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Study of the Efficacy of Irradiated Fungus Beauveria bassiana to Control Fig Moth Pupae.

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P A P E R I N F O

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A B S T R A C T

The ability of the fungus *Beauveria bassiana* was tested when exposed to ultraviolet light at a wavelength of 312nm for 4 and *8minutes.*A significant decrease was observed in the rates of adult emergence from fig moth pupae *Ephestia cautella* treated with fungi which was exposed to radiation with increasing fungal concentrations. Additionally, the percentage of albumin in the blood was decreased by 14.30 and 16.10µg.ml^{-1} for adults of the fig moth, that emerged from pupae at the age of (1-2) and (6-8)days, respectively, when sprayed to fungus solution irradiated for 4minutes at a concentration of 1.5×10^{7}spore.ml$^{-1.}$. This result was compared with adult insects resulted from pupae, which sprayed to a fungus solution that was not exposed to radiation. They reached 28.12 and 28.15µg.ml^{-1}, separately, and the amount of glucose in the blood also decreased, reaching 24.00 and 34.00µg.ml^{-1} for adult insects that resulted from pupae at the age of (1-2) and (6-8)days, respectively. The number of the hemocytes decreased 119 and 155cells, individually, for adult insects that resulted from pupae sprayed to the irradiated fungus, while 185 and 220cells to insects that resulted from pupae sprayed with the same concentration of non-irradiated fungal solution.

1. INTRODUCTION

Insects are one of the main causes of economic and qualitative losses that occur in stored and packaged foodstuffs, including dates, as they cause great economic damage.This destruction takes place as a result of the spoilage of those dates. The insect *Ephestia cautella* is one of the most common insects that infect dates, and the saw-grain beetle *Oryzaephilus surinamensis*(L.) also infects dates. Thus, insect infestation remains a constant and serious threat. Dates are regarded as one of the best food crops in Iraq, but stored dates are infested with a large number of insects, some of which belong to the genus *Ephestia* spp. of the order Lepidoptera. It was mentioned[1] that the larvae of the fig moth infect the dates falling from the palm tree and the dates are left after harvesting.More over the infection starts from the orchard and continues in stores throughout the months of the year markets and consumption. The types of date moths, which are *E. cautella*, *E. calidella* and *E. figulilella*, are among the species that spread globally.They are found to attack dates, raisins, figs, field pistachios and dried grapes, causing economic losses if they are not controlled in the field and in the store[2]. Two main factors make this pest danger to stored dates.These are the ability of the insect to develop resistance against chemical insecticides as well as the tendency of the larvae of this species to feed inside the date fruit. It was stated [3] that the genus Ephestia is one of the most important pests in flour factories in hot countries, as it causes severe damage to all stored winter products. Inaddition, the infection rates may reach about 42% if it is not effectively controlled. Thus, recent studies focused on this field, including searching for modern means to control insect pests in the Iraqi environment. Chemical control is one of the traditional methods of controlling insects that have shown a high ability to kill pests. However, the long-term negative effects of the use of pesticides and what they cause of pollution to the environment , the risks to human health and domestic animals and finally the imbalance they cause in the natural balance through their negative impact on vital enemies are the interests of research.The current study investigates using biological resistance elements in order to protect the crop without harming the environment. Among the most important elements that

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have proven efficient and received wide attention are fungi that are pathogenic to insects. The pathogenic fungus *B. bassiana* is utilized by contaminating males of the red palm weevil, *R. ferrugineus*, with the fungus and releasing it into palm groves. Males are released[4], and among the physical methods that are commonly used during the last decades of the last century and the current one in controlling store insects is the use of electromagnetic energy such as infrared, gamma and ultraviolet rays[5].

2. MATERIALS AND METHODS

2.1. Collecting and Breeding Insects

For the purpose of conducting experiments, a laboratory colony of *E. cautella* was used, which was raised on an artificial nutrient media (81% wheat groats, 12% glycerine, 6% date molasses, and 1%yeast) according to the method mentioned in[6]. 125g of industrial food was placed inside a sterile glass bottle with a diameter of 14cm and a height of 22cm, then 10 pairs of newly emerged insects were released into it. 25±2C°, relative humidity 50 ±5%, and lighting duration (8L:16D) hours for 25 days, which were monitored daily for the purpose of obtaining pupae up to adult insects. The larvae appeared and developed into the fifth larval stage, as they were observed in a wandering state for the purpose of preparation for pupation. Breeding lasted for two generations before being tested. Five replications were used for each concentration of fungus, and in each replication five pupae for each age group . The death rate was calculated from Equation (1) given in[7].

