00 b	41.33 b	62.88 a	51.33 b	68.59 b	77.88 b	78.59 b
3.0 × 10 ⁸	33.83 a	40.88 a	54.50 a	66.59 a	61.59 a	77.33 a	81.59 a	89.88 a
L.S.D	4.5482	4.1843	5.0939	5.4578	5.6397	3.2747	2.7289	2.365
Pr	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Mean in same column followed by the same letters is not significant

L.S.D., least significant difference; Pr., probability level

Table 2 Corrected mortality percentage of *Ephestia cautella* larvae treated with *Bacillus thuringiensis* at different exposure periods

Concentrations	Mortality % of <i>E. cautella</i> larvae after exposure periods			
	3 days	5 days	7 days	14 days
2.0 × 10 ⁸	6.67 d	17.59 d	31.50 d	44.59 d
2.0 × 10 ⁹	16.33 c	22.33 c	42.17 c	52.00 c
2.0 × 10 ¹⁰	26.17 b	28.17 b	56.00 b	63.17 b
2.0 × 10 ¹¹	30.50 a	36.00 a	68.88 a	72.50 a
L.S.D	4.0024	4.3662	4.912	5.2759
Pr	<.0001	<.0001	<.0001	<.0001

Mean in same column followed by the same letters is not significant

L.S.D., least significant difference; Pr., probability level

bassiana + *B. thuringiensis*, *M. anisopliae* + *B. thuringiensis*, and *B. bassiana* + *M. anisopliae* + *B. thuringiensis*) (Table 4). The corrected mortality percentages of *E. cautella* larvae were 67, 73, 63 and 86% for the LC₅₀ values of the previously mentioned combinations, respectively.

Discussion

Results of the present study showed that EPF and bacterium (*Bt*) had a potential effect on *E. cautella* larvae. Use of more than one pathogen to control the *E. cautella* larvae showed higher effects than using each one separately. The results are in agreement with those obtained by Alwaneen et al. (2020) who mentioned that *B. bassiana* isolates were screened against different developmental stages of *E. cautella*. The developmental stages were directly exposed to each fungus isolate at a concentration of 1 × 10⁷ conidia/ml. It was observed that all the fungus isolates affected the hatchability of eggs, causing significant larval and pupal mortality. Bahmani et al. (2020) investigated the effects of *B. thuringiensis kurstaki* bacteria and *B. bassiana* pathogenic fungi on the population dynamics of *Ephestia kuehniella* L., the most dominant pest of date stored in Khuzestan Province. It was mentioned that regarding the highest potential of *B. bassiana* and *Btk* in reducing the stock pest population, it is very possible to exploit this interaction for biocontrol. In another consensual study, *B. bassiana* caused a reduction in *E. cautella* egg hatching reached (15.3%) and (85.22%) larval mortality at the highest concentration of the

Table 3 LC₅₀ and LC₉₀ values with their confidence limits for eggs and larvae of *Ephestia cautella* treated with three entomopathogens at 14 days after *Beauveria bassiana* and *Metarhizium anisopliae* treatments and after 5 days for *Bacillus thuringiensis* treatments

Stages	Treatments	LC50 (Spores/ml)	LC90 (Spores/ml)	Confidence limits (Spores/ml)				Slope ± SD
				LC 50		LC90		
				Lower	Upper	Lower	Upper	
Eggs	<i>Beauveria bassiana</i>	6 × 10 ⁷	5 × 10 ¹¹	2 × 10 ⁷	2 × 10 ⁸	3 × 10 ¹⁰	2 × 10 ¹⁴	0.328 ± 0.059
	<i>Metarhizium anisopliae</i>	2 × 10 ⁷	4 × 10 ¹¹	7 × 10 ⁶	7 × 10 ⁷	2 × 10 ¹⁰	4 × 10 ¹⁴	0.296 ± 0.058
Larvae	<i>B. bassiana</i>	0.5 × 10 ⁶	4 × 10 ⁹	7 × 10 ⁴	1 × 10 ⁶	6 × 10 ⁸	2 × 10 ¹¹	0.329 ± 0.61
	<i>M. anisopliae</i>	1.2 × 10 ⁵	1 × 10 ⁹	8 × 10 ³	5 × 10 ⁵	2 × 10 ⁸	3 × 10 ¹⁰	0.326 ± 0.063
	<i>B. thuringiensis</i>	1.7 × 10 ⁹	1.6 × 10 ¹⁴	3 × 10 ⁸	5 × 10 ⁹	6 × 10 ¹²	5 × 10 ¹⁷	0.259 ± 0.058

