Journal of the Islamic University of Gaza, (Series of Natural Studies & Engineering), Vol.13, No.1, P.11-22, 2005

# A STUDY OF TREATMENT EFFECT WITH METARHIZIUM ANISOPLIAE AND FOUR TYPES OF DUSTS ON WHEAT GRAIN INFESTATION WITH RED FLOUR BEETLES (TRIBOLIUM CASTANEUM HERBS, COLEOPTERA: TENEBRIONIDAE)

**Yacoub A. Batta * and Daoud I. Abu Safieh*** Laboratory of Plant Protection, Dept. of Plant Production and Protection, Faculty of Agriculture, An-Najah National University, Nablus, Palestine, Tel/Fax: 09-2395105.

## دراسة تأثير المعاملة بفطر الميتاريزيوم أنيسوبليا وأربعة أنواع من المساحيق على إصابة حبوب القمح بخنفساء الطحين الصدئية

**ملخص:** تضمنت هذه الدراسة إجراء عدة معاملات لحبوب القمح المخزونة باستخدام أربعة أنواع من المساحيق منفردة أو مخلوطة مع كونيديا الفطر المضاد للحشرات ميتاريزيوم أنيسوبليا . تم في هذه المعاملات استعمال نسبة مزج مقدارها 4:1 بالوزن من كونيديا الفطر الى المسحوق الحامل للفطر على التوالي ، وكذلك نسبة استعمال مقدارها 2% بالوزن من المسحوق الحامل منفردا أو مضافا إليه كونيديا الفطر إلى حبوب القمح المعاملة . أظهرت النتائج التي تم الحصول عليها أن المعاملات التي اشتملت على مسحوق الفحم مضافا إليه كونيديا الفطر أو رماد الفرن والكونيديا أو مسحوق الطباشير والكونيديا قد أدت إلى الحصول على نسبة مئوية مرتفعة معنويا للوفيات ما بين أفراد خنفساء الطحين الصدئية(73.3، 53.3 ، 60.0 %) على التوالي مقارنة بالمعاملات باستخدام المساحيق الحاملة منفردة (13.3، 26.7، 13.3 % على التوالي) . كذلك تم الحصول على نسب مئوية منخفضة معنويا في معدلات الإصابة على حبوب القمح المعاملة بالمعاملات الواردة أعلاه حيث كانت أقل نسبة مئوية لمعدلات الإصابة عقب المعاملة برماد الفرن مضافا إليه كونيديا الفطر (0.5%) تلاها المعاملة بمسحوق الفحم وكونيديا الفطر (1.2%) ثم مسحوق الطباشير وكونيديا الفطر (2%) . أضف إلى ذلك الحصول على فترات أطول معنويا لدورة حياة الحشرة عقب المعاملة بمسحوق الفحم وكونيديا الفطر (54.7 يوم) ومسحوق الطباشير وكونيديا الفطر ( 60.0 يوم) مقارنة بالمعاملات باستخدام مسحوق الفحم والطباشير منفردة (48.0 و 53.7 يوم على التوالي) . وهذا يظهر بوضوح تأثير المعاملات السابقة في تأخير تطور الحشرة لمدة تزيد على ستة أيام. أخيرا ينصح بإجراء تجارب إضافية باستخدام مدى واسع من درجات الحرارة والرطوبة النسبية وعلى كميات أكبر من حبوب القمح قبل أن تتم التوصية باستعمال المساحيق الأكثر فعالية مضافا إليها كونيديا الفطر على نطاق واسع لمكافحة هذه الحشرة.

