Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2015; 3(6): 316-320

Received: 07-10-2015
Accepted: 12-11-2015

Abdel Raheem MA
Pests and Plant Protection Department, Agricultural & Biological Research Division, National Research Centre, 33rd ElBohouth St. – Dokki, Giza - Egypt.

IA Ismail
Pests and Plant Protection Department, Agricultural & Biological Research Division, National Research Centre, 33rd ElBohouth St. – Dokki, Giza - Egypt.

RS Abdel Rahman
Pests and Plant Protection Department, Agricultural & Biological Research Division, National Research Centre, 33rd ElBohouth St. – Dokki, Giza - Egypt.

NA Farag
Pests and Plant Protection Department, Agricultural & Biological Research Division, National Research Centre, 33rd ElBohouth St. – Dokki, Giza - Egypt.

IE Abdel Rhman
Department of Plant Protection, Faculty of Agriculture, Al-Azhar Univ. Egypt.

Correspondence
Abdel-Raheem MA
Assoc. Prof. Abdel-Raheem, M.A., Pests and Plant Protection Department, Agricultural & Biological Research Division, National Research Centre. 33rd ElBohouth St. - Dokki, Giza, Egypt.

Entomopathogenic fungi, *Beauveria bassiana* (Bals.) and *Metarhizium anisopliae* (Metsch.) As biological control agents on some stored product insects

Abdel-Raheem MA, IA Ismail, RS Abdel Rahman, NA Farag, IE Abdel Rhman

Abstract
The present work was evaluation of the efficacy of the entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* on *Rhyzopertha dominica*, *Sitophilus oryzae* and *Oryzaephilus surinamensis*. Each insect species was treated with formulated fungi *B. bassiana* and *M. anisopliae* at the concentrations of 0.12 x 10 ^{6} conidia /g, 0.22 x 10 ^{6} conidia /g, 0.32 x 10 ^{6} conidia / g, and 0.42 x 10 ^{6} conidia /g. *M. anisopliae* was more effective against tested insects than *B. bassiana*. Four concentrations of *B. bassiana* were tested against *R. dominica,* S. *oryzae* and *O. surinamensis*. LC_{50} were 1.2 x 10 ^{5} conidia / g, 1.6 x 10 ^{5} conidia / g and 1.4 x 10 ^{5}conidia / g, respectively. The same concentrations *M. anisopliae* were tested against *R. dominica, S. oryzae* and *O. surinamensis*. LC_{50} were 2.7 x 10 ^{5} conidia / g, 1.3 x 10 ^{5} conidia / g, and 3.5 x 10 ^{5} conidia / g, respectively.

Keywords: *Beauveria bassiana, Metarhizium anisopliae*, Stored Product, Control.

1. Introduction
Almost, all stored grains are subjected to be attacked by pests causing damage to stored [1]. It has been estimated that during storage 10 – 25 % of the worldwide grain crops are lost yearly mostly because of pests infestation. Losses caused by insects include not only the direct feeding damage resulting in loss of weight, but they also severely reduce nutrients, lowering percentage of seeds germination, reducing grade and lowering their marketing value due to accumulation of waste, webbing and insect cadavers. The favorable climatic conditions and relatively poor storage system in Egypt are in favor of increase and development of these pests, resulting in considerable crop losses. The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), the lesser grain borer *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.)(Coleoptera: Silvanidae), and cowpea weevil, *Callosobruchus maculates* F. (Coleoptera: Bruchidae) are among the most destructive pests of stored grain and seeds in the tropics [2]. Use of pesticides is one of the means for preventing some loss during storage. It causes problem such as; insecticide resistance, chemical residues in food stuffs, increasing cost, and environmental pollution [3].

