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Formulation of biopesticide from *Beauveria bassiana* as part of biological control of date palm stem borer (*Jebusaea hammershmidti*)

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Abstract. Biopesticide from *Beauveria bassiana* which were isolated from soil were obtained by using local materials. According to the number of spores/g of each material, two formulas were chosen. Formula A consist of 300 g (*B. bassiana*) grown on rice seeds + 400 g $CaCO_3$ + 100 of hornwort + 200 g of kaolinite. Formula B consist of 300 g (*B. bassiana*) grown on millet seeds + 400 g $CaCO_3$ + 100 g hornwort + 200 g kaolinite. Field experiment reveled that biopesticide formulated from *B. bassiana* was effective in control date palm stem borer but formula (B) which consisted mainly from *B. bassiana* grown on millet seeds was the best in control date palm stem borer larva as mortality percentage reached 72.7 % compared with 60 % of formula A.
Key Words: Date palm, stem borer, *Beauveria bassiana*, biological control.

Introduction. Date palms are very important trees in Iraq, they play critical role in the national economy, along with their great nutritive value (Abed-Alhassin 1985). Unfortunately the number of date palms reduced from 30 million tree before 1980 to less than 16 million during 2002 (Al-Jamali 2007). This reduction attributed to several reason like negligence and reduce care of date palm, increase salinity in irrigation water and soil and spread disease and insect (Fayyadh 2007). Palm tree may be infected by numerous number of insect, the most distributive insect is the stem borer (*Jebusaea hammershmidti*) and there is methods required to alleviate destructive damage caused by this insect (Al-Jboory et al 2007). Many strategies were used to control this insect like chemical control, but chemical control is not effective in addition to their harmful effects. In many instances alternative methods of insect management offer adequate level of pest control and pose fewer hazards. One such alterative is the use of microbial insecticides that contain microorganism or their by product. Microbial insecticides are especially valuable because their toxicity to non target animals and human is extremely low, they are safe for both pesticide user and consumers of treated crops (Weinzierl & Henn 1989; Al-Zubaidy 1992). Entomopathogenic fungi are ganging increased attention as environmental friendly insects control agent. Although over 750 species were reported to infect insects, few have received serious consideration as potential commercial candidate, *Beauveria bassiana* appears to have the broadest potential as a viable insect control agent (Feng et al 1994). The fungus *B. bassiana* has been found to be pathogens of many insect specially Lepidoptera and Coleoptera (Franklin & Julius 1999). It was also found that *B. bassiana* as conidial suspension was more effective for control of colorado potato beetle than parallel insecticide treatment (Poprawski et al 1997). Numerous studies indicated that *B. bassiana* was effective in control of many insects like jasmine white fly *Aleuroclava jasmini* (Mohammed et al 2008), *Plutella xylostella* (Cheol-Sik 1994) and grasshopper (Inglis et al 1997). Many attempts were carried out in Iraq to *use B. bassiana* as biocontrol agent. Jasim et al (1989) showed that spray of date palm with suspension of *B. bassiana* caused mortality of date palm stem borer reached to 95 %. Al-Bahely (2004) showed that spore suspension of *B. bassiana* was effective in control of

date palm stem borer (*J. hammershmidti)*. Fayyedh et al (2005) tested two isolates of *B. bassiana* and they found that the isolates had significant effect on reducing the number of different instars of the spider mites, therefore formulation of *B. bassiana* as biopesticide receiving some practical attention. Bextine & Thorvilson (2002) used *B. bassiana* as alginate pellets to control red ant *Solenopsis invicta*. Oil and water formulation of *B. bassiana* were found to be effective in control of cocoa weevil pest *Pantorhytes plutus* (Prior et al 1988). Mhdie & Fayyadh (2007) found that barley seed extract and corn cap was the best media for the growth and sporulation of *B. bassiana*.
B. bassiana achieved positive result in its control of group of harmful insects and was loaded as biopesticide on different media to be prepared as a pesticide to control date palms insects and other insects, but because poor available capabilities in universities the project did not continued (Al–Jboory 2007). However in order to introduce alternative methods of insect control like biological control as a key component in integrated pest management programs, to contribute with rehabilitation of date palm in Iraq and to facilitate use of this fungus by farmers this project proposed to achieve the following aims:

1- Formulation of biopesticide from entomopathogenic fungus *B. bassiana* by using local material.
2- To minimize the use of chemical pesticides.
3- To introduce the fungus *B. bassiana* as part of integrated pest control.

Material and Method

Isolation of the fungus from larva. Five date palms infected with stem borer *J. hammershmidti* were cut off and splited. Forty-four larvas were collected from inside date palms stem. Larva were transferred to the laboratory by polyethylene bags, after that 12 dead and weak larva were separated from them.