**Abstract** The present study included treatments of stored wheat grains with four types of dusts alone or in combination with conidia of the entomopathogenic fungus *Metarhizium anisopliae*. A ratio of 1:4 (w/w of the fungus conidia to the dust carrier, respectively), and a rate of application of 2.0% (w/w of the dust carrier alone or the dust carrier + fungus conidia to wheat grains) were used during the treatments. Results obtained have shown that the treatments with combination of charcoal + fungal conidia, oven ash + fungal conidia, and chalk powder + fungal conidia caused significant higher percentage of *Tribolium castaneum*-adult mortality (73.3, 53.3, and 60.0%, respectively) compared to treatments with charcoal, oven ash, and chalk powder alone (13.3, 26.7, and 13.3% respectively). Significant reduction in the percentage of insect infestation rate on wheat grains was also obtained when treatments with the above-mentioned combinations were compared with the dust treatment alone. The least significant percent of infestation rate was thus obtained with oven ash + fungal conidia (0.5%) followed by charcoal + fungal conidia (1.2%) then, chalk powder + fungal conidia (2.0%). Moreover, significant longer durations of *T. castaneum* life-cycle were obtained after treatment with charcoal + fungal conidia (54.7 days) and chalk powder + fungal conidia (60.0 days) compared with treatments with charcoal and chalk powder alone (48.0 and 53.7 days, respectively). This demonstrates clearly the effect of treatment on retardation of the insect development by > 6 days. Further experiments under a wide range of temperature and relative humidity and on larger quantities of wheat grains are recommended to be carried out before applying the effective combinations of the fungus and dust carriers at a large scale for control of this insect.

***Key words***: Red flour beetles, wheat grains, *Tribolium castaneum*, Adult mortality, Infestation rate, Life-cycle duration, Dust carriers, *Metarhizium anisopliae*

## 1. Introduction

The red flour beetle, *Tribolium castaneum* (Herbst) is a small insect (3-4 mm long) with red color and chewing mouth parts. It is cosmopolitan and considered one of the major stored-product insects of sound and processed grains with high rate of movement among food patches[1,2] .Significant losses were reported to be caused by this insect on grains and its by-products throughout the world [1,3,4].

The most frequent control measure of this insect and other stored-product insects is usually by fumigation of cereal-grain processing facilities with methyl bromide[5-8] .Although of its effectiveness, methyl bromide is considered by EPA (1993)[9] ozone-layer depleting substance and it is thus subjected to be prohibited or substituted with other substances. Insecticidal treatments with malathion [10,11] , deltamethrin [12], cyfluthrin [13-17], bioresmethrin [18] , and chlorpyrifos-methyl [19] gave also good control of red flour beetles and other stored-product insects. However, the resistance to repeatedly applied insecticides and some fumigants such as phosphine has developed, in addition to appearance of environmental and human health concerns as a result of insecticide application [8,10,11,20-22]. Heat treatment, controlled atmospheres, aeration and cooling technologies could be offered as alternative control measures of stored grain insects including red flour beetles [23]. A combination of heat and diatomaceous earth dust treatment, fore example, has demonstrated its effectiveness against the red flour beetles [24,25].

Until present, limited number of published articles on biocontrol of stored-grain insects using entomopathogenic fungi are available. *Beauveria bassiana*, for example, has proven highly effective against the major stored-grain insects: *Sitophillus oryzae*, *Rhyzopertha dominica*, and *Tribolium castaneum* [26-30]. In contrast, *Metarhizium anisopliae* has been less frequently reported for control of stored-grain insects although it has been used effectively to control other insects especially termites [31], black field crickets [32], grasshoppers and locusts [33], tobacco whitefly and red spidermites [34]. Mixtures of *M. anisopliae* conidial suspensions with those of *B. bassiana* have been reported to be used against *S. oryzae* on wheat grains [30]. In our recently published research, various combinations of *M. anisopliae* conidia with four types of dust carriers were used effectively against *S. oryzae* infesting stored wheat grains [35]. The use of diatomaceous earth (Insecto® and SilicoSec®) as an effective dust for suppressing *T. castaneum* has been reported by Subramanyan et al., 1994[4] and Athanassiou et al., 2003[36]. To the best of our knowledge, testing the effectiveness of *M. anisopliae* alone or in combination with dust carriers against *T. castaneum* on various types of stored-grains has not been investigated. Therefore, the objective of the present study was: to assess the treatment effect with four types of dust carriers (charcoal, oven ash, chalk powder and wheat flour) alone or in combination with *M. anisopliae* on adult mortality, infestation rate of wheat grains, and duration of life cycle of *T. castaneum*.

## 2. Materials and methods

### 2.1. Insect rearing

*T. castaneum* was reared on dried healthy mature wheat grains (cv: Anbar) under the insectary conditions (25±2$^{o}$C, 75±5%R.H. and 16h of illumination per day). The grains in the insect rearing were placed in plastic pots (15 cm diameter and 20 cm deep). The pots were then covered with muslin or cheese-cloth fastened by a rubber-band to prevent the escape of insects and to ensure the proper ventilation. Newly emerged adult insects (males and females) obtained from the culture were used in the experiments.