Therefore, it is necessary to find out safer alternative control strategies such as use of microbial control agents against stored –product insect pests. Using fungi and selected insecticides can potentially reduce the use of chemical insecticides and subsequently their residues and side effects in agriculture. *Beauveria bassiana* and *Metarhizium anisopliae* are naturally occurring entomopathogenic fungi with a wide host range [4]. Several studies have shown the effectiveness of *B. bassiana* and *M. anisopliae* for controlling the stored products pests such as; S. *oryzae*, *R. dominica*, *A. desobtectus, C. maculates* and *T. castaneum* [5-21]. *B. bassiana* and *M. anisopliae* were isolated from different insect hosts in Egypt [22, 23]. However, a variety of factors may determine or influence the host range and specificity of fungal pathogens. These include fungal strain, hosts physiological state, nutrition, defense mechanism, cuticle and epicuticular microorganisms [24]. Used entomopathogenic fungi to control *Tuta absoluta* and *Cassida vittata* Vill. The pathogen *B. bassiana* gave the highest effect on larval phase of *T. absoluta* during the evaluation time and similar to the effect of *M. anisopliae* followed by the effect of *Verticillium lecanii* [24, 25].

The aim of the present work was evaluation of the efficacy of the entomopathogenic fungi, *B. bassiana* and *M. anisopliae* on *Rhyzopertha dominica*, *Sitophilus oryzae* and *Oryzaephilus surinamensis*.

2. Materials and Methods
2.1 Study site
This study was carried out at Laboratory, National Research Centre. Dokki, Giza, Egypt.

2.2 Insect Rearing
Tested stored product insects species viz; *R. dominica* (F.), S. *oryzae* (L.) and *O. surinamensis* (L.) were obtained from laboratory cultures reared for several generations. The insects were reared on whole wheat grains. Insect cultures were maintained in plastic jars (1 liter) covered with muslin cloth. All insects were reared under laboratory conditions of 24±2 °C and 75±2% R.H.

2.3 Fungal Inoculation
The entomopathogenic fungi, *B. bassiana* and *M. anisopliae*, used in the experiments, were original isolated from the tortoise beetle, *Cassida vittata* (Vill.) and the sugar-beet moth, *Scrobipalpa ocellatella*. The fungi were grown on Potato dextrose agar (PDA) medium (1 Kg potatoes, 80 g Agar, 100 g Dextrose and 4 liter distilled water). The media was autoclaved at 120 °C for 20 minutes, and poured in Petri-dishes (9 cm diameter x 1.5 cm) and rice media. The fungi kept at 25 ±2 °C and 85± 5 % RH. The fungal isolates were sub-cultured every 14 – 30 days and kept at 4 °C.

2.4 Conidiospores Production
B. bassiana and *M. anisopliae* aerial conidia were produced using biphasic culture system [26]. Flasks (100 ml.) of liquid biomalt medium (25g biomalt and 2g yeast extract), were incubated for 3-4 days at 25 °C. The liquid cultures were then used to inoculate autoclaved white rice in sterilized plastic bags. The rice was first soaked in boiling water for 10 minutes and autoclaved for 30 min. in autoclavable plastic bags(30 x 50 cm) in the ratio of 1 kg rice/ bag. After cooling for one to three days, the old liquid culture of *B. bassiana* was mixed by hand with the substrate under aseptic condition, at the ratio of 25 ml/ bag. An absorbent cotton plug rolled around tube (10 cm long) had been used to plug each bag. The bags were connected with the source of filtered air through air valves. Solid substrate fermentation was kept for 11 days at 26 °C. Culture was observed daily and crumbled by hand within bags to prevent binding of the Substrate. Whole culture was then transferred to wooden screen shelves where it was dried for 7 days at 28 °C. Conidia were harvested by mechanical sieving.

2.5 Bioassay Procedures
The three tested insects, at mixed stages, were treated by the fungi to estimate the susceptibility of each species. Harvested conidiospores were mixed with talc powder and counted in the suspension by using a haemocytometer (Hirschmann 0.1 mm x 0.0025 mm2) to prepare four concentrations. Each insect species was treated with formulated fungi *B. bassiana* and *M. anisopliae* at the concentrations of 0.12 x 10^6 conidia /g, 0.22 x 10^6 conidia/g, 0.32 x 10^6 conidia / g, and 0.42 x 10^6 conidia /g. Insect diets were treated by different concentrations of entomopathogenic fungi, twenty five unsexed adult insects were introduced into the jars (the replicate was 25 insects /concentration / fungus /insect species). Non exposure and exposure of insects to contaminated diets with fungal spores were incubated at 26 °C and 70±5% R.H. [27]. Mortality rate was recorded at 3, 5, 7, 9 and 11 days after treatment. The percentage mortality and LC_{50}, LC_{90}, and LT_{50} were estimated.

