The Role of An Oil-Based Formulation for Protecting the Biocontrol Fungus *Beauveria bassiana* from Some Chemical Fungicides Under Labortory Conditions

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Abstract

The lab study included evaluation of oil formulation to protect the conidia of Beauveria bassiana from two fungicides; beltanol and basten50 and four insecticides; decis, quality 5, dynamec and supredimethoate after mixing and incubated for 1 and 9 days at relative humidity (RH) 0.0, 6, 33 and 75% and temperatures; 25 and 35 °C. Vitality percentage of conidia in aqueous suspension was negatively affected by recommended doses of the pesticides either the fungicides in which the vitality % of conidia kept at the lowest level (0.0%) over their exposure periods (1 and 9 Days) to the 25 and 35 °C under RH 0.0 and 6%. Not only that, but the insecticides had a negative effect under the same environmental conditions in which conidia recorded a vitality ranged 0.0-34.5%. On the other hand, vitality of the formulated conidia was more degraded under influence of extreme drought conditions (RH 0.0 and 6%) after mixing with a both fungicides over 9 days of exposing to the 35°C and was recorded 70 and 83.3% for 9 days of exposing to the 35 °C under RH 33% in compared with vitality of the fungicide-free formulated conidia. All the formulated conidia whether treated or untreated with chemical pesticides maintained their vitality in completely (100%) at all temperatures under RH 75%. These results have confirmed that it was not benefitted to use the oils only in protecting conidia from adverse physical and chemical environmental conditions, unless appropriate moisture was available, even at a low level (RH 33%).

Key words: *Beauveria bassiana*, Pesticides, Biocontrol, and Oil Formulation

دور المعاملة بالزيوت في حماية عامل المكافحة الحيوية الفطر *Beauveria bassiana* من بعض مبيدات الفطريات الكيميائية في ظروف المختبر

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الخلاصة

تضمنت الدراسة المختبرية تقييم معاملة الزيت لحماية كونيديا الفطر *Beauveria bassiana* من اثنين من مبيدات الفطريات؛ بيلتانول وباستن 50 وأربعة مبيدات حشرية decis و quality 5 وdynamec and supredimethoate بعد الخلط والتحضين لمدة 1 و9 أيام في رطوبة نسبية RH بلغت (0.0 و6 و33 و75%) ودرجات حرارة 25 و35 درجة مئوية. تأثرت النسبة المئوية لحيوية الكونيديا في المعلق المائي سلبًا بالجرعات الموصى بها من المبيدات سواء كانت مبيدات الفطريات التي بقيت فيها نسبة حيوية الكونيديا عند أدنى مستوى (0.0%) خلال فترات تعرضها (1 و9 أيام) إلى 25 و35 درجة مئوية تحت رطوبة نسبية 0.0 و6% ليس ذلك فحسب، بل كان للمبيدات الحشرية تأثير سلبي في نفس الظروف البيئية التي سجلت فيها كونيديا الفطر حيوية تراوحت بين 0.0-34.5%. من ناحية أخرى، كانت حيوية الكونيديا المعاملة بالزيوت أكثر تدهورًا تحت تأثير ظروف الجفاف الشديدة RH 0.0 و6% بعد الخلط مع كلا المبيدات الفطرية على مدى 9 أيام من التعرض لدرجة حرارة 35 درجة مئوية وتم تسجيل 70 و83.3% بالنسبة للمبيدات الفطرية 9 أيام من التعريض لدرجة 35 درجة مئوية تحت رطوبة نسبية 33% بالمقارنة مع حيوية الكونيديا المعاملة بالزيت والغير معالجة بالمبيدات الفطرية. حافظت جميع الكونيديا المركبة سواء المعالجة أو غير المعالجة بالمبيدات الكيماوية على حيويتها بالكامل (100%) في جميع درجات الحرارة ورطوبة نسبية 75%.

