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Establishment and detection of some Sudanease isolates of *Beauveria bassiana* (Balsamo) Vuillemin in Mejdhool seedling variety of date palm *Phoenix dactylifera* as prerequsit for prophyletic protection against pests and diseases

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Abstract: *Endophytic fungi, which live within host plant tissues asymptomatically, are important mediators of plant-herbivore interactions. two isolates of Beauveria bassiana (E=Sahar-1987OP616114 and M=Elbashir-1976OP616115) , an entomopathogenic fungus,are attempted to colonize the seedlings of date palm, Mejdhool variety using fungal spores suspension applied as foliar spray, injection and soil drench. The two isolates of B. bassiana were successfully established inside the aforementioned seedlings via all colonization techniques which were used. Mean of recovery was calculated by cutting the leaves of the treated plant and culturing it on selective media. The highest percent endophytic fungi recovery rate was recorded in the first months, where the mean of establishment of the three colonization techniques for recovery after 1 months were as follows 95.00±9.26 and 92.50±10.35 in case of spray method for isolate E and M consecutively. For injection, the record was 75.00±20.70 and 87.50±18.32 for E and M, consequently. For drench, 45.00±22.52 and 60.00±14.14 for E and M respectively. While for control all treatments registered 0.00±0.00, according to Duncan's Multiple Range test (DMRT). The study was continued for one year recording declining in establishment in second half of the year in all treatment and all protocols. Scanning electron microscope (SEM) images of the treated leaf surface revealed that numerous conidia inside the plant tissues . SEM of inner surface of treated leaf epidermis showed the spores and hyphae of the fungus. This work was carried out to establish the fungus into the plant hoping that this fungus would protect the plant against pests and diseases.*

Keywords: Date palm, *Beauveria bassiana*, endophyte, pests

1. Introduction

Date palm (*Phoenix dactylifera*L.), is considered one of the most important fruit crops, and provides a primary article of food and commerce in the great desert areas of Western North Africa to India, and many other subtropical areas**.** Constitute the most important agricultural crop in the area and provide highly nutritious food as well as a primary source of income to the majority of the inhabitants. The date palm offers a good food source of high nutritive value (Shinwari, 1993). In the Sudan the date palm trees are cultivated in the Northern state along the banks of the Nile over a distance of about 900 kilometers. According to FAO (2005) the, mean annual production of dates is 328.2 metric tons. This ranked Sudan as the 7th largest producer of dates among Arab countries. However, the date palm is facing many serious problems, including the insect pests and diseases. The use of beneficial microorganisms is one of the main pillars to provide a green turn in agriculture farming systems worldwide due to their noteworthy potential to increase crop health and fitness plus limiting negative impacts on the environment (Finkel et al, 2017). The entomopathogenic fungus, *Beauveria bassiana* is largely used as an alternative to chemical pesticides for the biocontrol of insect pests and the active ingredient of several commercial products used worldwide for sustainable pest management (Faria and Wraight, 2007)

The present study aimed to investigate the establishment of two Sudanese isolates of *B. bassianaas* as a biological control agent for pest and diseases of date palm under three different inoculation methods and using scanning electron microscopy (SEM) techniques to verify the internal colonization of Mejdhool variety by *B. bassiana*

2. Materials and Methods

The experiment was conducted in the laboratory and green house of Bio pesticides and bio fertilizers, Environment and Natural Resources and Desertification Research Institute (ENRRI), Khartoum. - North, Sudan, during the period 2018 – 2019.