2.6 Statistical Analysis
Mortality rates of the tested insects were analyzed using ANOVA test. Probit analysis was used to calculate values of the lethal concentration (LC_{50} and LC_{90}) and the lethal time (LT_{50}) for each fungus [27].

2.7 Statistics
LC_{50}s were calculated through probit analysis.

3. Results
R. dominica LC_{50} and LC_{90} values confirmed that *R. dominica* was more susceptible to *M. anisopliae* than *B. bassiana*, where LC_{50} and LC_{90} were 1.2 x 10^5, 2.3 x 10^5 conidia /g and 2.7 x 10^5, 0.01 x 10^6 conidia /g, respectively. The LT_{50}s were calculated as 4.6 and 6.6 days for the two respective fungi, indicating the superiority of *B. bassiana* over *M. anisopliae* (table 1).

Table 1: LC_{50}, LC_{90} and LT_{50} (days) [a] values of *R. dominica*, S. *oryzae* and *O. surinamensis* treated with *B. bassiana* and *M. anisopliae.*

Species	*B. bassiana*			*M. anisopliae*		
	LC_{50}	LC_{90}	LT_{50}	LC_{50}	LC_{90}	LT_{50}
R. dominica	2.7 x 10^5	0.01 x 10^6	6.6	1.2 x 10^5	2.3 x 10^5	4.6
S. *oryzae*	4.3 x 10^5	0.08 x 10^6	8.8	1.6 x 10^5	7.1 x 10^5	6.3
O. surinamensis	3.5 x 10^5	0.04 x 10^6	6.9	1.4 x 10^5	4.1 x 10^5	5.7

[a] at concentration of 0.12 x 10^6 and 0.22 x 10^6 for *B. bassiana* and *M. anisopliae.*

Data presented in table 2 show cumulative mortality percentage of *R. dominica* maximum mortality percentages adult were recorded 11 days after treatment with *B. bassiana* and *M. anisopliae*, where the mortality percentages were 25.2, 38.1, 45.2 and 50.3% for *B. bassiana* at the tested concentrations of 0.12 x 10^6conidia /g, 0.22 x 10^6conidia /g, 0.32 x 10^6conidia / g, and 0.42 x 10^6conidia /g, respectively, and 63.0, 72.3, 75.3, and 79.3% for *M. anisopliae* at the tested concentrations of 0.12 x 10^6conidia /g, 0.22 x 10^6conidia /g, 0.32 x 10^6conidia / g, and 0.42 x 10^6 conidia / g, respectively.

Table 2: Cumulative mortality percentages of *R. dominica* treated with *B. bassiana* and *M. anisopliae* at different concentrations.

Fungi	Conc. Conidia/g	Mortality percentages of *R. dominica* indicated days				
		3	5	7	9	11
B. bassiana	0.12 x 10^6	4±1	17±3.1	19±1.2	21±1.9	25.2±2.1f
	0.22 x 10^6	13±1.9	22±2.1	25±1.4	32±1.1	38.1±2.3ef
	0.32 x 10^6	15±7.5	25±1.9	28±1.6	39±1.9	45.2±2.7e
	0.42 x 10^6	22±3.8	27±4.1	32±1	47±1.9	50.3±2.2c
M. anisopliae	0.12 x 10^6	8.7±2.1	25±2.1	35±2.1	45±2.1	63.0±1.9b
	0.22 x 10^6	9.5±2.3	35±4	45±2.8	53±1.1	72.3±2.3 a
	0.32 x 10^6	20±1	36±2.3	55±1	61±1.9	75.3±2.2a
	0.42 x 10^6	33±2.3	41±1.2	60±1.3	70±1.2	79.3±1.2a