% killing =(treatment in killed insects No.)/(total insects No.)x100%	(1)

Corrected death rates were also calculated from Equation (2) given in[8].

%Corrected kill = (%kill in control - % kill in experiment) / (% kill in control -100)X100%	(2)

2.2. Preparation of the Main Concentrate of the Fungus

Use a container plate on the pure fungus colony to prepare the basic spore suspension (colony age 12days). After that, 5ml sterile distilled water containing Tween-20 solution as a moisture preservative was added to it .Then, the spores were mixed well.The contents have been filtered using a funnel with a small piece of medical gauze.After that, it was placed in a 50 ml glass beaker, after which 5ml from distilled water was added to remove all spores. It was shaken for 4minutes with a vortex device in order to separate the spores from the mycelium. After obtaining the filtrate, distilled water was added to complete the volume to 100ml, which represented the basic suspension.[9] Then, a spores counting slide was used by taking 0.1ml of the base suspension and placing it on a counting slide at 40× magnification. The number of spores inside the number of cells reached 60 spores. We multiplied it by the conversion number $2.5x10^5$ using the equation: $60x\ 2.5x10^5 = 1.5x10^7$ spore.ml^{-1} concentration of the basic solution[10]. Accordingly, it is considered a source for obtaining other concentrations.

2.3. Preparation of Different Concentrations of Fungus

A quantity of the basic fungus suspension was diluted to reach the required concentration for the fungus *B. bassiana* through the following equation: The quantity taken from the original fungal solution(ml)= The concentration of the fungicide solution required for the purpose of the experiment/ the concentration of the basic fungus solution.

For 30ml of fungal solution with a concentration of 10^6 spore.ml^{-1} of the basic solution, the relation: $10^6/10^7$ =0.1ml is used .Then the product of the relation, multiply by the amount of fungicide solution required, which is (30ml).Thus, the result becomes 3ml. This amount of the basic solution is taken and 27ml of distilled water is added to it. In this way, 30ml is obtained from the required concentration, which is 10^6 spores.ml^{-1}. In the same way, concentrations are obtained. The other (1.5×10^4,10^5,10^6 and 10^7spores.ml^{-1}) flasks are kept in the refrigerator at 4C^0.

2.4. To Obtain Pupae from Inside the Breeding Bottles

They were collected carefully with forceps, and divided into two groups, age (1-2) and (6-8)days. The pupae were placed in plastic dishes with a diameter of 10cm. It contains a filter paper that is moistened with distilled water, the conical flasks containing the concentrations of the fungal solution were exposed to UV rays for 4 and 8minutes from a distance of 20cm. After that, the pupae were sprayed in each repeater and for each age group with fungus. Regarding the control group, it was sprayed with a pathogenic fungal solution that was not exposed to radiation, and all samples were placed in volumetric flasks containing 50g of artificial food. All these samples were kept in the incubator at room temperature of 25 ±2C° and a humidity of 60± 5% for follow-up purposes. The results were analyzed by factorial method and using a completely randomized design (CRD), using the statistical analysis program GenStat2012.The differences between all samples were compared by using the L.S.D. At level of ($P<0.05$)[8].

3. RESULTS AND DISSCUTION

The results are shown in table 1and 2 the percentage of mortality of pupae increased, and consequently a decrease in the number of emerging pupae is noticed. The higher the concentration of the insect-pathogenic fungus *B. bassiana* exposed to ultraviolet radiation, the incidence of deformities increased in adult insects which resulted from pupae at the age of (1-2) and (6-8) days. They were sprayed to insect-pathogenic fungus exposed to ultraviolet radiation for periods of 4 and 8 minutes. It was also noted that the resulting insect movement was weak due to the emergence of fungus filaments. It was characterized by lethargy, and with the passage of time all parts of the insect's body became covered with fungal hyphae, and the hyphae of *B. bassiana* were white and smooth and grew externally after placing infected insects on the filter paper is moistened with distilled water. Also, death in newly spawned insects can be attributed to mycotoxins produced by fungal solution, experiments have shown that entomopathogenic fungi produce toxic substances that negatively affect the metabolic processes of insects[11]. Insects infected with the pathogenic fungus may have died because of the toxins of that fungus and not because of the fungus growing on those infected insects. In addition, the decrease in the numbers of immune cells of infected insects leads to a weakening of the immune system of emerging insects, and this is indicated by[12] when he studied the effect by fungal solution *B. bassiana* on the larvae of the lesser cotton leaf worm *S. exigua*.

