A NOVEL PATHOGENICITY OF NANO- *BEAUVERIA BASSIANA* AND *METARIHIZIUM ANISOPLIAE* AGAINST*SITOPHILUS ORYZAE* (L.)(COLEOPTERA : CURCULIONDAE)UNDER LABORATORY AND STORE CONDITIONS.

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Abstract—*Sitophilus oryzae* (L.) is a serious primary insect pest of the stored rice, wheat and maize grains **Objective:** The present studies aims to determine the efficacy of the nano-entomopathogenicity of the two nano-entomopathogenic fungi, *B. bassiana* and *M. anisopliae*against one serious pest of stored rice, wheat and maize.**Methods:** The effect of the two nano-entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* were tested under laboratory at concentrations ranged from 1X10 to 1X10^{8}spores/ml and experiments were conducted under laboratory conditions (26±2 ^{o} C and 65±5 %RH.), store (in 15 rice packages were sprayed by the nano-bio insecticides (*B. bassiana* and *M. anisopliae*) at concentration 8.25 x 10^8 conidia /ml for each fungus) **Results:**Data showed that the LC50' s of the rice weevil under laboratory conditions after treated with different concentrations of the nano entomopathogenic fungi which obtained 45X10^4 and 57 X10^4 conidia/ml after treated with Nano-*B. bassiana* and *Nano-M. anisopliae* respectively. under laboratory conditions, the infestations with the rice weevil *S, oryzae* were significantly decreased after the -nano- *B. bassiana* and *M. anisopliae* as compared to control. The infestations with *S. oryzae* under store conditions were significantly decreased after, nano- *B. bassiana*and *M. anisopliae* as compared to the control. Using of entomopathogenic fungi causing a significant reduction of the eggs number laid / female after being treated with nano entomopathogenic fungi, *B. bassiana* and *M. anisopliae*as compared the control. The emerged adults were significantly decreased in the store in the treated bags with nano-entomopathogenic fungi *B. bassiana* and *M. anisopliae*

keywords ., *Beauveria bassiana, Metarhizium anisopliae ,Sitophilus oryzae.*

1. Introduction

The rice *Sitophilus oryzae*weevil are pests of stored grain and seeds. They develop inside whole grain kernels as small, white, wrinkled, grub-like larvae. There is generally no external evidence that the larvae have been eating and growing inside the seed until after about one month when the adult weevil chews through the seed coat and emerges [1]. The adult weevils are 1/8th inch long and have slender, hard-shelled bodies that appear pitted or scarred with tiny holes. They are brown to reddish brown in color. The rice weevil has four faint yellowish spots on the back of the abdomen. The granary weevil is uniformly colored with no spots [2].

*Sitophilus oryzae a*dults feed on whole seeds or flour. Larvae develop in seeds or pieces of seeds or cereal products large enough to house larvae but will not develop in flour unless it has been compacted [3]. Feeding contributes to heating and infested grain is often damp due to moisture added by the insects' respiration [4].[5] rice is also one of the most important economic crops in Egypt. *Sitophilus oryzae* (L.) is a serious primary insect pest of the stored rice, wheat and maize grains. The effectiveness of many secondary plant metabolites for use against insects attacking stored products , was recorded to deter feeding and disturb insects as repellents due to their strong odoriferous nature [3,4, 5].

Currently, chemical control is the most commonly used strategy against the pests. There are many chemicals that are toxic to stored-grain pests, including insecticides such as organophosphates, pyrethroids and fumigants such as methyl bromide and phosphine [6, 7, 8, 9 ,10,11, 12,13] .

There is growing interest in the exploitation of naturally occurring entomopathogenic microorganisms for the control of crop pests. Biological control agents (BCA) may offer more environmentally safe alternatives to chemical pesticides. They could be also used where pests have developed resistance to conventional pesticides[2, 14]. Today many entomopathogens are used for the control of invertebrate pests in glasshouses, row crops, orchards, ornamentals, stored products and forestry [14] [16] found that the fungus *M. anisopliae* killed the insect pests*S. oryzae*through the cuticle and it was not needed to be consumed by them. They also mentioned that they somehow protected the rice.

The present work aimed to explore the protective potency of two nano entomopathogenic fungi as microbial agents against *Sitophilus oryzae* (L.) under laboratory and during storage.