2.1. Fungus culture and Preparation of conidial suspensions

Two Sudanese strains of *B. bassiana* (Sahar-1987OP616114 (E) and Elbashir-1976OP616115(M))provided by Department of Biopesticides and Biofertilizers- Environment and Natural Resources and Desertification Research Institute- Khartoum Sudan was grown on PDA media after autoclaved at 120 °C for 20 minutes, and poured in Petri-dishes and incubated about 2-3 weeks at 22

±2◦C and 85 ±5 R.H until the whole plate covered by fungal growth (plate.1).Fungal isolates were cultured for 14 days and kept at 4 °C. Conidia were harvested by scraping the sporulating colonies by soft-tipped sterilized spatula and placed the conidia into distilled water containing 5 ml Tween-80 (0.1% v/v).and filtered through cheese cloth to reduce mycelium clumps (Lacey, 1997). This stock solution was diluted and counts of the conidia were made using an improved Nuebauer haemocytometer (Goettel and Inglis 1997). Resulting conidia were diluted with sterile water containing 1.0% Tween to reach the appropriate concentrations (10^9 conidia /ml) and preserved at 5°C until used in the bioassay.

Plate.1. the culture of *B. bassiana*

2-2 The endophytism of *B. bassiana* in Mejdhool variety by spray, injection and soil drench methods.

the one year old date palm (*Phoenix dactylifera* cv. Mejdhool) was inoculated with *B. bassiana* in one of three ways (spray, Injection and soil drench by inoculating stems, leaves, and soil), For leaf inoculation a handheld sprayer (250 ml capacity) were used to spray each seedling with 3ml of conidial suspension (10^9 conidia/ml), the spray was directed to the leaves and to avoid conidial runoff to the soil, the top of each pot was covered with plastic bag. For Injection method the stems of date palm seedlings were injected with 3ml of 10^9 conidia/ml suspensions by 10 ml sterilized insulin injection needles and was planted in pots as described in the previous paragraph. For soil inoculation, a conidial suspension (10^9 conidia per ml) was applied around the root zone of each seedling. Control date palm seedlings were treated with sterile water containing a 0.01% Tween-80 solution and maintained under the same conditions as the treated seedlings. After inoculation, grown in pots containing sterile soil (approximately 2 kg) and covered with aluminium foil for 24 hrs to maintain a high level of humidity and setting in green house (27 ± 2°C under a 14 h/10 h (light/dark) photoperiod). A completely randomized design with five replicates per treatment was used. The plants were examined for entophytic presence of *B. bassiana*, one, 3, 6, 8, and 12 month post treatment.

Percentage colonization was calculated as number of samples exhibiting *B. bassiana* outgrowth per total number of samples, results are expressed as the percentage of plants positive for the presence of *B. bassiana* after inoculation

2.3 Scanning Electron Microscopy (SEM) examination

Adherence and germination of fungal conidia was examined by low temperature scanning electron microscopy (cryoSEM). A Philips XL20 SEM with an Oxford Instruments CT1500 cryo system was used. The plant (leaves) treated with fungus were positioned on an adhesive carbon disc and the samples were sublimated prior to sputter coating with gold/ palladium. For each plant treated with fungus, an assessment of the number of conidia present on three different areas of the plant was made. These areas were the prior, mid and hind of leaves and leaflets. The total observation of the fungus present was recorded

3. RESULTS

3-1 Means percent of *Beauveria bassiana* Establishment on Mejdhool after one, 3, 6, 8, and12 month with two isolates of fungus (E and M) used three methods of application (spray, injection and drench)

The spray methods of two isolate of *Beauveria* (E=Sahar-1987OP616114 and M=Elbashir-1976OP616115) after one month successfully introduced the *B. bassiana* isolate into Mejdhool plants with a ratio 95±9.26% and 92.50±10.35 % respectively followed by injection methods which recorded 75±20.70% and 87.50±18.32% respectively, whereas the drench methods of two isolate E and M, showed the lowest level 45.00±22.52% and 60±14.14% respectively (Fig 1). Both foliar spray and injection methods resulted in endophytic colonization by *B. bassiana* isolate E in post three months achieved 95±15.12% and 93.50±14.14% respectively. Followed by injection and foliar spray of Beauveria isolate M which recorded 75.50±21.21% and 93.50± 19.82% respectively, the drench methods showed the low level by recorded 40±23.90% and 50±20.70% in E&M respectively. After 6 month colonization in plant was detected a high significantly responded by result 92.00±9.26% and 77.50±0.00% respectively, of spray method by M and E isolates. The injection methods of *Beauveria* isolate M and E was recorded 67.50±14.88% and 75.00±17.73% respectively, on the other hand the least colonization level was observed in drench methods of *Beauveria* isolate M and E by 35.00±18.52% and