An increase in the killing rates in pupae was observed with the increase in the fungal solution concentration, as the highest rates of mortality were 72.30 and 62.33% by concentrate at $1.5x10^7$ spores.ml^{-1} for the pupae at the age of (1-2)days.More over, the irradiation period of the fungicide solution was 4 and 8minutes, respectively. As for the killing rates of pupae at the age of (6-8)days and with the same concentration and time periods, they showed a clear decrease, as they were 44.30 and 42.50%, respectively(Tables1 and2).This is due to the secretion of toxins from fungi that are pathogenic to insects and the growth of its filaments. As it is explained in [13] that the fungus tissues grow and replicate inside the body of the infected insects after penetration, and the development of the fungus within the blood lymph of the insect lead to a decline in the functioning of the immune system .Therefore, the fungal filaments penetrate all the internal tissues, after which they penetrate the body wall and exit on the outer surface.This leads to difficulty in movement, [14] stated that the effect of mycotoxins extended to insects produced from pupae that were sprayed to the fungus. The results also showed a clear decrease in the levels of blood glucose since the percentages of fungus concentrations increased. The decrease was more in pupae at (1-2)days while pupae at (6-8)days, as it reached 24.00 and 34.00µg.ml^{-1} in insects that emerged from pupae treated with age (1-2) and (6-8)days, respectively(Table1), treated with fungus at a concentration of 1.5×10^7spores.ml^{-1} for 4minutes, compared to 10.20 and 23.80 µg.ml^{-1}, respectively (Table2) for insects emerging from pupae. Treatment with the same concentration and the same age for a period of 8minutes also, albumin levels decreased in the blood of insects which emerging from pupae at the same concentrate and age, as it decreased significantly to 14.30 and 10.50 µg.ml^{-1} for pupae aged(1-2) days at a concentration of 1.5×10^7spore.ml^{-1}. The duration of exposure was 4 and 8minutes, separately, while to 12.20 and 16.10µg.ml^{-1}, exposure time of 4 and 8 minutes, respectively, for insects that emerged from pupae at (6-8)days and the same concentrate. As for the number the hemocytes in insects that resulted from pupae, they decreased clearly with the increase of the fungal solution. The number of cells was 119 and 155 cells.ml^{-1} for adults that emerged from pupae treated with a fungal solution of 1.5×10^7 spores.ml^{-1} at ages (1-2) and (6-8)days for 4 minutes(Table1). The number of cells was 70 and 86 cells/ml for adults that results from pupae that was treated by the same fungal concentrate itself for 8 minutes (Table2). [15]indicated that ultraviolet radiation causes changes in the composition of the genetic material inside the nucleus.Thus, the occurrence of various genetic mutations has a significant impact on the composition and vitality of the pathogenic fungus. [16] It was mentioned that the females of the desert locust insect, when treated with fungus exposed to ultraviolet radiation, their ovaries became smaller, reaching 50mm while 82mm in unexposed individuals, and there was also an extraction in the tissues of the digestive system of locust nymphs treated with fungus, compared to the control group. Through the results, it was found that the killing level did not reach 100%, except for $1.5\ x10^7$ spores.ml^{-1} exposed to radiation for a period of 4 minutes reached 72.30% for pupae aged (1-2)days after 12days (Table1). The insect's immune system may be able to defend whenever the solutions are low, and the system's effectiveness decreases whenever the solutions are high in density, and this is what he mentioned[17] as his results about effectiveness of the *B. bassiana* fungus on activity of the *P. operculella* insect explained the immune system the insect can only defend low densities. The tables1 and2 clearer that the pupae of age of (1-2)days were more affected by the fungus solutions than the pupae at the age of (6-8)days, as the statistical analyzes which used the clear differences between the percentage of death and a decrease in the percentages of glucose and albumin and the amount of blood cells in the blood, one of the possible reasons is that the cuticle of virgins at (1-2) days is less thick and more flexibile than those of pupae at the age of (6-8) days.Accordingly, it becomes easier to penetrate by fungal hyphae compared to other older ones.The reason behind that may be the different formations of the body wall insect, such as the presence of a waxy layer.In addition , there are several factors that depend on the type of insect and the insect's environment like humidity that help in germination of fungal spores.

