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A Study on the Efficiency of *Beauveria Bassiana* Isolate Inoculation Release for *Oryzaephilus surinamensis* Control in Date Store Condition

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ABSTRACT

In this research, *Beauveria bassiana* Bals mass release efficiency for microbial control of Saw-toothed beetle, *Oryzaephilus surinamensis* L. was studied in Date palm stored condition. For this propose, effects of treatments including *B. bassiana* fungus spore and methyl bromide fumigation on the Saw-toothed beetle population reduction was studied during the 25 weeks of storage period. Results showed that, population fluctuation of saw-toothed beetle in microbial control (χ^2 = 9752.72) and methyl bromide fumigation (χ^2 = 2281.47) treatments had significant difference. Freedman and Kendal concordance test also showed significant difference between microbial control and Methyl Bromide fumigation. The microbial control treatment acted in population density dependent manner and caused pest population reduction so that the control efficiency always was near to 60% and the highest level of it was 80% that occurred on the 3rd month of storage period. Maximum efficiency of methyl bromide treatment was about 90%. The efficiency of this treatment was reduced from the second month onwards so that the repetition of control operation was necessary.

Key words: Date palm, stored pests, microbial control, mass release

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is one of the oldest cultivated plants (Riad, 2006) and was certainly domesticated by 3000 B.C. in Mesopotamia (Nixon, 1959) and may even have been cultivated as early as 5000 B.C (Popenoe, 1924). Because of its high nutritional value, productivity and long yield-life (100 years), the date palm was referred to as the "tree of life" in the Bible (UN., 2003). Hussein (1974) published the most comprehensive recent account of insect pests in Iraq, based on large part of research done in that country; many papers in Arabic are cited. The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and the Saw-toothed beetles, *O. surinamensis* (Coleoptera: Silvanidae) cause both quantitative and qualitative damage to the stored grain. The main causes are reduction in weight, quality and commercial value of date fruits Latifian and Rad (2012). Residual insecticides have been employed to control insect pests of stored date palm but alternative control strategies are desirable because of the loss of insecticides due to pest resistance and consumer desire for pesticide free date fruit (Latifian and Rad, 2012). The biggest impetus for the growth of biopesticides comes from the growing awareness by farmers of the value of integrated pest management as a more environmentally sound, economical, safer and a selective approach to crop protection (Menn, 1996). Sayer, Zahedi and Deiri are the most widely cultivated Date palm varieties in Iran. They are also seriously affected by the Saw-toothed

Beetle (*O. surinamensis*) (Latifian and Rad, 2012). *Oryzaephilus surinamensis* has been controlled primarily by using fumigation of methyl-bromide.

Entomopathogenic fungi are generally considered to be safe in terms of low risks as compared to chemical pesticides. New areas for use of these fungal biocontrol agents include their use in close proximity to foods and feed, or even applied directly to stored as well as to other food commodities (Cox *et al*., 2003, 2004). Using of entomopathogenic fungi in stored is now considered as one of the most promising alternatives to residual pesticides and fumigants (Moore *et al*., 2000). Several fungal species have been tested in stored with often contradictory results (Moore *et al*., 2000). Among these species, *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes) seems to be the most effective fungal species against stored insect species (Moore *et al*., 2000; Lord, 2001; Akbar *et al*., 2004; Lord, 2005).

Three Iranian isolates of *B. bassiana* fungus include 441, 44 and 403°C had pathogenicity ability on different growth stages of Saw-toothed beetle. The minimum LC_{50} were 2.51×10^4 and 3.13×10^3 for isolate 441°C on adult and larvae, respectively. The results of these experiments showed that isolates 441°C had better mycelial growth and germination in the broader temperature range than the other two isolates. Therefore, it is more favorable for subsequent studies. For use in date storage condition which most of whom have been located in tropical and subtropical regions. It also has appropriate quickly control and LT_{50} was between 4-7 days (Latifian and Rahkhodaei, 2012). This entomopathogen had horizontal transmission ability between different developmental stages or among individuals of one stage. In addition it had vertical and intergenerational transmission. The host of this pathogen had ability to distributed pathogen on storage dates (Latifian and Rad, 2012). Pathogen had suitable activity between temperatures of 15 to 35°C and relative humidity higher than 56% that it is living conditions of Sawtoothed beetle in depots date. Fungus was formulated in oil vegetable because this action can increase their resistance to thermal shocks and thus increasing its toxicity at the higher temperatures (Latifian and Rahkhodaei, 2012). The present study aims to study the efficiency of *Beauveria bassiana* isolate inoculation release for *Oryzaephilus surinamensis* control in data store conditions.