الكلمات المفتاحية: *Beauveria Bassiana* والمبيدات والمكافحة الاحيائية والمعاملة بالزيوت

Introduction

The entomopathogenic fungus *Beauveria bassiana* (Deuteromycetes) has infected various types of insects. Lepidoptera (Moths and Butterflies) and Coleoptera (Beetles) were the most susceptible to this fungus and include many insects of agricultural economic importance inhabiting the soil or crops. The number of insects infected with this fungus have been estimated at more than 200 insects which were distributed among nine insect species (Storey and Gardner 1986; Li and yang, 1988). The fungus has been developed and commercially produced to control many agricultural insect pests (Amatuzzi, *et al*., 2017). *B*. *bassiana* has not cause any harmful effect on the environment components in compared with chemical pesticides (Sayed, *et al*., 2019), and it has been in a wide range of use due to its parasitic ability to most harmful insects that belong to different insect groups. The effectiveness of *B*. *bassiana* and its tolerance to environmental conditions were determined by its direct use as spore aqueous formulations against a specific insect pest (Prior, *et al*., 1988; Moslim, *et al*., 2004). It is worth noting that these formulations, which include biological control fungi, among them *B*. *bassiana*, remain in the place of developing and improving their biological performance based on the results achieved and the various environmental conditions including chemical ones that were exposed to them as these bio-pesticides were measured by the stability of their commercial use, and as far as provides positive results and rich economic returns. The presence of *B*. *bassiana* naturally in agricultural fields (Uma Devi, *et al*., 2008) or artificially released for biological control or integrated pest management made their spores susceptible to the chemical pesticides, either at the beginning of spraying or after that (Challa and Savinda, 2014) which leads to kill or inhibit their germination (Alves, 1998; Anderson and Roberts, 1983; Loria, *et al*., 1983; Benz, 1987), this eventually leads to the failure of the entire biological control. The success of using entomopathogenic fungi in the biocontrol or integrated programs for controlling the pest insect depended on the long survival of spores in the environment conditions (Benz, 1987) adapted from (Reddy, *et al*., 2018). Undoubtedly, the use of some environmentally friendly oils or those compatible with bio-control fungi in the bio-products is one of the main means of protecting them from harsh environmental conditions (inglis, *et al*., 2002; Sangamithra, *et al*., 2015). Although the positive properties of the water by lacking the toxicity, availability, cheap price, potential for using in bio-preparation and spraying by simple hydraulic pumps, a several previous studies have emphasized importance of an oils in bio-preparations in compared with the water for enhancement the work of fungi for insect pest infection, including the fungus *B. bassiana* (Prithiva, *et al*., 2017; Sridevi, *et al*. 2018; Sangamithra, *et al*., 2015). Chemical pesticides are still the first line in combating insect pests and fungi cause plant diseases in the short and medium term, as the use of chemical pesticides have been remained main strategy for agricultural pest control, giving farmers an opportunity to obtain large quantities of agricultural crops in good quality and at a lower cost, as the use of chemical pesticides may have inhibitory effects on natural enemies but in often leads in killing it (Bueno, *et al*., 2017). Fungicides and some insecticides have toxic effects on large numbers of their entomopathogens (De Oliveira and Neves, 2004).

This study objectived to evaluate the effect of oil formulation on protecting the

fungus *B. bassiana* from two fungicides; beltanol and basten 50 and four insecticides decis, quality 5, dynamic and superdimethoate at 25, 35°C under RHs; 0.0, 6, 33 and 75% after 1 and 9 days of exposure.

Materials and Methods

The Fungus *Beauveria bassiana*

The fungus *B. bassiana* isolate X5 obtained from the bio-pesticides department, former Iraqi atomic energy organization is still used by us in the bacterial pesticide laboratory, the biological control department of plant diseases, the integrated pest control center, the agricultural research directorate. The fungal isolate was refreshed by planting it in the medium potato extract dextrose agar (PDA), which was previously prepared and sterilized at the autoclave at a 121 °C/ 1.5 Kg/ cm^2 for 20 minutes and then placed inside sterile 9 cm petri dishes and incubated at 25 °C for seventeen days to form colonies and complete growth and production of many spores. The dishes were kept in the refrigerator for two weeks for the purpose of using them in carrying out the experiments.