Means followed with the same letter are not significantly different

Sitophilus oryzae

Obtained result presented in table 3 indicated that the cumulative mortality percentages started with low levels on the 3rd day after treatment. Then the Percent mortality of *S. oryzae* was increased gradually, till the maximum was recorded on the 11th days of observation, where the mortality percentages were recorded 23, 32, 40 and 54% for *B. bassiana* respectively. The *S. oryzae* was more susceptible to infection with *B. bassiana* than *M. anisopliae*. LC_{50} and LC_{90} values proved that *S. oryzae* was more susceptible to *M. anisopliae* than *B. bassiana*, where the LC_{50} and LC_{90} of, *B. bassiana* and *M. anisopliae* were 4.3 x 10 ^{5} and 0.08 x 10 ^{6} conidia /g, and 1.6 x 10 ^{5} and 7.1 x 10 ^{5}conidia/g, respectively (Table 1). LT_{50}s were calculated as 8.8 and 6.3 days for the two respective fungi, indicating that the conidial spores of *B. bassiana* were less efficient on *S. oryzae* than those of *M. anisopliae*.

Table 3: Cumulative mortality percentages of S. *oryzae* treated with *B. bassiana* and *M. anisopliae* at different concentrations.

Fungi	Conc. Conidia/g	Mortality percentages of S. *oryzae* indicated days				
		3	5	7	9	11
B. bassiana	0.12 x 10 ^{6}	4.4±2.1	8±2.1	18±1.1	21±1	23.0±1.3f
	0.22 x 10 ^{6}	8.2±2.3	18±3.2	25±2.1	30±1	32±7.1 c e
	0.32 x 10 ^{6}	11±1	25±3.3	27±2.1	38±1	40±1bc
	0.42 x 10 ^{6}	21±2.1	35±2.1	40±5.2	43±2	54±2.2ab
M. anisopliae	0.12 x 10 ^{6}	**5**±1	9±3	21±2.2	25±2.2	27±1.3f
	0.22 x 10 ^{6}	9±1.2	20±2.3	28±2	30±2.1	35±7.1e
	0.32 x 10 ^{6}	12±7.1	27±6.3	30±2.1	40±1.1	47±3ab
	0.42 x 10 ^{6}	23±3.2	37±9.1	45±1.0	47±1.3	54±1.1a

Means followed with the same letter are not significantly different

Oryzaephilus surinamensis

The same effective was found when the entomopathogenic fungi were used against *O. surinamensis* as shown in table 4. The concentrations 0.42 x 10 ^{6} conidia / g of fungi, induced increase the mortalities rates. The highest cumulative mortalities were recorded after 11 days of treatment when mortality percentages were recorded 25, 35, 43 and 51%for *B. bassiana* at the tested concentrations of (0.12 x 10 ^{6}conidia /g, 0.22 x 10 ^{6}conidia /g, 0.32 x 10 ^{6}conidia / g, and 0.42 x 10 ^{6} conidia / g, respectively). and 40, 45, 55 and 65 % for *M. anisopliae* at the tested concentrations of (0.12 x 10 ^{6} conidia /g, 0.22 x 10 ^{6} conidia /g, 0.32 x 10 ^{6} conidia / g, and 0.42 x 10 ^{6} conidia / g, respectively). The findings in the table showed that the mortality percentage of *O. surinamensis* treated with *B. bassiana* and *M. anisopliae* differed significantly among different concentrations. Also mortality percentage differed significantly between *O. surinamensis* treated with *B. bassiana* and with *M. anisopliae*.

LC_{50} and LC_{90} values confirmed that *O. surinamensis* was more susceptible to *M. anisopliae*, than *B. bassiana* where the LC_{50} and LC_{90} were (1.4 x 10 ^{5}and 8.1 x 10 ^{5}conidia /g) and (3.5 x 10 ^{5}and 0.04 x 10 ^{6}conidia /g), respectively. The LT_{50}**'**s were calculated as 5.7 and 6.9 days for the two respective fungi, indicating that *O. surinamensis* adults were more susceptible to *M. anisopliae*, than *B. bassiana* (Table 1).

Table 4: Cumulative mortality percentages of *O. surinamensis* treated with *B. bassiana* and *M. anisopliae* at different concentrations.