37.50±15.12% respectively. (Fig. 1)**.** Colonization was highest with the foliar spray method of the two isolate E and M by 77.50±12.82% and 62.50±12.82% rate. Establishment by injection method of isolate M&E recorded 67.00±20.70% and 47.50±14.88. On other hand the least level observed by soil drench caused 35.00±14.14% and 20.00±15.12% of isolate M and E subsequently post 8 month of inoculation. The lower result of the colonization compared of all previous treatments observed after 12 month of colonization. The fungus was responded by weak result 42.50±12.82% and 40.00±10.69% of foliar spray method recorded by the two isolate M and E respectively. Whereas the injection method of two isolate E and M gave the same result by achieved 25±17.73% and the least one was observed in drench methods of isolate M and E which causeed 20±15.12% and 12.50±14.88% respectively. No growth of two isolates were observed in the non-inoculated control.

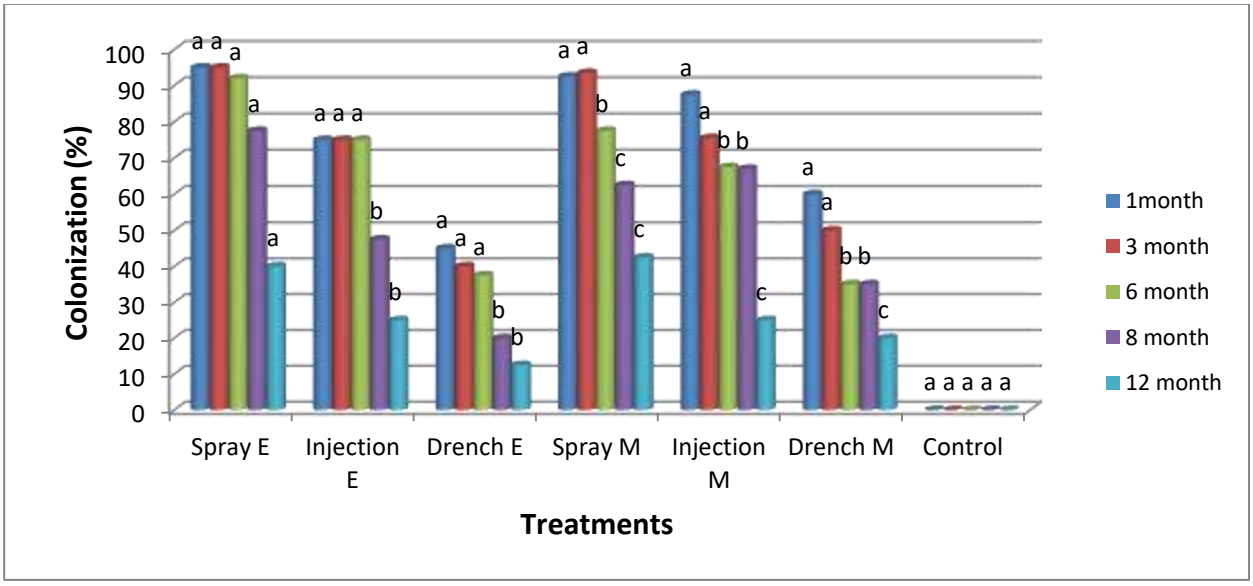

Fig 1: Establishment of *Beauveria bassiana* (isolates E and M) in *Phoenix dactylifera* cv. Mejdhool seedling at different intervals post-treatment, using three application techniques (leaves spray, stem injection and soil drench).
E=Sahar-1987OP616114 & M=Elbashir-1976OP616115

3.2 Scanning electron microscopy of entomopathogenic fungi, *B. bassiana* on Mejdhool variety

The growth was indicated by the apperence of the fungal spores of isolate E & M associated on the leaf surface (fig.1).fig.2 clearly revealed the beginning of interance and interaction of seedlings tissue through the opening stomata. fungal inner plant as colonization of seedlings tissues also were found in isolate M (fig. 3). The fine hyphae of *B. bassiana* as decleared by SEM is shown in Fig. 4 and fungal inside plant and interaction of Date Palm seedlings without any damage of tissue was also shown (fig. 5)