MATERIALS AND METHODS

Insect rearing: A colony of *O. surinamensis* was used in this research obtained from Plant Protection department of Date palm and tropical fruits Research Institute of Iran. The insects were reared for four months at 27±5°C, 60±5% RH and 12:12 (L: D) photoperiod in plastic box (7.5 cm diameter and 8.5 cm height) and fed with Sayer Date cultivar for 10 generations.

Preparation of fungi: Strain Iran 441°C of *B. bassiana* was obtained from fungi collection of Iranian Research Institute of Plant Protection. The isolate cultured in SDA medium and kept 10 days at 26±1°C, 100% RH and 12:12 (L:D) photoperiod, Then conidia were harvested and suspended in Tween 80 (0.2 ml L^{-1}) in sterile distilled water and vortexes for 3 min to produce a homogenous suspension. Then the suspension was filtered through several layers of cheesecloth to remove mycelia and debris. By using a Haemacytometer, the spore concentration was determined and adjusted 10^3 conidia mL^{-1}.

Conidia formulation: Conidia were formulated in Soy vegetable oil. Conidia were mixed with vegetable oil and wetting/spreader agents prior to the addition of distilled water to obtain homogeneous suspensions. The stock formulation was filtered using a sterilized muslin cloth then

mixed using a Whirli Mixer (FSA Laboratory, U.K.) for 3 min to break down conidial chains and to reduce clumping. All conidial formulations were calibrated at a concentration of 10^4 conidia mL^{-1} using an improved Neubauer's chamber. Then formulations after a 2 h rest were thoroughly agitated for 10 sec using the Whirli Mixer. Aliquots of 0.1 mL from each formulation were then pipetted by an Eppendorf Research piston-stroke pipette and thinly spread over the SDA in petri dishes.

Conidial germination tests were carried out after 24 h. Conidia were examined at 400x magnification and germination was recorded when the germ tube was visible. All the conidia in each field of view were counted to obtain at least a total of 300 conidia in a range between 300 and 400, for each replicate.

Experiment treatments

Treatment by *B. bassiana*: The Backpack suspension Portable spraying machines were used for treating. The Sprayer unit volume was 10 L. Treated date received 1.49×10^4 spore mL^{-1} suspension of the fungal spores.

Fumigation by methyl bromide: Twenty four grams per cubic meter of methyl bromide gas were used for the treatment at temperature 25±5°C. First treatment date was placed on pallets and a container with 100 mL volume was placed in the center of them. methyl bromide was fumigated by it entered into the container through a special tube that was connected to a reservoir tank of methyl bromide. The date was blocked by plastic coating sand bags. The sandbags and plastic coating were removed 24 h after fumigation operations.

Control: Control treatments were the same date as A and B treatment that they did not had any protection operations on it. Storage and environmental conditions were similar for all treatments. On each replicate was treated by 200 Saw-toothed beetle adult with lifetime less than a week as infection source. Each treatment had four replicates. The experiments were performed by plastic boxes. Box size was 45×30×15 cm. Three rows of holes were created at a distance of 5 cm from each others in the one side of wall box. The hole was closed with a cork. Boxes were filled by 25 kg of dates.

Sampling: Sampling was conducted by randomly selecting 25 g of dates form holes of each row in the vertical surfaces of boxes. Three samples were taken from each box. The wet funnel was applied for assessment density of different beetle life stages. Each date sample was transferred to 250 mL Arleen then 20 g of NaCl, 30 mL HCl and 40 mL H_2O was added to it. The resulting mixture transferred to a to mixer heater. After going through 2 min of boiling the mixture is filtered for removing the date waste of sample. Then forty milliliter of odorless oil was added to Arleen. Different Saw-toothed beetle life stages of populations were concentrated between the polar phase (sodium chloride, caustic soda and water) and non-polar (odorless oil) of mixture. The middle phase that carried the life stages were separated by using a funnel. The various life stages densities were assessed by a stereomicroscope (Brader *et al.*, 2002).