2. MATERIALS AND METHODS

2.1 Study site: This study was carried out at Lab. In N.R.C. Dokki, Giza, Egypt.

2.1. Insect rearing
Adults of S. oryzae (strain RW1) collected frominfested stored wheat grains were reared on healthywheat grains (CV: Anbar) held in cloth mesh coveredplastic pots (15 cm diameter by 20 cm high) at 2872!C,7075% RH, and 16:8 L:D cycle. Newly emerged adults(males and females) were used in the experiments.

2.4 Cultivation of the fungi: The fungi *B. bassiana* and *M. anisopliae* were kindly obtained from Prof. Dr Alain Vey, Mycology unite, National De La Research Sientifique, Univ. Montpellier. (Apopka strain 97 and reproduced in Microbiology Dept., N.R.C. Cairo, Egypt. The fungi were primarily purified using the mono-spore technique. They were propagated in Petri-dishes (10cm) on potato dextrose agar medium (PDAM) enriched with 1% peptone, 4% glucose, and 0.2% yeast and incubated at 26 °C. Seven-days old cultures with well-developed spores were harvested by washing with 10 cc sterilized water, then added 3ml, Tween-80 and completed to 100 ml water and used as stock suspension with known spore concentration and kept in a refrigerator at 4 °C, from which the fungi were sub-cultured to be used in laboratory evaluation tests (infectivity and bioassay tests). Then adjusted as conidiophores concentration of $1X10^8$ spores /ml. Large amount of conidia spores, if needed, were produced by culturing the fungus on liquid medium in 1L cell culture glass bottles according to [16].

2.5 Evaluation of the fungi effects on the target insect pest: The fungi, *B. bassiana* and *M. anisopliae*at concentrations ranged from $1X\ 10^2$ to $1X10^8$ spores /ml were prepared and tested against *P. operculella* third instar larvae. Experiments were conducted under laboratory conditions (26 ± 2 ^{o} C and 65± 5 %RH.). Fresh leaves of potatoes were sprayed with the desired diluted suspension to the point of run off, left to dry, then put in 1 L plastic container(5 containers were used/concentration/ treatment). Twenty newly larvae of each species were placed in each container and covered with muslin. Untreated leaves were sprayed by water only and used as control. The leaves were changed every other day. The experiment was repeated 4 times. The percentages of mortality were calculated after seven days and corrected according to Abbott (1925) [16] while LC_{50}s were calculated through probit analysis of [17].

2.2. Fungal formulations
Five fungal formulations were prepared using strainMeta. 1 of *M. anisopliae* (obtained Prof. Dr Alain Vey, Mycology unite, National De La Research Sientifique, Univ.

Montpellier. Each formulation contained fungal conidiaand a dust carrier at 1:4 ratio (W/W). The dustcarriers that were obtained from local sources are wheatflour (milled durum wheat grains of CV: Anbar), ovenash (completely burned paper sheets), chalk powder(finely ground board chalks) and charcoal (finely groundcoal pieces). Comparative treatments were unformulatedconidia and fungus-free dust carriers. Conidiaharvested from 14-day-old cultures of M. anisopliaegrown on oat meal agar medium (OMA) plates werethoroughly mixed with the carrier in screw cappedbottles. The formulations contained 5.2"108 conidia/g determined by dilution of 1 g of the formulation in 50mlof sterile distilled water held in screw-capped vialsfollowed by vigorous shaking before counting byhemacytometer. The same stock fungal conidia wereused in preparing the formulations to ensure that nodifferences were present among the individual formulations.The initial water content measured on a dryweight basis was 6% for unformulated conidia and3–4% for the formulations, according to the type of dustcarrier.

2.3. Conidial viability

Suspensions of 0.5% (W/W) of formulations or 0.1%(W/W) of unformulated conidia in sterile distilled waterwere prepared and then diluted 100-fold. Twenty-fivemicroliters of diluted suspensions were spread onto thesurface of glass slides held in Petri dishes under humidconditions and scored for germination after 24 h ofincubation at 2572!C. No wetting agent or agar medium for coating the slides was used in this test. The assessment of conidial viability was determined each week for a 22-week period. The formulated and unformulated conidia were stored during the study in tightly closed screw capped bottles under a constant temperature of 2071!C. This was to exclude the effect of variable environmental conditions on conidial viability during the assessment. Three replicates representing three glass slides per formulated or unformulated conidia were used and three counts were performed on each. The mean percent conidial germination was calculated. Log-linear regression analyses of viability (% conidial germination) versus time were performed to determine the viability half-life of each formulated and unformulated conidia treatment.