Table 4 Effects of LC₅₀ combined values of *Beauveria bassiana*, *Metarhizium anisopliae* and *Bacillus thuringiensis* on the percentage mortality of *Ephestia cautella* larvae

Treatments	Mortality % of <i>E. cautella</i> larvae after exposure periods			
	3 days	5 days	7 days	14 days
<i>B. bassiana</i> + <i>M. anisopliae</i>	56.66 c	63.33 b	67.00 b	67.00 c
<i>B. bassiana</i> + <i>B. thuringiensis</i>	66.66 b	66.66 ab	73.33 a	73.33 b
<i>M. anisopliae</i> + <i>B. thuringiensis</i>	53.33 d	53.33 c	60.00 c	63.33 c
<i>B. bassiana</i> + <i>M. anisopliae</i> + <i>B. thuringiensis</i>	70.00 a	70.00 a	76.66 a	86.29 a
L.S.D	1.8193	3.6385	5.4578	5.8217
Pr	<.0001	<.0001	<.0001	<.0001

Mean in same column followed by the same letters is not significant

L.S.D., least significant difference; Pr., probability level

fungus (1×10^8). On the other hand (Büda and Pečiulytė 2008) tested the effect of 4 fungal species (*Beauveria bassiana* (*B.b.*), *Lecanicillium* (*Verticillium*) *lecanii* (*L.l.*), *Metarhizium anisopliae* var *anisopliae* (*M.a.*) and *Paecilomyces farinosus* (*P.f.*) isolates on mature larvae of Indian meal moth, *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae). Under laboratory conditions, during the first-three-day period after spraying, the highest mortality (35–40% versus control) caused by *P.f.* and *M.a.* There was non-significant difference in the survival rate than the control when *B.b.* and *L.l.* were sprayed. The median lethal time period when mortality reached 50% (LT₅₀) or 100% (LT₁₀₀) varied depending on the fungus species from 1 to 5 days and from 9 to 12.3 days, respectively. *P.f.* was effective to *P. interpunctella* adults, but not to the larvae of the species, as during 14 days of testing 50% of mortality was recorded.

Conclusions

It was concluded that the combination of *B. thuringiensis* with *B. bassiana* and *M. anisopliae* increased the mortality percentages of *E. cautella* than using each alone. The mortality reached its maximum by using the mixture of the 3 entomopathogens. Thus, the mixture could be recommended for controlling *E. cautella* stages in stored date fruits.

Abbreviations

Btk: *Bacillus thuringiensis* Subsp. *kurstaki*; Bt: *Bacillus thuringiensis*; ARC: Agricultural Research Center; CZA: Czapek-dox agar; MCAs: Microbial Control Agents; *r*: Correlation coefficient of regression line; SD: Standard deviation of the mortality regression line; L.S.D.: Least significant difference; Pr: Probability level; EPF: Entomopathogenic fungi.

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Author contributions

WKM provided the stages of the insect that were used in the experiment, as well as providing isolation of the: *M. anisopliae*, fungus, examined and recorded the data, and analyzed the mortality percentages statistically and was a major contributor in writing the manuscript. RHM Rania helped prepare insects for biological experiments and check plagiarism. SSA Participated in providing the isolate of the *B. bassiana*, and *B. thuringiensis* the isolate of the bacteria used in the evaluation, making their concentrations and writing the discussions. All authors read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate

Not applicable, because our manuscript report neither studies involving Human participants nor human data or human tissue.

Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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