Fungi	Conc. Conidia/g	Mortality percentages of *O. surinamensis* indicated days				
		3	5	7	9	11
B. bassiana	0.12 x 10 ^{6}	4±1	10±1.1	17±1.1	20±1.7	25±1.3f
	0.22 x 10 ^{6}	10±1.3	17±2.2	22±1.2	30±1.4	35±2.1fg
	0.32 x 10 ^{6}	13±1.5	22±1.3	25±1.1	35±1.1	43±1.5b
	0.42 x 10 ^{6}	20±3.1	25±1.1	28±1.1	38±1.4	51±2ab
M. anisopliae	0.12 x 10 ^{6}	8.1±2.1	21±2	25±2.1	35±2.1	40.0±1.7g
	0.22 x 10 ^{6}	9.1±2.1	30±1	35±2.2	43±1.1	45±2.3 g
	0.32 x 10 ^{6}	18±1	32±2.2	45±3	51±1.4	55±2.3ac
	0.42 x 10 ^{6}	30±2.1	37±1.1	50±1.6	60±1.5	65±1.3ac

Means followed with the same letter are not significantly different

The recent study revealed that *M. anisopliae* was more efficient against *R. dominica* and *O. surinamensis* than the fungus *B. bassiana* and proved that *M. anisopliae* had higher virulence than *B. bassiana*. *M. anisopliae* and *B. bassiana* were less efficient against *S. oryzae*.

The entomopathogenic fungi *B. bassiana* and *M. anisopliae* have potential for effective and economically feasible control of stored product insects.

4. Discussions

We find in our study that *M. anisopliae* was more efficient against *R. dominica* and *O. surinamensis* than the fungus *B. bassiana* and proved that *M. anisopliae* had higher virulence than *B. bassiana*. *M. anisopliae* and *B. bassiana* were less efficient against S. *oryzae*.

The entomopathogenic fungi *B. bassiana* and *M. anisopliae* have potential for effective and economically feasible control of stored product insects according (Salem & Abdel-Raheem; Magda Sabbour & Mohamed Abdel-Raheem and Silvia Mudrončeková, *et al.* [28-30].

(Hendrawan Samodra and Yusof Ibrahim) mentioned that the isolate of *Beauveria bassiana* (BbGc) was the most infectious against the rice weevil adults, *S. oryzae* than *Metarhizium anisopliae* (MaPs) [31]. According with Kassa, *et al.* mentioned that *B. bassiana* and *M. anisopliae* were virulent against *S. zeamais* when used concentration 1x10 ^{7} and 1x10 ^{8} conidia ml^{-1}(92-100% mortality) [32].

5. Conclusion

M. anisopliae was more efficient against *R. dominica* and *O. surinamensis* than the fungus *B. bassiana* and proved that *M. anisopliae* had higher virulence than *B. bassiana*. *M.*

anisopliae and *B. bassiana* were less efficient against S. *oryzae*. The entomopathogenic fungi *B. bassiana* and *M. anisopliae* have potential for effective and economically feasible control of stored product insects.