2.4. Bioassays

The application of fungal treatments was carried out by adding 0.2 g of each formulation containing 1.04"108 conidia to 200 wheat grains (mean weight= 10.02 g) held in a small, cloth-mesh covered plastic can (9.5 cm diameter, height 5 cm and area 70.85 cm2). This resulted in an application rate of 2.8 mg/cm2 of treated area or 2.0% (W/W of formulation to wheat grains). As control treatments, 0.16 g of each fungus-free dust and 0.04 g of unformulated conidia were applied versus 0.2 g of prepared formulation. Ten newly emerged adults (five males and five females) of S. oryzae were introduced using a small brush into each replicate can. Infested cans were then incubated at 2872!C, 7075% RH, and 16:8 L:D cycle. Three replicate cans per treatment and 1–2-week-old preparations of formulated and unformulated conidia were used in all tests.

2.4.1. Adult insect mortality and wheat grain damage

To assess efficacy against S. oryzae, treatments were applied either before or after pest infestations. Accordingly, introduction of insects was done either on thesame day of the treatment or 14 days before the treatment. The 14-day period was sufficient for the insect to cause damage to the grains. Dead and livingadults were counted 7 days after the treatment and used to the percentage of adult mortality. The damage rate to wheat grains by the insect was assessed with the treatments applied before pest infestation. The number of damaged grains was counted 21 days after the treatment and percentage of damage was calculated for each replicate can. 2.4.2. Development time and mortality of F1 adults To assess treatment effects o n subsequent S. oryzae development time, all adults surviving treatment were removed from cans 14 days following treatment. The 14-day period was sufficient for egg deposition andsubsequent

hatching. All replicate cans were then incubated as indicated above until peak emergence of F1 adults. Development time was defined as the time period from egg deposition to peak emergence of F1adults. The effect of the previously applied treatments on the mortality of emerged F1 adults was also assessed. Dead and living F1 adults were counted 7 days after treatment and peak emergence, and the percentage of adult mortality was calculated.

3. RESULTS

Table 1 show that the LC50' s of the rice weevil *S. oryzae* under laboratory conditions after treated with different concentrations of the nano entomopathogenic fungi which obtained that $45X10^4$ and $57\ X10^4$ conidia/ml after treated with Nano-*B.bassiana* and *Nano-M. anisopliae* respectively.

Under store conditions the LC50's of both nano-fungi recorded that, $66X10^4$ and $77\ X10^4$ conidia/ml after the rice weevil treated with different concentrations of both nano entomopathogenic Nano-*B. bassiana* and *Nano-M. anisopliae* ,respectively **(Table 2).**

Table 3, show the effect of the nano-particles against the rice weevil biology under laboratory conditions, the number of eggs laid per female significantly decreased 22±3.7 and 34±3.9 after nano- *B. bassiana*and *M. anisopliae* as compared to 286±8.3 eggs/female. The percentage of egg hatching reduced to 2% after nano *M. anisopliae* treatments as compared to 100% in the control .the malformations among the adult emerged reached to 97 and 98% after nano- *B. bassiana*and *M. anisopliae* as compared to100% in the control.

Table 4; Accumulative mortality of *S. oryzae* during the first week of rice seeds exposed to treated foam with Nano-*B.bassiana,* and*Nano-M.anisopliae*. After seven days from treated rice seeds the accumulative mortality were 69.2, 57.2, respectively compared with 0.0 in the control (untreated). Also after 15 days the accumulative mortality recorded, 88.1, 77.9 after treated withNano-*B. bassiana* and *Nano-M. anisopliae* respectively as compared to 1.1 in the control (untreated).after 45 days of rice storage period , the accumulative reached to 92.3 and 93.3 of the corresponding treatments as compared to 1.2 in the control (untreated).
Figure 1, show under laboratory conditions, the infestations with the rice weevil *S, oryzae* were significantly decreased after the -nano- *B. bassiana*and *M. anisopliae* as compared to control.