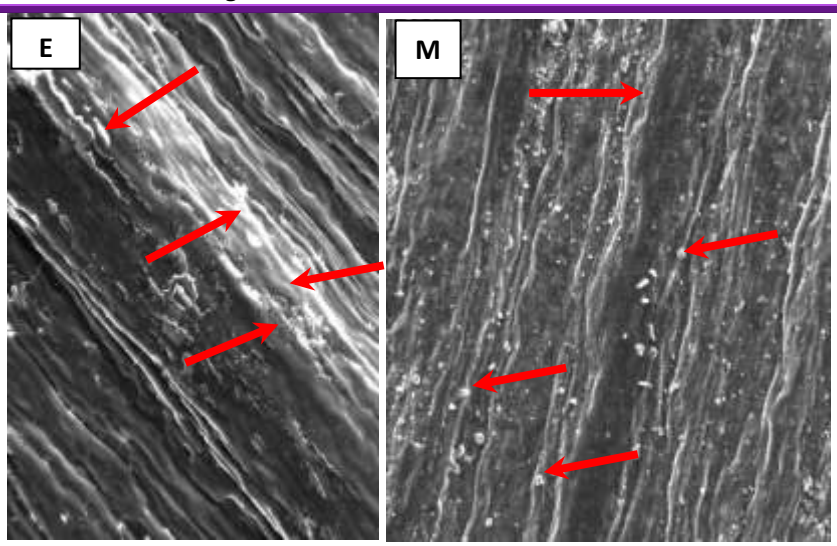

Fig.1 SEM of Date Palm seedling, showing fungal isolate (E&M) its close association to the leaf surface(arrow) at 100 µm

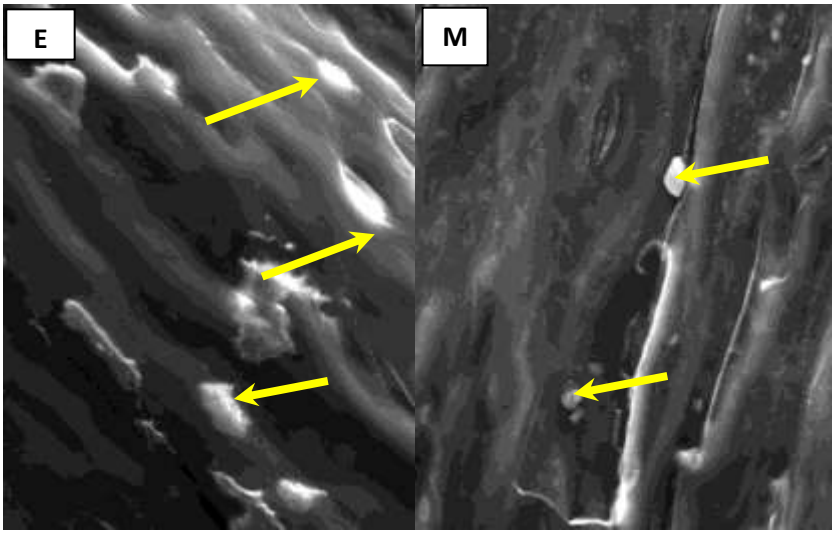

Fig.2. SEM of fungal beginning of interance and interaction of Date Palm seedlings through the opening stomata(arrow) at 20 µm