Statistical analysis: Population fluctuations of each life stages of Saw-toothed beetle were drawn against as time (duration of storage) as x^- axis and mean±SE of population density as y^- axis by

excel software. Decrease in the pest population was used to determine the effectiveness of the treatments with. Chi-square test and Friedman and Kendall Coefficient of Concordance had been used for comparing population density in the different treatments as statistical methods. The results with the treated samples were corrected with Abbott's formula (Eq. 1) for calculating the efficiencies of treatments:

$$\text{Mortality (\%)} = \frac{X - Y}{100 - Y} \times 100 \quad (1)$$

where, X is the percentage mortality in the treated sample and Y is the percentage mortality in the (untreated) control.

RESULTS AND DISCUSSION

Variations pest population density in different treatments during the storage: There were four periods of activity in the curves of pest population density fluctuation. In all cases, the pest population density variation curves showed sinus pattern in fungus, *B. bassiana* treatment. So that the population developed to a certain stage of development that it was defined as the epizootic threshold. This stage was known as the pre-epizootic. Then epizoot occurred at the peak of the curve, then the population decreased and the post- epizootic phase begins. During the 25 weeks of storage, six waves of epizootics were occurred at, at week fourth and fifth, tenth and eleventh, thirteenth and fourteenth, seventeenth and eighteenth, twentieth and twenty-first and twenty-second and twenty-fourth weeks after storage. The population density of pests in *B. bassiana* treatment had been less than control during the 25 weeks of storage conditions (Fig. 1). The pest population of methyl bromide treatment was a less than *B. bassiana* and control until nine weeks treatment. The pest density of methyl bromide treatment was under the control between the ninth to the eighteenth week after storage. But it was higher from the eighteenth to the twenty-fifth week.