Dead and weak larva were surface sterilized by 10 % of sodium hypochlorite (NaClO) and washed with sterilized distilled water, sterilized larva were cut to small pieces (0.5 cm) then 3 pieces from each larva were transferred to sterile Petri dishes contain sterilized potato dextrose agar (PDA) amended with antibiotic (Chloramphenicol 250 mg/L) to prevent growth of bacteria. Petri dishes were incubated at 25 °C for 4 days, after incubation the colonies was examined with dissecting microscope. Colonies which gave appearance like *B. bassiana* were transferred to Petri dishes contain PDA and purified by using single spore technique (Alexopoulos & Beneke 1962). The fungus *B. bassiana* was identified according to (Domsch et al 1980).

Isolation from soil. Soil samples were collected from date palms and alfalfa orchard, the soil samples packed in 10 plastic pots in average of 1 kg soil for each pot. Soil samples were moistened with tab water, after one day surface was sterilized and wax worm larva were buried in the soil for 5 days. The wax worm larva was take off from the soil and surface sterilized with NaClO, then larva was cut to small pieces (0.5 cm), three pieces of them were transferred to each Petri dishes contain PDA, and incubated at 25 °C for 4 days (10 Petri dishes were used).

Purification and identification of the fungus *B. bassiana* were done as in paragraph (I).

Preparation of spore suspension. 10 ml of sterile distilled water was added to the surface of three Petri dishes containing colonies of 7 days old *B. bassiana*, surface of colonies was scraped with a rod glass. Spore suspension was transferred to test tube and vibrated by vortex in purpose to homogenize the suspension, after that 1 mL of suspension was transferred to test tube containing 9 mL of sterile distilled water to obtain 10^{-1} dilution and 1 mL of the later dilution was transferred to test tube containing 9 mL of sterile distilled water to obtain 10^{-2} dilution and thus series of dilutions was made up to 10^{-6}, later 1 mL of the last dilution was transferred to Haemocytometer to calculate the number of spore per mL.

$2x10^6$ spore/mL was used in pathogenicity experiment

Pathogenicity of B. bassiana. Two isolates of *B. bassiana* and two species of insects (larva of date palms stem borer and larva of wax worm) were used in this test. Larva were surface sterilized by 1 % sodium hypochlorite by dipping its for two minutes then

washed by sterile distilled water and dried on filter paper. Six larva of wax worm and four larva of date palm stem borer were transferred to each Petri dish containing moistened double filter papers or water agar. Five replicates were used to each species and to each isolate, after that larva were sprayed with spore suspension ($2x10^6$ spore/mL) of *B. bassiana* by using sterilized small sprayer (1/2 L capacity). Sterile distilled water was used for control treatment. Mortality percentage were calculated after 1, 2, 3 days of incubation at 25 °C. The fungus was re isolated from died larva.

Formulation of biopesticide from B. bassiana by using local materials.

Sporulation media. In aim to obtain the best media for sporulation of the fungus *B. bassiana* the following materials were tested: rice seeds, wheat seeds, millet seeds (*Panicum miliaceum*), wheat bran, and corn cap.

One kg of each material were washed under tap water and cooked for 30 minutes. After that 200 g of each material were transferred in to flasks (250 mL volume), four flasks of each material were used. Flasks containing the materials were sterilized in autoclave under 121 °C, 1.5 kg/cm^2 pressure for 30 minutes. Re sterilization were done after 24 hours after the temperature of flasks reduced to 40-45 °C approximately. Each flask inoculated with four discs (0.5 cm) of the fungus *B. bassiana* grown on PDA disc was taken by cork borer. Flasks were shaken in order to mix the fungus inoculated with the materials, and then incubated at 25 °C for 14 days. After incubation period the numbers of spores per gram of each material were calculated by haemocytometer.

Formulation of biopesticide by using local materials. Different materials were used in order to obtain biopesticide from *B. bassiana* suitable for field application. The materials include the following: calcium carbonate ($CaCO_3$), kaolinite, hornwort (*Ceratophyllum demersum*), talic (medical powder for kids, used after bath), date seed powder.

Materials were mixed in different quantities and kept for one month under room temperature (22 - 26 °C). Numbers of viable spores was calculated by using dilution method. According to the result of the number of viable spores, two mixtures (formula) were used. The formula of biopesticide consists of the following materials:

Formula A consists of: 300 g *B. bassiana* grown on rice seed + 400g $CaCO_3$ + 100 g hornwort + 200 g of kaolinite;

Formula B consists of: 300 g *B. bassiana* grown on millet seeds + 400 $CaCO_3$ + 100 g hornwort + 200 g kaolinite.

Each formulate were grinded two time in order to obtain fine powder. The powder of each formula were packaged in polyethylene bags and kept in the refrigerator for one month.

Evaluation the efficiency of biopesticide formulate in control of date palm stem borer (filed experiment). 18 date palms highly infected with date palms stem borer were used in this experiment. 6 date palms were considered for each formula and 6 serve as control treatment (control treatment were dusted with all materials consist the formula A and B free from fungus *B. bassiana*).

Head of each date palm were dusted with 8-10 g of formula A or B by using small duster.