Figure 2, show that the infestations with *S. oryzae* were significantly decreased after, nano- *B.bassiana*and *M. anisopliae* as compared to the control.

4. DISCUSSIONS

Similar results were found by [17, 18] who storage the different stored materials under laboratory conditions. The same results obtained by [20, 21]who controlled the stored product Insect pest by the different bioagent under laboratory and store conditions..

Table (1):: Evaluation of the two entomopathogenic fungi, *B. bassiana* and *M. anisopliae*, *on S. oryzae* under laboratory conditions at 26 ± 2 ^{o} C and 65± 5 %RH. In this respect, [21,21] applied different doses of bio insecticide against stored product pests under laboratory and store conditions and found that they protect the seeds from pest infestation.

The increase in the pathogenicity of *B. bassiana* combined with mustard oil to *C. maculatus* beetles may be attributed to some degradation occurring at the structural level of the integument, which could have facilitated the penetration of the cuticle by the germ tube of the fungus. Similar results were obtained by [23] in *Manduca sexata* treated with *M. anisopliae* and the chitin-synthesis inhibitor dimilin. Synergistic effects of a combined application of *B. bassiana*

and the chloronicotinyl insecticide Imidacloprid on the Curculionid *Diaprepes abbreviates* were reported by [24]. The same results obtained by [25 &26] .
[27],recorded that the LD_{50} for some formulations of *B.bassiana* was reduced to 97 % after the addition of coconut oil. It was suggested that the cutinophilic properties of the oil could allow a greater number of fungal conidia to penetrate the mouth parts of insects. Oil carriers can also distribute the inoculum over the thin intersegmental membranes, which are more readily penetrated by entomopathogic fungi [27, 28], applied different doses of the essential oils *Acorus calamus* to seeds of green gram *Viga radiate* to protect them against *Callosobruchus chinensis* and found that 1 ml/kg offered a high degree of protection up to a period of 135 days. Prolonged protection of the seeds was mainly due to a high adult mortality besides reduced oviposition and low hatching. [29] reported that foam sprayed with clove oil (5%) and placed between sacks caused the highest mortality. [30] reported that edible oils are potential control agents against *S. Oryzae* and play an important role in stored-grain protection. [29] mentioned that clove and eucalyptus oil vapours impaired the fecundity of brachid beetles. [30] reported that edible oils are potential control agents against *S. oryzae* and play an important role in stored-grain protection. [31] mentioned that clove and eucalyptus oil vapors impaired the fecundity of brachid beetles. Data proved promising oviposition deterrence toxicity and suppression of eggs and adult emergence.