Inoculum Preparation of The Fungus *B. Bassiana*

The inoculum of *B. bassiana* (Isolate 5x) was prepared for many times as needed. Several glass bottles (size 200 ml) were used in this preparation. A rice boiled with distilled water was added into these bottles at 30 g/ bottle and were plugged with cloth stoppers. The glass bottles were sterilized in an autoclave at 121 ° C/ 1.5 Kg/ cm^2 for 15 minutes. After sterilization, the glass bottles were cooled and inoculated with plugs of the fungal culture was kept on the refrigerator using one plug/ bottle. The bottles were incubated for two weeks at 25 °C with continuous manual shaking until completing fungal growth. The bottles were left two weeks under lab conditions for more sporulation and fungal cultures were dried. In order to collect the flying spores' dust, an empty and sterilized glass bottle of the same size was placed inverted so that the openings of the two bottles coincided, as they were shaken together vigorously and simultaneously to stir up the spore dust, and it took five minutes to collect the dust. To obtain a suitable aqueous spore suspension, 100 ml of sterile distilled water was added. Several spores were counted using a haemocytometer and a light compound microscope. The concentration of the spores was calculated using the following equation.

Evaluation of the Efficiency of Oil Formulations in Protecting *B. Bassiana* Spores from Chemical Pesticides Under Temperatures, and Different Exposure Periods

To evaluate the efficacy of some oil formulations in protecting *B. bassiana* spores from some chemical pesticides, the two fungicides were used betanol (Chinasol, Spanish) and basten 50 SC (Carbendazim, Chinese) and four insecticides; Quality 5, Superdimethoate (a.i. is Dimethoate, Ugandan), decis 2.5 EC (a.i. is Deltamethrin, German) and dynamec (a.i. is Abamectin, the Manufacturer is Syngenta). We followed the methods described by Wyss (2004), Lopes, *et al*., (2014), Sangamithra, *et al*., (2015), Perez-Gonzalez and Sanchez-Pena (2017). The corn oil (20%) and Tween 20 (2%) were used with distilled water and a number of formulations were prepared after mixing with *B. bassiana* spores (6.6 x 105 spores/ml) that were previously prepared and dried in the sterile dishes supplied with hand-made sterile cardboard covers at rate 1ml / dish as above-mentioned method, and three replicates (Dishes) were used for each treatment. These formulations contained distilled water with Tween 20 (TW),

corn oil with Tween 20 (COT), and spores with distilled water. The pesticides were prepared by concentration (X) according to the instructions of the companies produced , while a double concentration (2X) was prepared, as the pesticides of concentration (X) were added to the aforementioned formulations at 1: 1 (v/v) in each dish base, they were mixed under sterile conditions using sterile microscopic slides, and the following treatments were obtained : corn oil +tween20+ spores + beltanol (Co +Tw+ Sp + Belt) , corn oil +tween20 +spores + basten 50 (Co + Tw+ Sp + Bast), corn oil + tween20+spores + Quality 5 (Co+ Tw + Sp + Qual), corn oil+tween20 + spores + superdimethoate (Co + Tw+Sp + Supe), corn oil +tween20+ spores + decis (Co + Sp + Dec), corn oil+tween20 + spores + dynamec (Co + Tw+ Sp + Dena), It is also the pesticides with double concentration (2X) were put onto dishes containing distilled water at a rate of ml/ plate. In addition, pesticides untreated formulations; corn oil + Tw 20 + spores (Co + Tw + Sp), spores + distilled water + Tw, and spores + distilled water were used as control treatments, those combinations were added onto dishes at a rate of 2 ml/ dish. Three replicates (Dishes) were used for every treatment and the dishes were distributed in the two incubators at 25 °C, 35 °C. These dishes were divided into two groups, where the first group was incubated for1 day, and another for 9 days of exposing. After the exposing period ended, the dishes were removed from the incubators and the viability percentage of spores was calculated as described by Lacey (1997).