6. References

1. Haq T, Usmani NF, Abbas T. Screening of plant leaves as grain protectants against *Tribolium castaneum* during storage, Journal of botany. 2005; 37:149-153. httpciteseerx.ist.psu.eduviewdocdownloaddoi=10.1.1.560.5623&rep=rep1&type=pdf
2. AbdEl Aziz SE. Control strategies of stored product pests. Journal Entomology. 2011; 8(2):101-122.
3. Sullivan P. Stored grain pest management, 2002. (http://www.attra.ncat.org/attra-pub/PDf/storegrain.PDF).
4. Wakil W, Ghazanfar MU. Entomopathogenic fungus as a biological control agent against *Rhyzopertha dominica* F. (Coleoptera Bostrychidae) on stored wheat. Archives of phytopathology and plant protection. 2010; 43(12):1236-1242.
5. Sheeba G, Sundaram S, Raja N, Janarthanan S, Ignacimuthu S. Efficacy of *Beauveria bassiana* for control of the rice weevil *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Applied Entomology Zoology 2001; 36(1):117-120.
6. Lord JC. Desiccant dusts synergize the effect of *Beauveria bassiana* (Hyphomycettes; Moniliales) on stored–grains beetles, Journal Economic Entomology. 2001; 94(2):367-372.
7. Lord JC. Low humidity, moderate temperature and desiccant dust favor efficacy of *Beauveria bassiana* (Hyphomycettes; Moniliales) for the lesser grain borer, *Rhyzopertha dominica* (Coleoptera Bostrychidae), Biological. Control 2005; 34:80-186.
8. Lord JC. Desiccation increases the efficacy of *Beauveria bassiana* for stored–grain pest insect control, Journal Stored product Research. 2007; 43:535-539.
9. Sabbour MM, Abd-El-Aziz SE, Sherief MA. Efficacy of three entomopathogenic fungi alone or in combination with diatomaceous earth modification for the control of three pyralid moths in stored grains, Journal plant protection Research. 2012; (52):359- 369.
10. Sabbour MM, Abdel-Raheem MA. Repellent Effects of Jatropha curcas, Canola and Jojoba seed oil, against *Callosobruchus maculates* (F.) and *Callosobruchus chinensis* (L.), Journal of Applied Sciences Research. 2013; 9(8):4678-4682. http://www.aensiweb.com/old/jasr/jasr/2013/4678-4682.pdf
11. Sabbour MM, Abdel-Raheem MA. Evaluations of *Isaria fumosorosea* Isolates against the Red Palm Weevil *Rhynchophorus ferrugineus* under Laboratory and Field Conditions. Current Science International 2014; 3(3):179-185. https://www.academia.edu/9384251/Evaluations_of_Isaria_fumosorosea_Isolates_against_the_Red_Palm_Weevil_Rhynchophorus_ferrugineus_under_Laboratory_and_Field_Conditions
12. Abdel-Raheem MA. Impact of Entomopathogenic Fungi on Cabbage Aphids, *Brevicoryne brassica* L. in Egypt. Bulletin National Research Centre 2011; 36(1):53-62.
13. Abdel-Raheem MA. Susceptibility of The red palm weevil, *Rhynchophorus ferrugineus* Olivier to some Entomopathogenic Fungi. Bulletin National Research Centre 2013; 38(1):69-82.
14. Abdel-Raheem MA, KH Sabry, Zakia Ragab A. Effect of different fertilization rates on control of *Bemisia tabaci* (Genn.) by *Verticillium lecanii* and *Beauveria bassiana* in potato crop Journal Biological Pest control. 2009; 19(2):129-133.
15. Abdel-Raheem MA, Zakia Ragab A. Using Entomopathogenic Fungi to Control the Cotton Aphid, *Aphis gossypii* Glover on Sugar Beet. Bulletin National Research Centre, 2010; 35(1):57-64.
16. Abdel-Raheem MA, Zakia Ragab A, Abdel-Rahman IE. Effect of Entomopathogenic Fungi on the Green Stink Bug, *Nezara viridula* L. in Sugar Beet. Bulletin National Research Centre, 2011; 36(2):145-152.
17. Abdel-Raheem MA, Abdel-Rhman IE, Zakia Ragab A. Virulence of some Entomopathogenic Fungi on *Schistocerca gregaria* (Forskal). Bulletin National Research Centre, 2011; 4:337-346.
18. Zaki FN, Abdel-Raheem MA. Using of entomopathogenic fungi and insecticide against some insect pests attacking peanuts and sugar beet. Archives of phytopathology and plant protection10 December 2010; 43(18):1819-1828.
19. Golshan H, Saber M, Shilsar FM, Karimi F. Efficacy of nine isolates of *Beauveria bassiana* against adults of *Tribolium castaneum* (Coleoptera Tenebrionidae). Acta Entomologica Sinica, 2013; 56(2):201-206.
20. Sewify GH, El Shabrawy HA, Eweis ME, Magda Naroz H. Efficacy of Entomopathogenic Fungi, *Beauveria bassiana* and *Metarhizium anisopliae* for controlling certain stored Product Insects Egypt, Journal of biological Pest Control. 2014; 24(1):191-196.
21. Elsufty RA, Boraei HA, El-Gremi SH. Isolation of Beauveria bassiana (Bals.) from the Egyptian alfalfa weevil Hypera brunneipennis (Boheman) and its habitat in Egypt, Journal of biological Pest Control. 1994; 3(2):149-156.
22. Abdel-Raheem MA. Possibility of Using the Entomopathogenic Fungi *Beauveria bassiana* and *Metarhizium anisopliae* for controlling the Sugar-Beet Insects *Cassida vittata* Vill. And *Scrobipalpa ocellatella* Boh. In Egypt, Ph.D. Faculty of Agriculture, Cairo University, Cairo, 2005.
23. Wan H. Molecular biology of the entomopathogenic fungus *Beauveria bassiana*, insect-cuticle degrading enzymes and development of anew selection marker for fungal transformation. Ph. D. Thesis, Combined faculties for the Natural Science and for Mathematics, Ruperto-Carola University, Heideiberg Germany 2003; 125.
24. Abdel-Raheem MA, Ismail IA, Abdel-Rahman RS, Abdel-Rhman IE, Naglaa Reyad F. Efficacy of Three Entomopathogenic Fungi on Tomato leaf miner, *Tuta absoluta* in Tomato crop in Egypt, Swift journals of Agricultural Research. 2015; 1(2):015-020. http://www.swiftjournals.org/sjar/abstract/2015/june/Abdel.php
25. Salem SA, Abdel-Raheem MA, Abdel-Salam AMA, Farage NA. Lab-Field Evaluation of Some Egyptian isolates of Entomopathogenic Fungi *Metarhizium anisopliae* and *Beauveria bassiana* Against Sugar Beet Beetle *Cassida vittata* Vill. Swift journals of Agricultural Research. 2015; 1(2):009-014. http://www.swiftjournals.org/sjar/pdf/2015/august/Salem%204.pdf
26. Kassa A, Brownbridge MI, Parker BL, Skinner M, Gouli V, Gouli S *et al.* Why for mass production of *Beauveria bassiana* and *Metarhizium anisopliae* Mycological Research 2008; 112(5):583-591.
27. Finney DJ. Probit analysis. 2nd Ed, Cambridge. Univ.