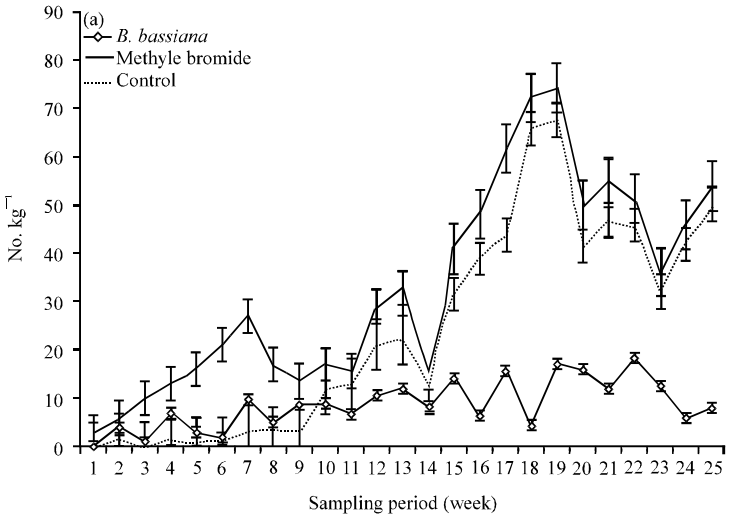

Fig. 1(a-d): Continue

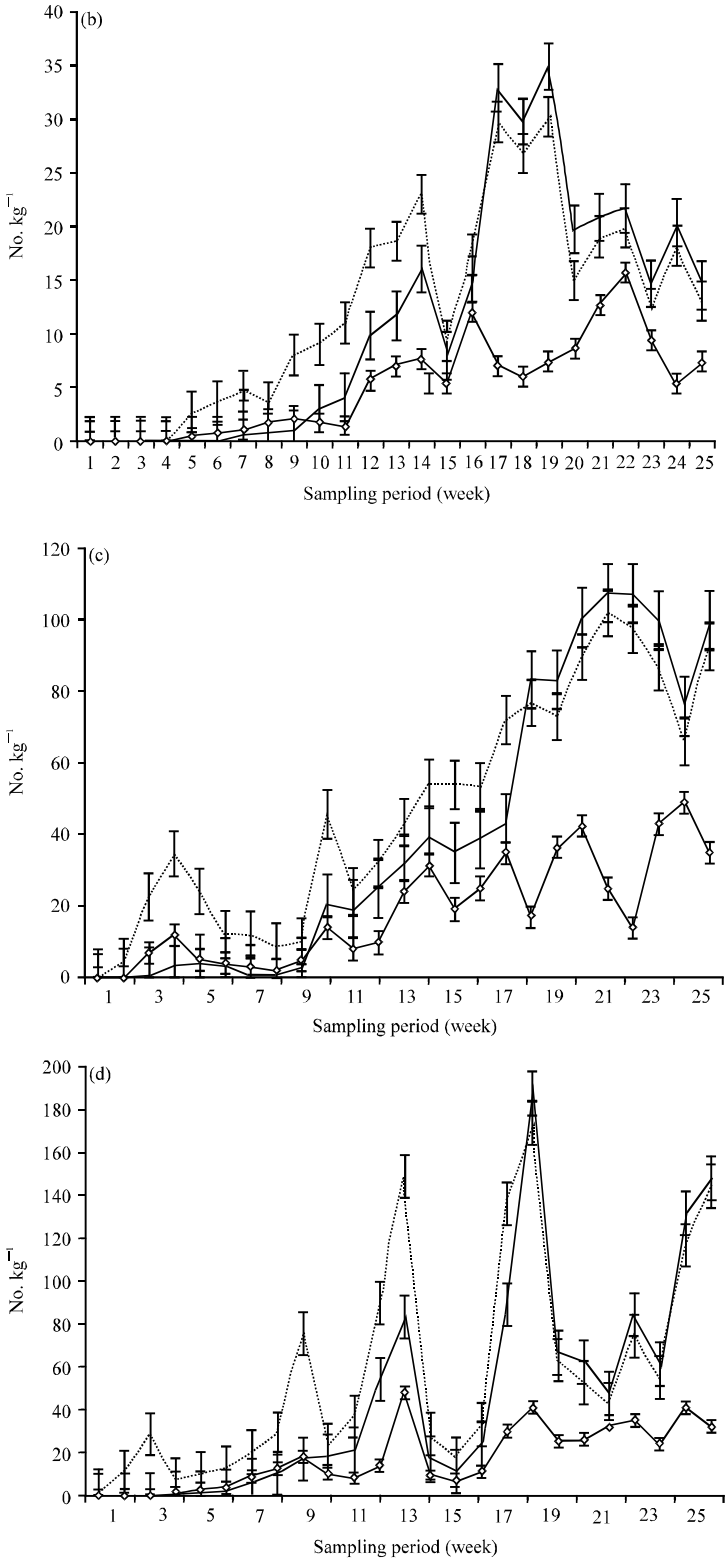

Fig. 1(a-d): Population fluctuation of Saw-toothed life stages (a) Adult, (b) Pupa, (c) Larval and (d) Egg during the 25 weeks of storage in conditions

Pest population reduction rates of different treatments relative to control: *Beauveria bassiana* and methyl bromide treatments pest population densities had significant difference with control at 0.01% probability level (χ^2 = 72.97 and χ^2 = 47.23). The synchronization test results showed that the trends in population density of *B. bassiana* and methyl bromide had significant differences over control at 0.01% probability level (Coeff. of Concordance = 0.45). The Differences in population density of *B. bassiana* and methyl bromide to control pest during the storage periods are illustrated in Fig. 2.

Density differences of methyl bromide treatment were more than *B. bassiana* until ninth week. The effects of *B. bassiana* disease epizoot were started at nine weeks so that population density difference increased more than methyl bromide treatment a week later.

Comparison efficiency of control treatments: Maximum Efficiency of methyl bromide treatment was about 90%. The efficiency of this treatment was reduced from 8th so that the repetition of control operation was necessary. But the *B. bassiana* treatment control efficiency was often about 60% and it reached to the highest level (80%) at 12th week (Fig. 3).

Studies of other researchers had shown that various fungal and microbial factors cannot be used to control stored product pests. Among the most important factors in using *M. anisopliae*, *B. bassiana* fungi are their saprophytic nature (James and Jeffrey, 2003) besides the toxemia which may affect the food quality. The first study on application of entomopathogenic fungi as formulated materials for control of storage pests was carried out by Hluchy and Samsinakova (1989). They used Boverosil® a formulation as wettable powder (containing 5.92×10^9 conidia g^{-1} powder) from *B. bassiana* for control of *Sitophilus granarius* adults. They noted that Boverosil® can cause mortality in this insect, since 90% mortality was recorded at 5.92×10^8 conidia mL^{-1}. But, they added, effective treatment requires a period of high humidity at dew point that is a critical parameter in use of entomopathogenic fungi in storage facilities and it is not accessible. There is