Statistical analysis

The obtained results were statistically analyzed using the Ready-genstatistical Program. In the statistical software package, a calculation of the mean vitalities of three replicates of each treatment, standard error of each mean (S.E.M.) and standard deviation (SD) of the tested variables were determined. A T-test was used to assess individually the significant difference of vitality% between chemical treated and untreated formulated conidia within one column under the 25 or 35 °C, RH, and one period of exposure. We used analysis of variance (ANOVA) at one-way ANOVA (No Blocking). On the other hand, a T-test was applied to assess the significant the difference of vitality% between every chemical treated and untreated formulated spores within every row which reflected the impact of interaction of all different temperatures, RHs after periods of exposure using Two-way ANOVA (No Blocking). Lowest significant difference (LSD) was used at ($P = 0.05$) assessment between the treatments, whereas the value had probability less < 0.05 was more significant.

Evaluation of the Efficacy of Oil-Formulations in Protecting the *B. Bassiana* Spores from Some Chemical Pesticides Under Different Temperatures and Different Exposure Periods

Table (1) reveals the results of all the pesticides that killed spores of fungus B. bassiana in which spores recorded vitality 0.0% except superdimethoate vitality of spores showed superiority (28%) after one day of treatment at 25 °C. Spores showed high vitality (34.5%) after 9 days of treatment with the decis at 25 °C in compared with the other chemical treatments. Spores were treated with qality 5 registered high level of vitality (19.7%) after one day of incubation at 35 °C related to other chemical-treated spores.

In the Table (2), the results discovered that the formulated spores were superior ($p = 0.05$) in their keeping vitality in

which ranged 91.1% (178.1 Colony) to 100% (193.3 Colony) after 1 day of exposing to the 25 ° C under RH 0.0 % either with or without pesticides presence in compared with their vitality which ranged 0.0% (0.0 Colony) - 80% (154.64 Colony) after 9 days of exposing to the 25 ° C, 9.1% (17.6 Colony) - 98% (189.4 Colony) after 1 day of exposing to the 35 °C, and 0.0% (0.0 Colony) to 72.4% (139.94 Colony) after 9 days of exposing to the 35 °C. The spores of control treatment (Spores Only + Distilled Water) recorded a very significant reduction in their vitality was ranged 0.0% (0.0 Colony) to 42.5% (Colony) over all the heat exposure periods. Spores of the formulations (Co+Tw) showed non-significantly differences in their vitality after 1 and 9 days of exposing to the 25 °C related to the vitality of all chemical-treated spores except to the formulated spores treated with beltanol and superdimethoate in which vitality amounted 91.1 and 0.0%, respectively. Formulated spores recorded a high percentage of vitality under RH 6%. Vitality revealed a relative rise after 9 days of exposing to pesticides at 25 °C which ranged (71.4 – 100 %), but it decreased when it was exposed to the 25 °C for the same period, whereas reached 36.7 – 77.1 %. Vitality was significantly (p = 0.05) declined after 1 day of exposing to the beltanol and basten 50 at 35 ° C and the percentage was 25.9 and 39%, respectively. While the vitality remained steadfast after 1 day of exposure to the insecticides at 25 °C (93.4 – 100%). Formulated spores were recorded full vitality (100%) under RH 33% after 1 and 9 days of exposing to the two temperatures, but they recorded 70 and 83.3% after 9 days of exposinf to the beltanol and basten 50, respectively at 35°C.

Table (1) The effect of fungicides and insecticides on vitality% of fungus Beauveria bassiana under two exposure periods.