Press. England. 1964; 318.
http://www.abebooks.com/book-search/title/probit-analysis/

28. Salem SA, Abdel-Raheem MA. Interrelationships among some aphids and their host plants Swift journals of Agricultural Research. 2015; 1(4):041-046.
http://www.swiftjournals.org/sjar/pdf/2015/august/Salem%204.pdf
29. Magda Sabbour, Mohamed Abdel-Raheem. Toxicity of the fungus *Beauveria bassiana* and three oils extract against *Sitophilus granaries* under laboratory and store condition, American Journal of Innovative Research and Applied Sciences. 2015; 1(7):251-256.
http://www.americanjiras.com/SabourManuscriptRef.1-AJIRAS300715.pdf
30. Silvia Mudrončeková, Marián Mazáň, Marek Nemčovič, Ivan Šalamon. Entomopathogenic fungus species Beauveria bassiana (Bals.) and *Metarhizium anisopliae* (Metsch.) used as Mycoinsecticide effective in Biological control of IPS Typographus (L.), Journal of Microbiology, Biotechnology and Food Sciences. 2013; 2(6):2469-2472.
31. Hendrawan Samodra, Yusof Ibrahim. Effects of dust formulation of three entomopathogenic fungal isolates against *Sitophilus oryzae* (Coleoptera: Curculionidae) in rice grain, Journal Biosains. 2006; 17(1):1-7.
httpwww.tlsr.usm.mytlsr1701200617012006_01.pdf
32. Kassa A, Zimmermann G, Stephan D, Vidal S. Susceptibility of *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculionidae) and *Prostephanus truncates* (Horn) (Coleoptera: Bostrichidae) to Entomopathogenic Fungi from Ethiopia. Biocontrol Science and Technology 2002; 12:727-736.
httpwww.jki.bund.dedownloadFatPdf.phpfile=02_0174.pf