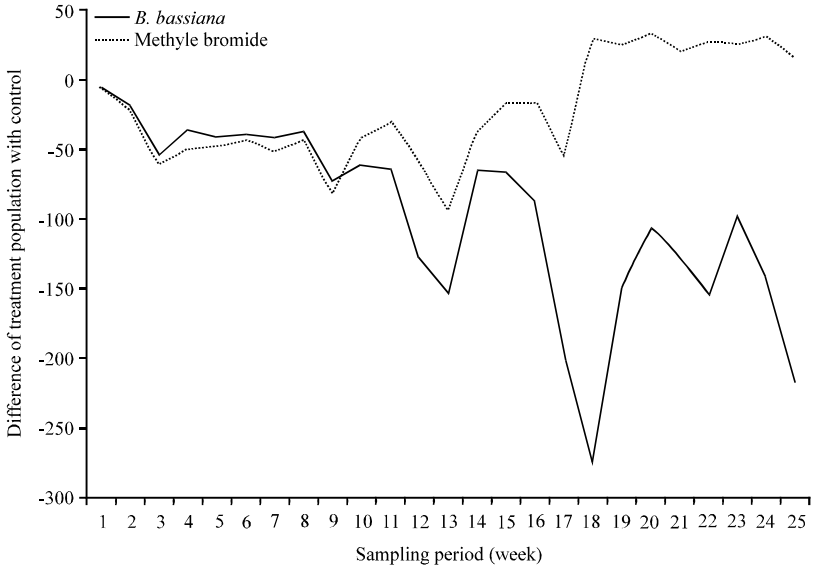

Fig. 2: Sawtoothed beetle population density difference in *B. bassiana* and methyl bromide treatments during the 25 weeks of storage

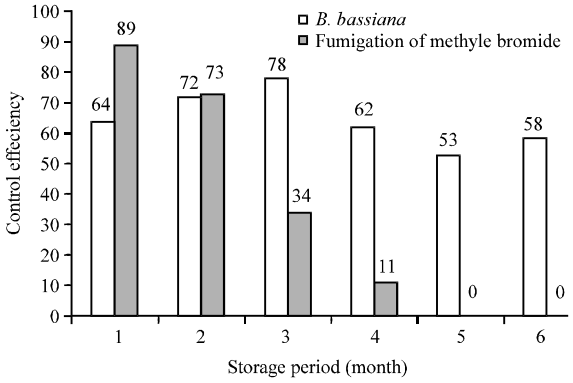

Fig. 3: Comparison the efficiency of *B. bassiana* and Methyl bromide treatment in Date palm storage condition

a predominant perception that fungi require a moist atmosphere. While condition requires atmospheric moisture near saturation, conidial germination and initiation of the process of insect infection is less demanding. Studies on the relationships between moisture and fungal efficacy for insects show great variation (Lord, 2005). The longevity of conidia of *B. bassiana* is generally more stable at cool and dry conditions (Hong *et al.*, 1997). It was also proved that this fungus is more effective at moderate temperatures with an optimum around 25°C (Walstad *et al.*, 1970; Ekesi *et al.*, 1999; Lord, 2005). Akbar *et al.* (2004) in their investigations demonstrated that adults of *Tribolium castaneum* exhibited very little susceptibility to *B. bassiana*. They showed that in commercial products of this fungus, technical powder contained 9.4×10^{10} conidia-g^{11} (strain GHA, Emerald BioAgriculture, Butte, MT) even in 2,000 mg kg^{-1} could only control 8.3±2.5% of this pest after 7 days of treatment. This result is similar to ours research and indicated that 64.99±4.4% mortality of adults *T. castaneum* occurred in 1,000 mg kg^{-1} (corresponding to 2.9×10^{9}). Few more data exists concerning the effects of entomopathogenic fungi on progeny production in storage commodities. Athanassiou and Steenberg (2007) used *B. bassiana* against *S. granarius* in stored wheat. They indicated that this fungus at rate of 0.72×10^{12} spores per kg grain can cause 52% mortality 7 days after treatment and 64% reduction in progeny production (143.8 insect/vial for control group and 52.5 insect/vial for treated one) 65 days after treatment at 55% of R.H. and 25°C.

Jassim *et al.* (1998) showed that *B. bassiana* conidia (300,000/cubic meters) reduced *Cadra cautella* population up to 96 percent in date storage conditions. Overall, the results of this study showed that biological control had application potential for pest management in date storage condition.

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