Table1. Demonstrates effect of fungus treated with ultraviolet light for 4minutes on pupae at the age of (1-2) and (6-8) days and its development of E. cautella.

(6-8) days				(1-2) days				fungus concentrate
No. hemocyte /ml	The glucose µg.ml^{-1}	The albumin µg.ml^{-1}	The death rate %	No. hemocyte /ml	The glucose µg. ml^{-1}	The Albumin µg. ml^{-1}	The death rate %	spore .ml^{-1}
220	55.10	28.15	26.00	185	42.05	28.12	42.10	Cont. (10^6 ×1.5)
202	52.00	27.00	25.02	180	40.20	1225.	45.05	10^4 × 1.5
180	45.10	23.30	39.33	162	36.15	22.10	52.50	10^5 × 1.5
164	39.10	19.10	42.10	143	30.01	20.00	68.20	10^6 × 1.5
155	34.00	1016.	44.30	119	24.00	14.30	72.30	10^7 × 1.5
0.48	0.85	0.88	9.00	8.20	11.60	5.19	5.98	L.S.D.

Table2. Demonstrates effect of fungus exposed to ultraviolet light for 8minutes on pupae aged(1-2) and (6-8)days and its development to E. cautella.

(6-8) days				(1-2) days				fungus concentrate
No. hemocyte /ml	The glucose µg. ml^{-1}	The albumin µg. ml^{-1}	The death rate %	No. hemocyte /ml	The glucose µg. ml^{-1}	The albumin µg. ml^{-1}	The death rate %	spore. ml^{-1}
242	42.00	32.90	33.30	208	40.10	16.15	44.00	Cont. (10^6 ×1.5)
210	34.50	2.102	38.20	198	36.20	18.30	48.30	10^4 × 1.5
183	33.00	18.20	34.00	176	30.33	16.20	54.10	10^5 × 1.5
125	28.00	14.50	38.66	115	28.15	12.20	58.00	10^6 × 1.5
86	23.80	12.20	42.50	70	20.10	10.50	62.33	10^7 × 1.5
3.69	5.60	0.51	12.476	6.41	1.03	12.10	13.34	L.S.D.

4. REFERENCES

1. Abid, I. , Laghfiri, M., Bouamari, R. and Bourioug, M., "Integrated Pest Management (IPM) for *Ectomyelois ceratoniae* on Date Palm", Current Opinion in Environmental Science and Health, Vol.19, (2021). 576- 582.
2. Abdul-Hussein, A., "Date Palms and Dates and their Pests", Dar Al-Kutub for Printing and Publication Al-Basra University, (1985). 576pp.
3. Fatma, O. A. ,Leyla, A. ,Bulent, B. and Zekiye, S., "Isolation and Characterization of Native *Bacillus thuringiensis* Strain From Soil and Testing the Bioactivity of Isolates Against *Ephestia kuehniella*(Lepidoptera: Pyralidae) Larvae", Turkish Journal Biochemistry Vol.33, No.4, (2008). 202-208.
4. Al-Bagham, S. H. and Musa, S. A., "Conditions of Biological Control of Agricultural Pests to Reduce Environmental Pollution in the United Arab Emirates", National Workshop on the Use of Biological Control of Agricultural Pests to Reduce Environmental Pollution. Damascus Syrian Arab Republic. (2002). 32-42.
5. Al-Iraqi, R. A., Khaleda, A. S. and Sumaya, A., "The Effect of Low Temperatures on Four Types of Storage Insects. Jordan Journal Applied Science, Vol.1, No.10, (2008). 40-48.
6. Ahmad, M. S. H., Hameed, A. A. and Kadhum, A. A., "Disinfestation Commercially Packed Dates by Combination