### 2.2. Types of experimental treatments

Five types of treatments with *M. anisopliae* (strain Meta 1) were applied in the experiments, they were: fungal conidia + charcoal, fungal conidia + oven ash, fungal conidia + chalk powder, fungal conidia + wheat flour, and fungal conidia only. In the first four treatments, a ratio of 1:4 (w/w of fungal conidia to dust carrier, respectively) was used. Moreover, additional four types of treatments using the above-mentioned dust carriers alone were also used in the experiments as control treatments for comparing their treatment effects with and without fungal conidia. The dust carrier, oven ash, used in the experiments was obtained from the completely burned papers.

To prepare the ingredients of the above-mentioned fungus treatment, a thorough mixing of the dry fungal conidia harvested from 14-day old culture of the fungus grown on oatmeal agar medium plates with each dust carrier. Such mixing is necessary to ensure the homogeneous distribution of the conidia in each treatment mixture. The concentration of the fungal conidia after introduction into the homogenized mixtures was $6.5x10^8$ conidia/gram of the mixture. This concentration was measured in the suspension resulted from suspending and homogenizing 1 gram of the mixture in 50 ml of sterile distilled water.

The treatment with the fungal conidia only was done by applying directly the dry pure form of the conidia. The same quantity of fungal conidia was used in this treatment as that used in the fungal-dust mixtures.

**2.3. Technique of fungal treatment and insect infestation**

The application of fungal treatments was accomplished by adding then mixing 0.2 gram of each one of the treatment mixtures described in section 2.2 per each replicate. Thus, 0.2 gram which contains $1.3x10^8$ conidia (original concentration=$6.5x10^8$ conidia/gram of the mixture) was added and then mixed with 200 wheat grains (average weight=10.0 grams). The application rate was therefore 2.0% (w/w of the fungal treatment to wheat grains). The same application rate was used during application of the control treatments or dust carriers alone. The preventive effect of the above-mentioned treatments was bioassessed by infesting wheat grains in each replicate with *T. castaneum* immediately after application of each treatment. This infestation was carried out by transferring the newly emerged adult insect (males and females) obtained from insect-pupae reared in the culture into each replicate using a small smooth brush. Each replicate was consisted of 200 healthy dried mature wheat grains (average weight=10.0 grams; cv: Anbar) placed in plastic can (9.5 cm diameter and 5 cm deep). Each can was covered after the treatment with muslin or cheese-cloth fastened by a rubber-band to prevent the escape of the transferred insects and to permit the proper ventilation.

**2.4. Assessment of fungal treatment effect on mortality of adult insect, infestation rate of grains and insect life-cycle**

Ten newly emerged adult insects of *T. castaneum* (5 males and 5 females) were introduced into each treatment replicate immediately after application of each treatment type to assess the preventive effect of fungal and dust-carrier treatments as indicated in section 2.3. The plastic cans which represent replicates of the different treatments were then kept under the insectary conditions (25±2$^{o}$C, 75±5% R.H., and 16h per day of illumination) for 7 days before being evaluated. Mortality assessment was then made by counting dead and living adult insects and percentage of adult mortality was calculated. Evaluation of the treatment effect on infestation rate of wheat grains by *T. castaneum* was done 21 days after the treatment. The infestation rate was then assessed by counting the damaged grains which manifest boring holes with varying size on the surface especially in the embryo and endosperm region of the Kernels in proportion to the total number of grains (200 grains/replicate) subjected to the insect at the beginning of infestation time. Percentage of damaged grains were calculated. Evaluation of the treatment effect on life cycle of *T. castaneum* was done by removing the surviving adult insects from cans in all replicates 21 days after the treatment. This time period is enough for egg deposition in all replicates. The plastic cans which represent the treatment replicates were then incubated under the insectary conditions until emergence of adult insects of new generation at the end of insect life cycle. The duration of life cycle or time period needed for production of one generation of *T. castaneum* in each treatment type was calculated. Mean duration of the insect life cycle was calculated. Three replicates represented by 3 plastic cans which contain 200 healthy dried mature wheat grains each were used per treatment type in each treatment effect indicated above.

**2.5. Statistical analysis**

The mean percentage of adult mortality and damaged wheat grains was calculated. Mean duration of the life cycle was also calculated. Analysis of variance (ANOVA) and mean separation by Duncan's multiple range test were conducted for the three treatment effects.