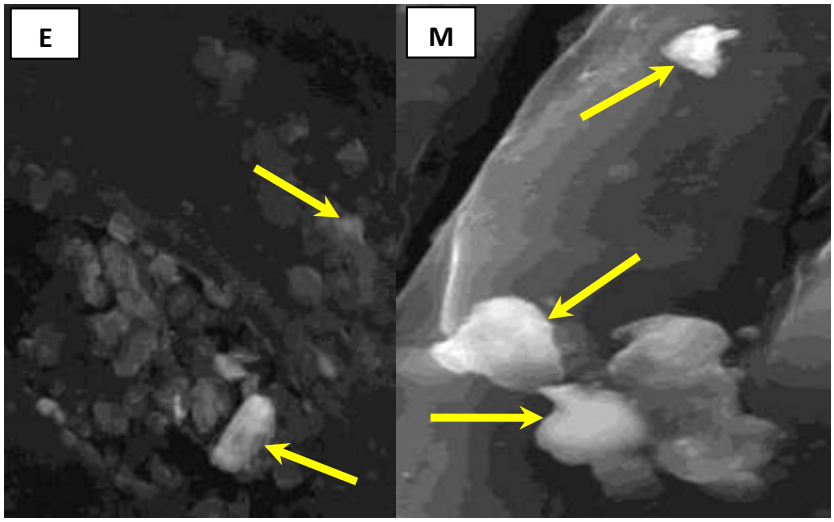

Fig.3. showing entrance and penetrates of fungal through stomata, M fungal inner plant as colonization of Date Palm seedlings of tissue plant(arrow) at 5 µm