Treatments", Iraq Atomic Energy Commission, Baghdad. Nuclear Research Institute), Vol.15, No.3, (1986) .221- 226.
7. Abbott, W. S., "A Method of Computing the Effectiveness of An insecticides", Journal Economic Entomology, Vol.18, No.1,(1925). 265- 267.
10. Ixodidea) to the Entomopathogenic Fungi *Beauveria bassiana* and *Metarhizium anisopliae*" Biological Control, Vol.31, No.3, (2004).414 – 421.
11. Hansen, P. J., "Use of a Haemocytometer", www. animal. Ufl.edu/ Hansen/ protocols /haemocytometer.hum",University of Florida, Departmentf Animal Science, (2000).
12. Garry, T. and Kendrick, B., "Biology of Conidial Fungi", Department of Botany, University of Texas,(1981).659pp.
13. Hung, S. and Boucias, D. G. "Influence of *Beauveria bassiana* on the Cellular Defense Response of the Beet Armyworm, *Spodoptera exigua*", Journal of Invertebrate Pathology,Vol.60, (1992).152-158.
14. Lazgeen, H. A., Feyroz, R. H. and Gehan, H. Y., "Effect of the Entomopathogenic Fungus, *Beauveria bassiana* (Bals.) Vuill. On the Reproductive Potential of Poplar Leaf Beetle *Melasoma populi* L.", J. Duhok University, Vol.14, No.1, (2011). 9-15.
15. Al-Habib, A., Faisal, D., Hashim, N. and Ali, Y. A. "The Pathogenicity of Two Local Isolates of the Fungus *Beauveria*
8. Al-Rawi, K. M. and Khalaf Allah, A. M., "Design and Analysis of Agricultural Experiments", Dar Al-Kutub Printing and Publication House Press, University of Mosul, Iraq,(2000).488pp.
9. Kirkland, B. H., Cho, E. and Keyhani, N., "Differential Susceptibility of *Amblyomma maculatum* and *Amblyomma americanum* (Acari) *bassiana* (Balsmo) on the Prepupae Larval and Adult Stages of the Olive Fruit Fly *Bactrocera oleae* (Rossi)", Arab Journal Plant Protection, Vol.36, No.1, (2018). 312-320.
16. Prakash, S., Vinici, V. A. and Strobel, R. J., "Improvement of Microbial Strains and Fermentation Process", Applied Microbiology Biotechnology, Vol.54, (2000). 287-301.
17. Mitch, B. D. and Basaed, F. Al-Zahra "A Study of the Effect of *Metarhizium anisopliae* Var. Acridium in ther Desert Locust *Schistocerca gregaria* in Algeria", Arab Journal Plant Protection, Vol.25, No.1, (2007). 123-130.
18. Hafez, M., Zaki, F., Moursy, A. and Sabbour, M., "Biological Effects of the Entomopathogenic Fungus *Beauveria bassiana* on the Potato Tuber Moth *Phthorimaea operculella* Zeller", Journal Islamic Academy of Science, Vol.7, No.4, (1994). 211-214.

Arabic Abstract

تم اختبار قدرة الفطر *Beauveria bassiana* عند تعريضه للأشعة فوق البنفسجية عند طول موجي 312 نانومتر لمدة 4 و 8 دقائق. ولوحظ انخفاض معنوي في نسب بزوغ البالغات من عذارى عثة التين *Ephestia cautella* المعاملة بالفطر المعرض للإشعاع مع زيادة التراكيز الفطرية، وانخفضت نسبة الألبومين في الدم بمقدار 14.30 و16.10 ميكروغرام.مل$^{-1}$ في بالغات فراشة التين التي بزغت من العذارى بعمر (1-2) و(6-8)يوم على التوالي، عند رشها بمحلول فطري مشعع لمدة 4 دقائق بتركيز 1.5×10^{7} بوغ.مل$^{-1}$ مقارنة بالحشرات البالغة الناتجة عن العذارى التي تم رشها بمحلول فطري غير معرض للإشعاع بلغت 28.12 و28.15 ميكروغرام.مل$^{-1}$ على التوالي. كما انخفض مستوى الجلوكوز في الدم ليصل إلى 24.00 و34.00 ميكروجرام.مل$^{-1}$ في الحشرات البالغة الناتجة عن العذارى بعمر (1-2) و(6-8) أيام على التوالي. انخفض عدد الخلايا الدموية 119 و 155 خلية على التوالي للحشرات البالغة الناتجة عن العذارى المرشوشة بالفطر المشعع، بينما انخفض عدد الخلايا الدموية 185 و 220 خلية للحشرات الناتجة عن العذارى المرشوشة بنفس التركيز من المحلول الفطري غير المشعع.