## 3. Results

**3.1. Effect of fungal and dust treatment on adult mortality of *T. castaneum***

The preventive effect of *M. anisopliae* treatments on adult mortality of *T. castaneum* demonstrated significant higher percentage of adult mortality compared to the effect of dust carriers or control treatments (Table 1).

Therefore, the treatments with the fungus mixtures using fungal conidia + chalk powder, charcoal + fungal conidia, and oven ash + fungal conidia resulted in significant higher mortality percent in the adult-insects than that in the control treatments with chalk powder alone, charcoal alone, and oven ash alone (Table 1). This indicates the advantage of using the fungus mixtures with the above-mentioned carriers over using the dust carriers alone in the treatments of the insect. Accordingly, using chalk powder, oven ash or charcoal in the fungus mixtures resulted in the highest percent of adult mortality (60.0, 53.3 and 73.3%, respectively), thus they may be used in biocontrol of *T. castaneum* adults by mixing them with *M. anisopliae* conidia.

**3.2. Effect of fungal and dust treatment on infestation rate of wheat grains by *T. castaneum***

The effect of preventive treatment with the fungus mixtures mentioned-above on the infestation rate with *T. castaneum* on wheat grains stored at 25±2$^{o}$C, 75±5% R.H. and 16h of illumination per day indicated that percentage of infested grains was significantly less than that of infested grains in the dust carriers or control treatments (Table 1). Thus, the treatment with the fungus mixtures using oven ash + fungal conidia, charcoal + fungal conidia, and chalk powder + fungal conidia resulted in the least percentage of infested grains (0.5, 1.2, and 2.0%, respectively) compared to the control treatments with oven ash alone, charcoal alone, and chalk powder alone (3.0, 4.8, and 7.5%, respectively) (Table 1).

**3.3. Effect of fungal and dust treatment on life cycle of *T. castaneum***

The treatment with certain fungus mixtures have shown significant effect on duration of *T. castaneum* life-cycle (Table 1). Therefore, the treatment with chalk powder + fungal conidia and charcoal + fungal conidia significantly prolonged duration of the insect life-cycle (60.0 and 54.7 days, respectively) with > 6 days compared to duration of the insect life-cycle when treated with the control treatments using chalk powder or charcoal alone (53.7 and 48.0 days, respectively) (Table 1). The time period of 6 days represents the delay in life-cycle duration which is extremely important when conditions are

**Table 1:** Effect of treatments with *M. anisopliae* (strain Meta 1) on the adult mortality, infestation rate and life-cycle duration of *Tribolium castaneum* on wheat grains; incubation of treated grains at 25±2$^{o}$C, 75±5%R.H. and 16h of illumination per day

| Fungus treatments and control [1)] | Mean percent mortality of adult insect [2)] | Mean percent infested grains with the insect [3)] | Mean duration of insect life-cycle in days [4)] |
|---|---|---|---|
| Chalk powder only | 1.3.3 ab [5)] | 7.5 e [5)] | 53.7 b [5)] |
| Wheat flour only | 6.7 a | 11.3 f | 42.7 a |
| Charcoal only | 13.3 ab | 4.8 cd | 48.0 a |
| Oven ash only | 26.7 ab | 3.0 bc | --- [*] |
| Chalk powder + fungal conidia | 60.0 de | 2.0 ab | 60.0 c |
| Wheat flour + fungal conidia | 33.3 bc | 5.3 d | 46.0 a |
| Charcoal + fungal Conidia | 73.3 e | 1.2 ab | 54.7 bc |
| Oven ash + fungal Conidia | 53.3 cd | 0.5 a | --- [*] |
| Fungal conidia only | 46.7 bc | 1.2 ab | 48.0 a |

[1)] The fungus treatments are consisting of 1 part of fungal conidia mixed with 4 parts of the carrier (chalk powder or charcoal or oven ash or wheat flour). The concentration of the fungus in the treatments was 20% w/w. The control treatments are consisting of the carriers only.

[2)] Ten newly emerged adults of *T. castaneum* (5 males and 5 females ) were introduced per replicate which contains 200 grains of wheat immediately after application of each treatment. Mortality of adult insects in each replicate (per 10 adults) was determined 7 days after infestation and treatment.