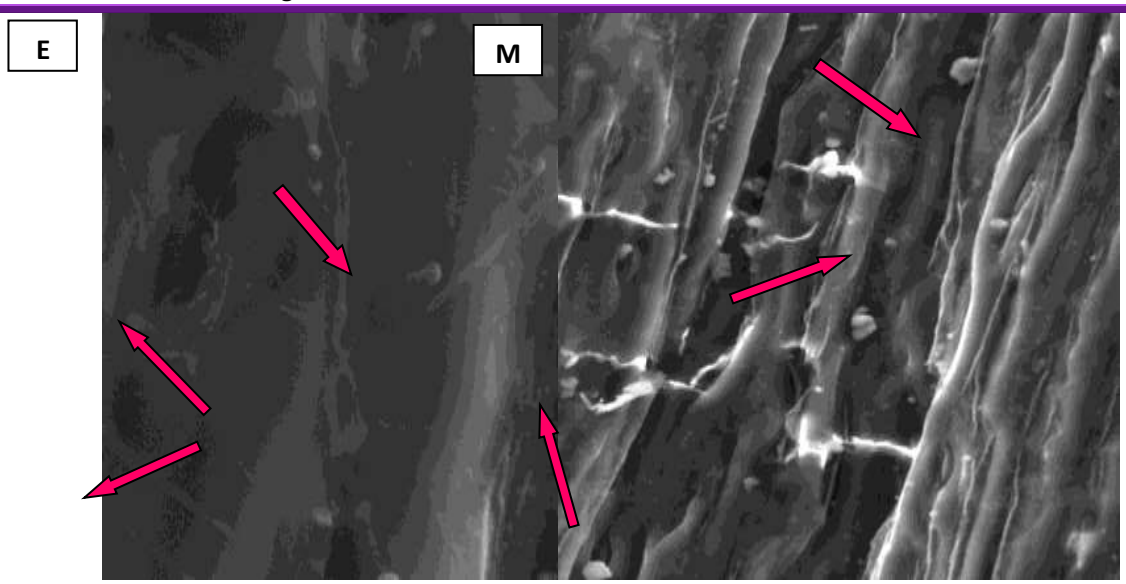

Fig. 4 . SEM of Date Palm seedlings, showing fungal hypha

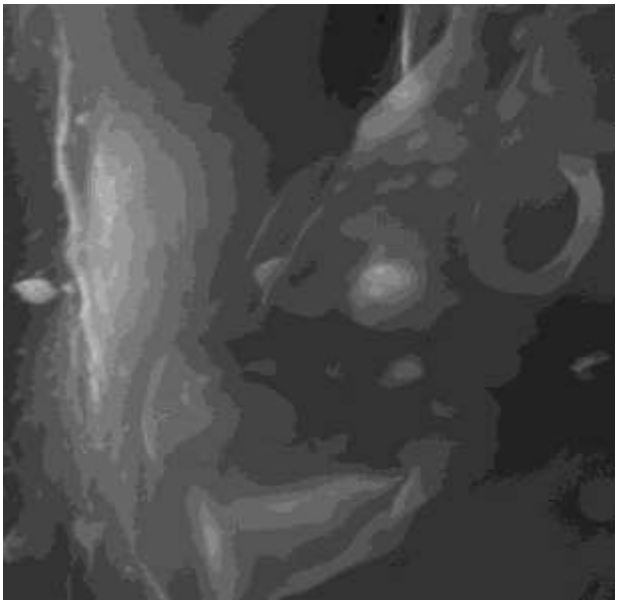

Fig.5. fungal inside plant and interaction of Date Palm seedlings without any damage of tissue plant at 5 µm

4. Discussion

B. bassiana is naturally fungus that is found in the soil of most fields. In this work, tow isolates of B. bassiana were tested for their capacity to colonize date palm Mejdhool, as biocontrol agents

The Present study demonstrates that *B. bassiana* can be established as an endophyte in Mejdhool seedlings by foliar spray, injection and soil drench. colonization by *B. bassiana* depended upon the inoculation method the current results explained that the spray and Injection caused the highest level in plant inoculated when compare with soil drench methods, this agree with the data obtained by Väanninen et al (2000) who mentioned that the *B. bassiana* has been reported to have lower soil persistence when applied as unformulated conidia using the soil drench method. Also this observation is similar to previous findings in other crops such as coffee by Posada et al. (2007) who found that the highest post-inoculation recovery of *B. bassiana* occurred after direct injection.

SEM allowed observing B. bassiana adhesion and penetration structure on the seedling date palm. SEM of Mejdhool with B. bassiana revealed adhesion and penetration structures in the infected tissues. Growth of the fungus on the tissues and signs of hyphal penetration were also appearing. These observation agree with the SEM observation of Saurabh et al (2016) who found the penetration of B. bassiana hyphae into the leaf tissues of inoculated cauliflower leaves

5. Conclusion and recommendation

The local strain of *B. bassiana* used in this study was successfully colonized the Date palm seedlings inoculated with its conidia but further studies on more technique of endophyte are still wanted.

6. Reference

1- FAO (2005). Agro-Statistics Databa (http://faostat.fao.org/site/340/default .aspx).
2- Faria, M.R.d.; Wraight, S.P. Mycoinsecticides and Mycoacaricides: A comprehensive list with worldwide coverage and international classification of formulation types. Biol. Control **2007**, 43, 237–256.
3- Finkel, O.M.; Castrillo, G.; Paredes, S.H.; González, I.S.; Dangl, J.L. Understanding and exploiting plant beneficial microbes. Curr. Opin. Plant Biol. **2017**, 38, 155–163.
4- Goettel, M.S. and G.D. Inglis (1997). Fungi: Hyphomycetes. In: Manual of techniques in insect pathology, Lacey, L.A. (Ed). Academic Press, New York, San Diego, USA,pp: 213- 250.
5- Lacey, L. A. (Ed.) (1997). Manual of techniques in insect pathology. Academic Press, San Diego.
6- Posada, F; Aime, M.C; Peterson, S.W; Rehner, S.A; Vega, F.E; (2007). Inoculation of coffee plants with the fungal entomopathogen Beauveria bassiana (Ascomycota: Hypocreales). Mycol. Res. 111, 749–758.
7- Saurabh Gautam, S Mohankumar, JS Kennedy. (2016). Induced host plant resistance in cauliflower by Beauveria bassiana. *Journal of Entomology and Zoology Studies* ; 4(2): 476-482
8- Shinwari, M.A. (1993). Date palm. *In Encyclopedia of Food Science, Food Technology and Nutrition*, Macrae, R.; Robinson, R.k. and Salder, M.(eds.). Academic Press Ltd, London, UK, pp.1300-1305.
9- Väanninen, I.; Tyni-Juslin, J.; Hokkanen, H. (2000). Persistence of augmented Metarhizium anisopliae and Beauveria bassiana in Finnish agricultural soils. BioControl 45, 201e222.