A RH 6% caused increase in the vitality of superdimethoate-treated formulated spores after 1 and 9 days of an exposing to the 25 °C, as it reached 96 and 66.6%, respectively. On the other hand, the results showed that a raising the RH to 33% led to an increase in the level of vitality (100%) of the formulated spores after 1 and 9 days of treating with pesticides and exposing to 25 and 35 °C, except beltanol-treated formulated spores that recoded 90 and 75 % after 9 days of exposure to the 25 and 35 °C, respectively . All the pesticides-treated formulations provided completely protection from thermal and chemical effects using RH 75% in which spores achieved vitality 100%.

Table (1) The Effect of Fungicides and Insecticides on Vitality% of Fungus *Beauveria bassiana* Under Two Exposure Periods

Treatment	Temperature			
	25 °C		35 °C	
	1 Day	9 Days	1 Day	9 Days
Distilled Water + Conidia (Control)	42.5	0.0	37.5	0.4
Beltanol	0.0	0.0	0.0	0.0
Basten 50	0.0	0.0	0.0	0.0
Decis	0.0	34.5	3.7	2.4
Quality5	0.0	0.0	19.7	0.0
Dynamec	0.0	2.5	0.0	0.0
Superdimethoate	28.3	0.0	0.0	0.0

Table (2) Vitality Percent of Formulated and Unformulated Spores of Fungus *Beauveria Bassiana* at Different Chemical and Thermal Conditions

Treatment	Standard Number of Colonies = 180 Colony or Standard Vitality% = 100				Standard Number of Colonies = 170 colony or Standard Vitality% = 100				Standard Number of Colonies = 122.5 Colony or Standard Vitality% = 100			
	RH 0.0%				RH 6%				RH 33%			
	25 °C		35 °C		25 °C		35 °C		25 °C		35 °C	
	1 Days	9 Days	1 Days	9 Days	1 Days	9 Days	1 Days	9 Days	1 Days	9 Days	1 Days	9 Days
	Vitality %	Vitality %	Vitality %	Vitality %	Vitality %	Vitality %	Vitality %	Vitality %	Vitality %	Vitality %	Vitality %	Vitality %
Co+tw	100	80	80	72.4	100	100	100	100	100	100	100	100
Co+tw+bel	91.1	72.9	9.3	5.1	82.3	83.5	25.9	36.7	100	100	100	70
Co+tw+bas	92.4	68.9	9.1	4.6	100	75.5	39	41.42	100	100	100	83.3
Co+tw+dec	100	67.8	98	58.5	94.3	100	96.6	61.8	100	100	100	100
Co+tw+qua	100	67.6	95	59	100	83.3	100	73.3	100	100	100	100
Co+tw+dyn	99.2	78	85.7	78	81.7	100	93.4	77.1	100	100	100	100
Co+tw+sup	96	0.0	76.6	0.0	100	71.4	95	66	100	90	96.7	80
l.s.d* at P = 0.05	8.23	20.26	11.16	8.24	4.98	5.18	8.4	13.53	0.0	6.62	2.82	10.12
l.s.d1 at P = 0.05	10.1	25.7	26.9	12.8	12.1	10.3	27.6					
l.s.d2 at P = 0.05	7.19	11.8	12.5	16.7	16.4	10.2	35.3					
l.s.d3 at P = 0.05	7.2	35.6	34.3	11	7.2	8.2	9.5					

- L.s.d* between treatments under thermal period and RH
- L.s.d1 between periods of thermal exposure under different RHs
- L.s.d2 of thermal –humidity interaction for every exposure period
- L.s.d3 between periods of humidity exposure under every temperature