[3)] Percent infestation rate = number of infested wheat grains manifesting typical insect damage 21 days after infestation date per 200 grains per replicate. Ten newly emerged adult-insects ( 5 males and 5 females) were used per replicate.

[4)] Life-cycle duration = time from infestation date to adult emergence. Infestation was done at the same time of fungus treatment for 21 days before the adults being removed, then grains were incubated until adult emergence.

[5)] Means within the same column followed by different letters are significantly different at p<0.05 according to ANOVA table and Duncan's multiple range test.

[*] No adult emergence in these treatments during 65 days of regular observation following infestation, thus no duration of insect life-cycle was cited.

favorable to insect development. It is noteworthy to mention that treatment with either oven ash + fungal conidia or oven ash alone (as control treatment) resulted in inhibition of the insect development where no emergence of adults was observed after 65 days of regular observation following infestation and treatment of the insect (Table 1).

## 4. Discussion

Results obtained here on adult mortality of *T. castaneum* infesting wheat grains treated with *M. anisopliae* conidia + dust carriers indicate that this mortality was relatively high (53.3 to 73.3% when fungal conidia were combined with oven ash, chalk powder and charcoal). Comparable results (up to 80% adult mortality) were obtained by treatment of *Sitophillus oryzae* infesting three types of food substrates with conidial powder of another entomopathogenic fungus: *Beauveria bassiana* [29]. Also, the treatment of stored wheat grains with formulated *B. bassiana* in milled rice significantly reduced the total grain weight loss due to *S. oryzae* infestation during 4 months of storage [28]. To the best of our knowledge, *M. anisopliae* has not been reported as biocontrol agent of *T. castaneum* infesting stored grains or their by-products. Certain investigators studied the effect of diatomaceous earth dust treatment of stored grain beetles especially *T. castaneum* where high adult mortalities of *T. castaneum* were obtained[4,25,36,37]. Higher mortalities of *T. castaneum* adults (up to 100%) were obtained when combination treatments of heat (50$^{o}$C for 30 min) with the most effective diatomaceous earth dust formulation were applied [25]. The dusts applied in the present research especially oven ash, charcoal and chalk powder demonstrated its effect against *T. castaneum* infesting wheat grains alone or in combination with *M. anisopliae* conidia. Moreover, synergistic effect between the three types of dusts and *M. anisopliae* for *T. castaneum* adult mortality was statistically obtained in this work. This is in agreement with the synergistic effect obtained by us [35] for *M. anisopliae* + oven ash and *M. anisopliae* + charcoal applied to *S. oryzae* on stored wheat grains. The mechanism of this synergism needs to be explained by conducting further experiments.

In conclusion, overall results presented here on adult mortality of *T. castaneum*, its duration of life cycle, and infestation rate on stored wheat grains demonstrated effectiveness of treatment with *M. anisopliae* in combination with charcoal, oven ash, or chalk powder compared to the control treatments with these dust carriers alone. The results are promising and encouraging, and there are no adverse effects of using *M. anisopliae* on treated grains. However, further experiments are recommended to be carried out to determine the following : 1) the temperature and relative humidity range required for full effectiveness of the most proper combination of the fungus and dust carriers since obtained results were realized under one set of experimental conditions (25±2$^{o}$C and 75±5% R.H.); 2) the insecticidal potential of the most proper and most effective combination of the fungus and the dust carrier; and 3) the interaction between the proper and effective combination of the fungus and dust carrier and other components in *T. castaneum* management. These experiments are necessary to be done before applying the treatment with the recommended combination on a large scale or before integrating them in managing of this insect in cereal-grain processing facilities.