We find in our study that the two entomopathogenic fungi due to reduction the number of eggs laid / female after being treated with *B. bassiana* and *M. anisopliae*as compared the control. The emerged adults were decreased and the yield weight of potatoes increased in plots treated with*B. bassiana* and *N. rileyi*.
Many studiesfound that the fungi *B. bassiana, M. anisopliae, Pacilomyces fumosoroseus Verticillium lecanii;* reduced insect infestations of cabbage and tomato pests under laboratory and field conditions [19, 20]. Also, found that, in all treatments the number of corn pests were significantly decreased [21]. Moreover, these results agree with, [22, 23]. The same results obtained by [32] Sabbour(1992),who find that the potato tuber moth affected by the different formulations of the *Bacillus thuringiensis* and the fungus *B. bassiana* causes a higher mortality to the target pests [33]. The same findings recoded by[34], who control *Earias insulana* by the microbial control agents [35]. Fadel and [36, 37]could to produce the microbial control agents on the coffee and Dairy media [38, 39]. [10],Control potato tuber moth by the combinations between the microbial control agents and the plant extract [26]. [26.]. [41, 42, 43,44,45] studied the effect of terpines and microbial control agents against cotton bollworms can find that the cotton bollworms decreases after treatments in both laboratory and field conditions [29, 30, 31, 26,27,41,46,47], Used two entomopathogenic fungi alone or in combination with modified diatomaceous earth to Control of *Bruchidius incarnates* and *Rhyzopertha Dominica*. [33, 42]. Usingof *Nomuraea rileyi* and *Isaria Fumosorosea* on some serious pests and the pests' efficient predator prevailing in tomato fields in Egypt.[32, 33].
The results obtained by [49,50].[48.49].,Also studding the nanotechnology and microbial control agents against stored products under laboratoy and store conditions [34].
[48.49, 50], also Sabbour, et al., (2012), Used UV to enhance the bacteria *B. thuringiensis*against the potato tuber moth [35].[48.49], used entomopathogenic fungi to control some insects and the results according that results [36, 37, 38, 39]. According with[48] mentioned that biological insecticides in particular were more effective in preventing losses by insects in stores in cases where the initial level of infestation was relatively low [41]. Our results meet with [50]who find that, the effect of the nanoparticles (Silica gel Cab-O-Sil-750 , silica gel Cab-O-Sil-500) against *S. oryzae* under store conditions showed after treatments with 0.2% treatments with nano Silica gel Cab-O-Sil-750 , silica gel Cab-O-Sil-500, the number mortality of *S. oryzae* were significantly increased to 14.1±2.3, 28.2±8.8 and 29.8±3.9 individuals after nano Silica gel Cab-O-Sil-750 , silica gel Cab-O-Sil-500 treatments as compared individuals in the control. At higher concentrations of 3% the mortality percent were significantly increased to 89.0±1.1 individuals after 45 days of applications as compared to 3.0±2.6 individuals in the control. [52] reported that, the effect of the nanoparticles (ZnO) against *S. oryzae* under store conditions at concentration, 0.2% treatments with nano ZnO, the number mortality of *S. oryzae* were significantly increased to 14.1±2.3,

28.2±8.8 and 29.8±3.9 individuals after nano ZnO treatments as compared individuals in the control.At higher concentrations of 3% the mortality percent were significantly increased to 89.0±1.1 individuals after 45 days of applications as compared to 3.0±2.6 individuals in the control. [52]

Table 1: Evaluation of the two entomopathogenic fungi, *B.bassiana*and *M. anisopliae*, *onS. oryzae* under laboratory conditions at 26 ± 2 ^{o} C and 65± 5 %RH.

Target pathogen	LC_{50}	*S*	*V*	95% C*onfidence limits*
Nano-*B. bassiana*	45X10^4	0.1	1.4	39-139
Nano-M. anisopliae	57 X10^4	1.1	1.1	47-99

Table2: Evaluation of the two entomopathogenic fungi, *B. bassiana*and *M. anisopliae*on, *S. oryzae* under store conditions.

Target pathogen	LC_{50}	*S*	*V*	95% C*onfidence limits*
B. bassiana	66X10^4	0.1	1.1	44-109
M. anisopliae	77 X10^4	1.1	1.0	48-100

Table 3: Effect of the two nano- entomopathogenic fungi, *B. bassiana* and *M. anisopliae*tested against *S. oryzae* biology.

Target pest	No of eggs laid/female	% of egg hatching	% of larval mortality	% of malformed larvae	% of malformed pupae	% of emerged adults	% of malformed adults
B. bassiana	22±3.7	3	65	69	71	1	97
M. anisopliae	34±3.9	2	70	79	79	1	98
Control	286±8.3	100	-	-	-	100	-
F value	35.0	2	4	4	20	20	20
Lsd5%	12.1	1	3	3	12	12	11

Table 4: Accumulative mortality of *S. oryzae* during the first week of rice seeds exposed totreated foam with the two nano entomopathogenic fungi . Means within columns followed by the same letter are notsignificantly different (LSD test p > 0.05

Time (days*)*	*Accumulative mortality*		
	Nano-*B. bassiana*	Nano-*M. anisopliae*	Control (untreated)
0.0	29.9	28.8	0.0
2	51.8	44.9	0.0
4	56.2	49.5	0.0
7	69.2	57.2	0.0
10	72.1	67.9	0.0
15	88.1	77.9	1.1

30	91.1	90.1	1.1
45	92.3	93.3	1.2

Figure 1: The infestation percentage under laboratory conditions after nano entomopathogenic fungi treatments.