Results and Discussion

Generally, all the tested fungicides and insecticides used in this study showed their negative effect on the viability of *B. bassiana* spores after treated directly with them according to the recommended concentrations; 1.5, 2.5, 1.25, 1, 1, and 2 ml/ L. for beltanol, basten 50, desic 2.5 ES, quality 5, dynamic, and superdimethoate, respectively. The fatal effect (100%) of the basten 50 on spores was into line with the findings of Deb, *et al*., (2017) during their study the effect of some chemical fungicides, one of them was bavistin (Carbendazim 50 WP) on the germination of *B. bassiana* spores as it caused significant reduction in percentage (0.0%) after 7 days of treatment with all concentrations of the fungicide at a 25 °C. In relevant to this study, (Challa and Savinda, 2014) explained that the complete inhibition (100%) in germination spore was resulted of susceptibility of the spores of *B. bassiana* to the active ingredient (Carbendazim), which constitutes 50% of this fungicide, as the spindle formation disrupted in one stage of the mitosis, so it prevents the emergence of germination tube and the appresorium adhesion and thus the mycelium of the fungus was not formed. As for the negative effect of beltanol that had contributed to the viability declining (0.0%) did not differ from counterpart (Basten 50), only mechanism for killing the spores of the fungus *B. bassiana* differed in its influential role due to chinosol (8-Hydroxyquinoline Sulfate; 71.42% Hydroxyquinoline and 24.15% Sulfate) which was believed to have a chelating role in extracting minor essential elements from the surface of any fungus and thereby negatively affecting the balance of the mineral elements of this organism which inhibits their growth (EMEA, 1998), and this may applied to the fungus *B. bassiana*. In fact, there are no previous studies that dealt with the effect of beltanol on entomopathogenic fungi but confined to phytopathogenic fungi. The treatment of spores with the insecticide superdimethoate, significantly caused reduction in their vitality which

amounted to 28.3% after 1 day of exposure to the temperature 25 °C. These results were disagreed with findings of some previous studies that showed the compatibility of some insecticides with the spores of the fungus *B. bassiana* at different concentrations depending on the isolates were used. De Oliveira and Neves (2004) had studied the effect of three concentrations (three MC concentrations, half MC and twice MC) of 12 acaricides; Abamectin (Dynamec), and Dimethoate (Superdimethoate) incorporated in different formulations on some growth parameters of *B. bassiana*, found that the Abamectin was had moderate compatibility with the spores, where germination was (53.9%), after 1 day of incubation at the 25 °C, while the dimethoate was incompatible with the spores as they attributed the compatibility variation to the different chemical nature of these pesticides and to the concentrations were used. On the whole, many of authors have explained in their previous studies the main possible reasons behind the inhibition of some insecticides for the *B. bassiana* spores, which may be due to the complete obstruction of the biological pathways responsible for metabolism of fungal spores (Moore-Landecker, 1990) or to the action of some chemical (Ionic or Molecular) pesticides in the equation of the electrostatic charges of the mucosal coated layer of spores or the removal of those charges from them, which an imbalance in the communication of instructions or signals responsible for stimulating germination (Boucias, *et al*., 1988). Our results were also consistent with the findings of (Filho, *et al*., 2001) they have confirmed the incompatibility of Decis® 25 CE (it's a.i. is), as the fungus recorded a significant decrease in colonies form units (0.0 Colony) after 1 day of treatment at 30 ° C, they gave no explanation for the reason for the deltamethrin decline in this growth parameter except that they noted what Morris (1977) showed that the pesticides belonging to the pyrethrins group had an highly bacteriostatic of the bacteria parasiting insects. RH and temperature are important environmental factors that determine longevity of the entompathogenic fungi and efficiency for infection the insect pests (Lazzarini, *et al*., 2006; Inyang, *et al*., 2000). Corn oil has fallen short in efficacy under the conditions of interference between RH 0.0% and two periods (1 and 9 Days) of exposing spores to the temperature 35 °C in the presence of chemical pesticides. The benefit of corn oil in protecting B. *bassiana* spores from temperature 35 °C and RH 0.0% may take a few hours followed by the damage spores, whereas a one-day exposure period to those adverse environmental conditions was sufficient to kill or inhibit their vitality. Adverse effect of pesticides on the spore's vitality, may be increased due to the rising in the pesticides concentrations as a result of water evaporation at a high temperature (35 °C) and low RH (0.0%). This interpretation supported by Franklin, 2007 article explained that the high temperature and low RH will make the water in the pesticide droplets evaporated quickly, reducing the droplet size of the bush pesticide 2, 4-D, and the ester volatilization rate of the pesticide will had been accelerated three times after heat rising from 60 to 80 Fahrenheit. The corn oil had more instrumental when the RH was available, even if it was at a scarce level (6%). The long-term exposure (9 Days) has a bad effect on the spore vitality, despite the temperature was ideal (25 °C) at RH 0.0%. With the presence of these pesticides, as perhaps this period under these conditions is sufficient to volatile the hydrocarbons of the oil, which leads to an increase in its density and viscosity, and consequently, the dissolution of the