**References**

1. Ziegler, J.R., *Evolution of the migration response: emigration by Tribolium and the influence of age*. Evolution ,Vol.30.p:579.(1976).
2. Campbell, J.F., Hagstrum, D.W., *Patch exploitation by Tribolium castaneum: movement patterns, distribution, and oviposition.* J. Stored Prod. Res.,Vol.38.p:55.(2002).
3. Vrba, C.H., Arai, H.P., Nosal, M., *The effect of silica aerogel on the mortality of Tribolium confusum (Duval) as a function of exposure time and food deprivation.* Canadian J. Zool.,Vol.610p:1481.(1981).
4. Subramanyan, B.H., Swanson, C.L., Madamanchi, N., Norwood, S., **Effectiveness of Insecto®, a new diatomaceous earth formulation, in suppressing several stored-grain insect species.** In: Highley, E., Wright, E.J., Banks, H.J., Champ, B.R. (Ed.), Proceedings of the Sixth International Working Conference on Stored-Product protection, Canberra, Australia, p650(1994).
5. Tebbets, J.S., Vail, P.V., Hartsell, P.L., Nelson, H.O., *Dose response of codling moth (Lepidoptera: Tortricidae) eggs and nondiapausing and diapausing larvae to fumigation with methyl bromide*. J. Econ. Entomol.,Vol.79.p:1039.(1986).
6. Yokoyama, V.Y., Miller, G.T., Hartsell, P.L., *Methyl bromide fumigation for quarantine control of codling moth (Lepidoptera: Tortricidae) on nectarines.* J. Econ. Entomol.,Vol.80.p:840.(1987).
7. Banks, H.J., **Fumigation-an endangered technology** ? In: Highley, E., Wright, E.J., Banks, H.J., Champ, B.R. (Eds.), Proceedings of

the Sixth International Working Conference on Stored-product Protection, 17-23 April 1993, Canberra, Australia, Vol. 1, CAB International, Oxon, p2(1994).

8. Zettler, J.L., Arthur, F.H., *Chemical control of stored product insects with fumigants and residual treatments*. Crop Prot.,Vol.19.p:577.(2000).
9. EPA (Environmental Protection Agency**), Regulatory action under the clean air act on methyl bromide.** United States Environmental Protection Agency, Office of Air Radiation, Strategic Protection Division, Washington, D.C.,p 10(1993).
10. Arthur, F.H., Zettler, J.L., *Malathion resistance in Tribolium castaneum (Coleoptera: Tenebrionidae): differences between discriminating concentrations by topical application and residual mortality on treated surfaces.* J. Econ. Entomol. ,Vol.84.p:721.(1991).
11. Arthur, F.H., Zettler, J.L., *Malathion resistance in Tribolium confusum (Coleoptera: Tenebrionidae): correlating results from topical application with residual mortality on treated surface*. J. Stored Prod. Res.,Vol.28.p:55.(1992).
12. Arthur, F.H., *Differential effectiveness of deltamethrin dust on wood, concrete, and tile surfaces against three stored-product beetles.* J. Stored Prod. Res.,Vol.33.p:167.(1997).
13. Arthur, F.H., *Residual efficacy of cyfluthrin emulsifiable concentrate and wettable powder formulations on porous concrete and on concrete sealed with commercial products prior to insecticide application*. J. Stored Prod. Res. ,Vol.30,p:79(1994a).
14. Arthur, F.H., *Degradation of cyfluthrin EC and WP residues on painted steel: effects of commercial sealants on residual persistence.* J. Stored Prod. Res.,Vol.30,p:163.(1994b).
15. Arthur, F.H., *Residual toxicity of cyfluthrin wettable powder against Tribolium confusum exposed for short intervals on treated concrete*. J. Stored Prod. Res.,Vol .34.p:19.(1998a).
16. Arthur, F.H., *Residual studies with cyfluthrin wettable powder: toxicity towards red flour beetles (Coleoptera: Tenebrionidae) exposed for short time intervals on treated concrete.* J. Econ. Entomol. Vol.91.p:309.(1998b).
17. Arthur, F.H., **Efficacy of cyfluthrin as a residual surface treatment on concrete against *Tribolium castaneum* and *T.***

***confusum***. In: Zuxum, J., Quan L., Yongsheng, L., Xianchang T., Linaghua, G. (ed.), Proceedings of the Seventh International Working Conference on Stored Product Protection, Beijing, China, October 1998. Sichuan Publishing House of Science and Technology, chengdu, p 891(1999).