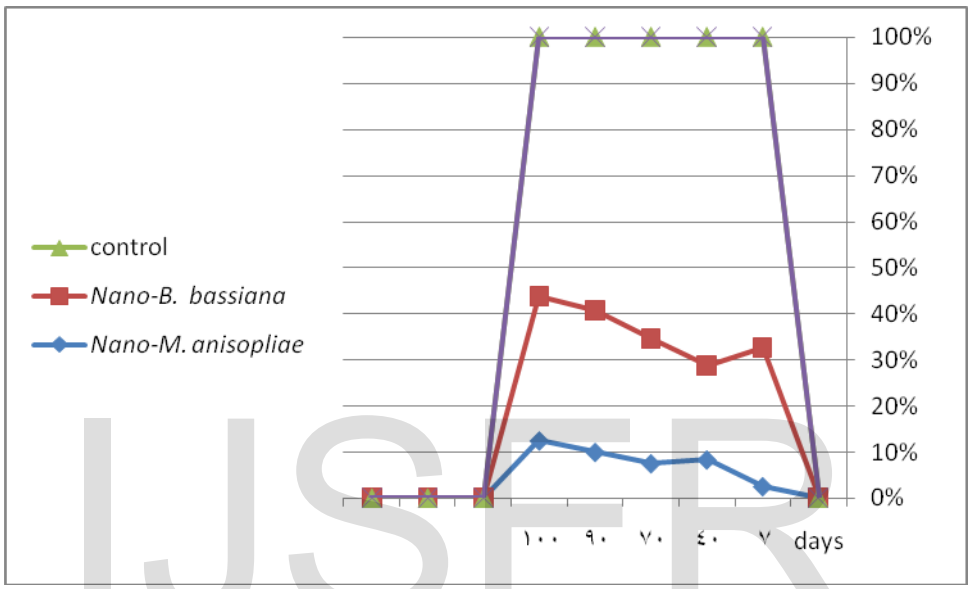
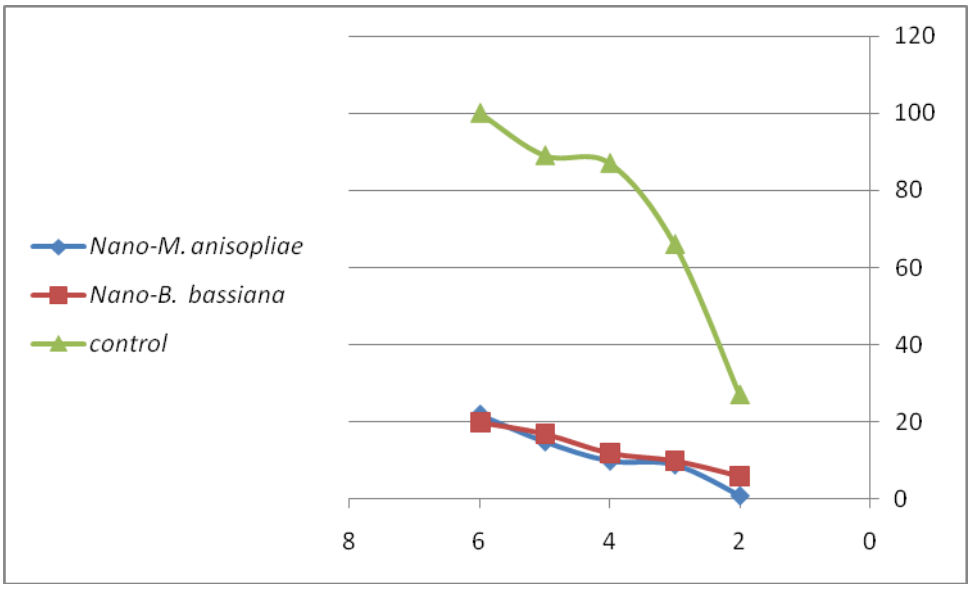


Figure 2: The infestation percentage under store conditions after nano entomopathogenic fungi treatments.



5. CONCLUSION

The Using of the nano-entomopathogenic fungi *B.bassiana*and *M. anisopliae* against the rice weevils leads to reduction of the number of eggs laid / female after being treated with both nano *B. bassiana*and *M. anisopliae*as compared the control.The emerged adults under store conditions decreased after treated with*B. bassiana*and *M. anisopliae*.

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