After 20 days four date palms of each treatment were cut. Date palms leaves were removed and the stem were spilt. The percentage of died larva of stem borer were calculated by using the following formula:

$$\text{Mortality } \% = \frac{\text{number of died larva}}{\text{total number of larva}} x100$$

Re-isolation of the fungus *B. bassiana* were done from died larva.

Results and Discussion

Isolation of B. bassiana and pathogenicity test. Three isolates of *B. bassiana* were obtained. Tow isolates (Bb_1, Bb_2) of *B. bassiana* were obtained from the soil and one isolate (Bb_3) from the larva of date palm stem borer. Pathogenicity test (Table 1 - 2) showed that Bb_1 isolate was more effective against wax worm and date palm stem borer

as mortality percentage reached 100 and 72.1 % respectively compared with 83 and 66.6 % for Bb_2 isolate. Isolate Bb_3 was slightly pathogenic only for wax worm, so data for this isolate is not presented on tables. Figures 1 and 2 showed the pathogenicity of Bb_1 isolate on date palm stem borer and wax worm.

Table 1

Mortality percentage of wax worm larva treated with *Beauveria bassiana*

Isolate	*Time after treatment (days)*		
	1	*2*	*3*
Bb_1	22.2	66	100
Bb_2	11.1	50	83
Control	0	0	0

Table 2

Mortality percentage of date palm stem borer artificially treated with *Beauveria bassiana*

Isolate	*Time after treatment (days)*		
	1	*2*	*3*
Bb_1	49.9	66.6	72.1
Bb_2	50	33.3	66.6
Control	0	0	11.1

Figure 1. Date palm stem borer artificially infected with *Beauveria bassiana.*

Sporulation media. Results showed that rice and millet seeds gave highest number of spores/g (Table 3). According to this results this materials (rice and millet seeds) were chosen as a source of inoculum in formulation of biopesticide.

Table 3

Number of spores/g media

Materials (media)	*Number of spores/gx10^6*
Rice seeds	4.5
Millet seeds	4.1
Wheat seeds	2.1
Wheat bran	1.7
Corn cap	1.2

L.S.D. 0.05 = 0.6x10^6

Formulation of biopesticide from local materials. Formula A and B were chosen in this experiment according to the number of viable spore/g after one month of storage (Table 4). Formula A content: 300 g fungus grown on rice seeds + 400 g $CaCO_3$ + 100 g hornwort + 200 g kaolinite. Formula B content: 300 g fungus grown on millet seeds + 400 g $CaCO_3$ + 100 g hornwort + 200 g kaolinite.

Table 4

Number of viable spore/g of each formula

Type of formulate	*Number of spore/gx10^6*
Formula A	2.7
Formula B	2.2

L.S.D. 0.05 = 0.3, Note: Other type of formulate (mixture) were excluded because the no. of viable spore/g were least than 0.9 x 10^6 spore/g.

Evaluation of the efficiency of biopesticide formulate in control of date palms stem borer palm stem borer. Table 5 showed that biopesticide formulated from *B. bassiana* was effective in control date palm stem borer but formula B which consisted mainly from *B. bassiana* grown on millet seeds was the best in control date palm stem borer larva, were mortality percentage reached 72.7 % in contrast with 60 % of formula A.

Table 5

Mortality percentage of stem borer larva after 20 days of treatment

Type of formulate	*Average no. of larva/palm*	*Average no. of died larva/palm*	*Mortality (%)**
A (60 %)	15	9	60
B (72.7 %)	22	16	72.7
Control (11.1 %)	18	2	11.1

L.S.D. 9.6, * - each number represent the result of 4 replicates.

The entomopathogenic fungus *B. bassiana* was effective against many economic insects like cocoa weevil (Prior et al 1988) *Spodoptera exigua* (Studdert & Kaya 1990), red fire ant (Bextine & Thorvilson 2002), *Aleuroclava jasmini* (Mohammed et al 2008), *Cydia pomonella* (Khlaywi et al 2006), *Microcerotermes diversus* (Al-Jassany & Al–Salehi 2011) and date palm stem borer (Al-Bahely 2004). Application of *B. bassiana* in the filed challenge many problems like viability and longevity. However many attempt were done in order to make this fungus more practicable. Prior et al (1988) used water and oil suspension of *B. bassiana* in control *Pantorhytes plutus*. Alginate pellet was also used to formulate *B. bassiana* against red fire ant (Bextine & Thorvilson 2002). Mhdie & Fayyadh (2007) found that barley seeds extract and corn cap were the best media for growth and sporulation of *B. bassiana*.

Conclusions. In this project we used different materials available in Iraq to formulate *B. bassiana.* Results of this project indicate that millet and rice seeds were the best media

for fungus sproulation and these materials can be mixed with other materials like kaolinite and hornwort (water weed) to formulate *B. bassiana.* Further study must be done to evaluate the effect of some ecological factors (temperature, humidity, light) on longevity of the fungus in the filed.

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