18. Ardley, J.H., *Synergized bioresmethrin as a potential protectant*. J. Stored Prod. Res. ,Vol.12,p:253.(1976).
19. LaHue, D.W., *Chlorpyrifos-methyl: doses that protect hard winter wheat against attack of stored grain insects*. J. Econ. Entomol.,Vol.70.p:734.(1997).
20. Zettler, J.L., Haliday, W.R., Arthur, F.H., *Phosphine resistance in insects infesting stored peanuts in the southeastern United States.* J. Econ. Entomol.,Vol.82.p:1508.(1989).
21. Zettler, J.L., Cuperus, G.W., *Pesticide resistance in Tribolium castaneum (Coleoptera: Tenebrionidae) and Rhyzopertha dominica (Coleoptera: Bostrichidae) in wheat.* J. Econ. Entomol.,Vol.83.p:1677.(1990).
22. Zettler, J.L., *Pesticide resistance in Tribolium castaneum and T. confusum (Coleoptera: Tenebrionidae) from flour mills in the United States.* J. Econ. Entomol.,Vol.84.p:763.(1991).
23. Donahaye, E.J. *Current status of non-residual control methods against stored product pests.* Crop Prot.,Vol.19.p:571.(2000).
24. Fields, P., Dowdy, A., Marcotte, M., Structural pest control: the use of an enhanced diatomaceous earth product combined with heat treatment for control of insect pests in food processing facilities. Canada-United States Working Group on Methyl Bromide Alternatives, http://www.res.agr.ca/winn/Head-DE.htm.(1997).
25. Dowdy, A.K., *Mortality of red flour beetle, Tribolium castaneum (Coleoptera: Tenebrionidae) exposed to high temperature and diatomaceous earth combinations*. J. Stored Prod. Res.,Vol.35.p:175.(1999).
26. Padin, S.B., Bello, G.M., Vasicek, A.L., Dal-Bello, G., *Bioinsecticide potential of entomopathogenic fungi for stored-grain pests.* Revista de la Facultad de Agronomia Universidad de Buenos Aires ,Vol.15.p:1.(1996).
27. Padin, S.B., Dal-Bello, G., Vasicek, A.L., *Pathogenicity of Beauveria bassiana for adults of Tribolium castaneum (Coleoptera: Tenebrionidae) in stored-grains.* Entomophaga ,Vol.42.p:569.(1997).

28. Padin, S.B., Dal-Bello, G., Fabrizio, N., *Grain loss caused by Tribolium castaneum, Sitophilus oryzae, and Acanthoscelides obtecus in stored durum wheat and beans treated with Beauveria bassiana.* J. Stored Prod. Res.,Vol.38.p:69.(2002).
29. Rice, W.C., Cogburn, R.R., *Activity of the entomopathogenic fungus Beauveria bassiana (Deuteromycota:Hyphomycetes) against three coleopteran pests of stored-grain*. J. Econ. Entomol.,Vol.92.p:691.(1999).
30. Dal-Bello, G.. Padin, S., Lopez-Lastra, C., Fabrizio, M., *Laboratory evaluation of chemical-biological control of the rice weevil (Sitophillus oryzae L.) in stored grains*. J. Stored Prod. Res.,Vol.37.p:77(2001).
31. Quarles, W., *New technologies for termite control.* IPM Practitioner ,Vol.17.p:1.(1995).
32. Milner, R.J., Miller, L., Lutton, G.G., Driver, F., *Biological control of black field cricket Teleogryllus commodus Walker (Orthoptera: Gryllidae) using fungal pathogen Metarhizium anisopliae (Metsch.) Sorokin (Deuteromycotina: Hyphomycetes).* Plant Prot.,Vol.11.p:9.(1996).
33. Lomer, G.J., Prior, C., Kooyman, C., **Development of *Metarhizium* spp. for the control of grasshoppers and locusts**. In: Goettel, M.S., Johnson, D.L. (Eds.), Microbial Control of Grasshoppers and locusts, Vol. 171, Mem. Entomol. Soc., Canada, p265(1997).
34. Batta, Y.A., *Production and testing of novel formulations of the entomopathogenic fungus Metarhizium anisopliae (Metsch.) Sorokin (Deuteromycotina: Hyphomycetes).* Crop Prot.,Vol.22.p:415.(2003).
35. Batta, Y.A., *Control of rice weevil (Sitophillus oryzae L., Coleoptera: Curculionidae) with various formulations of Metarhizium anisopliae.* Crop Prot.,Vol.23(in press,2004).
36. Athanassiou, C.G., Kavallieratos, N.G., Tsaganou, F.C., Vayias, B.J. Dimizas, C.B. Buchelos, C.T., *Effect of grain type on the insecticidal efficacy of SilicoSec against Sitophillus oryzae (L.) (Coleoptera: Curculionidae).* Crop Prot.,Vol.22.p:1141.(2003).
37. Quarles, W., and Winn, P.S., *Diatomaceous earth and stored product pests*. IPM Practitioner ,Vol.18.p:1.(1996).