oil or its cracking into smaller units (Degrémont, 1991), which may allow the pesticides to enter the spores .This study showed that the benefit of the corn oil is limited to a specific time and that its positive role depends on the availability of RH. Increasing RH 0.0% to 6% to 33% helped spores for keeping their survivalability at highest attainable standard in all thermal and chemical periods, as the RH can alleviate a high temperature by evaporative cooling (Schroeder, 2000), lowering the amount of oil evaporation, and retardation its dehydration, as the oil has a slowest evaporation rate of water (Fingas, 2012). With this mechanism the RH at different levels maintained the physical properties of the corn oil and thus the oily film coated the spores acted as a barrier preventing the pesticides from crept into the spores. Besides the positive role of corn oil in maintaining vital of B. bassiana spores from extreme conditions (RH 6% and High Temperature 35 °C), water vapor surrounded spores worked as enhancement factor in mitigation of heat Intensity (Arauz and Sutton, 1989). Concerning the reduction in spores vitality of the control treatment (Only + Distilled Water), it was much more likely to the adversely impact of the water imbibition on the dry spores, as (Faria, *et al*., 2010) found that a high percentage of spores of *B. bassiana* that had already been exposed to environmental stress showed very slow germination, but lethal significant impact, as a result of hypersensitivity to the imbibition, in compared with the non-stressed spores (Meaning Spores That Were Prepared in the Form of an Aqueous Suspension from Fungal Colonies and Used Without Going Through The Drying Stage), showed a large germination rate on the PDA, only 2% were destroyed after 24 and 72 hours of incubation at 25 °C, while stressed- spores recorded a low percentage of germination (14 and 44%) after 24 and 72 hours of incubation at a 25 °C, respectively, and the shattered of them reached more than 40%. On the contrary, spore of the formulation corn oil + tween 20 achieved a vital percentage of 100% in the first period (One Day Only) of exposure to the 25 °C at RH 0.0%, and the first and second periods of exposure to the 25 and 35 °C at RH 6% spores were also recorded 100% vitality. Mixing the oil- treated dried spores with the added distilled water has staved them off direct contact with water, avoiding the spores from destruction effect of imbibition. Paraffin oil prevented spores of imbibitional damages, and only 40% of them were destructed. This result consistent with the Faria's finding (2009), that the oil-based formulation supplied with the paraffin oil protected the spores from imbibition damage. Formulation of conidia in pure (Non-emulsifiable) paraffinic oil provided considerable protection from imbibitional damage. This study underscores a need for establishing standard protocols for preparing aqueous suspensions of sensitive fungi for both research and commercial. Referring to these results, corn oil was the better oil in inducing the *B. bassiana* spores to germination and forming fungal colonies. And this vegetable oil has an ability alleviate the suffering of these fungal spores from the chemical pesticides hazards used in this study and mitigate the early drought as a result of exposing spores to the high temperature. Vitality of the fungus spores is directly proportional to the increasing RH exposed it. Because of corn oil succession in protecting the *B. bassiana* spores from the used chemical pesticides, we recommend expanding the test to the largest possible number of chemical pesticides preferably those are more important and commonly used in Iraq.

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