RESEARCH

LABORATORY TRIALS OF *Metarhizium anisopliae* VAR. *acridum* (GREEN MUSCLE®) AGAINST THE SAXAUL LOCUST, *Dericorys albidula* SERVILLE (ORTHOPTERA: DERICORYTHIDAE)

Heydar Valizadeh[1, 2], Habib Abbasipour[2], Mohammad Mahmoudvand[3*], Hassan Askary[4], and Vahid Reza Moniri[5]

The saxaul locust, *Dericorys albidula* Serville (Orthoptera: Dericorythidae) is a major pest of saxaul plants in Qom province of Iran. During 2005-2006, different nymphal instars of bands of *D. albidula* were treated by aerial spraying of *Metarhizium anisopliae* var. *acridum* (Green Muscle®). The gasoline formulation of *M. anisopliae* var. *acridum* isolate IMI 330189 was applied in different conidial concentrations (10^6, 10^7, 10^8, 10^9, 10^{10} and 10^{13} spores mL^{-1}) that were prepared in sterile distilled gasoline. Results showed that various concentrations significantly affected the 2nd, 3rd, 4th and 5th nymphal instars of *D. albidula* compared to control. In addition, there were no differences in the effects of the different concentrations in 2005, but the differences were significant in 2006. Concentration 10^{10} killed 100% of tested insects 15 d after treatment. Comparing the results of the two years showed that the susceptibility of nymphs in the second year (2006) was higher than in the first year (2005). In conclusion, the results of this study indicated that the fungal insecticide *M. anisopliae* var. *acridum*, diluted in gasoline, was efficacious with the nymphal instars of locust *D. albidula* in 2005 and 2006.

Key words: Saxaul plants, fungal insecticide, nymphal instars, gasoline formulation.

The saxaul locust, *Dericorys albidula* Serville (Orthoptera: Dericorythidae), is a major pest of saxaul plants in Qom province in central Iran. To prevent shifting sands, the desert areas in Qom are covered by two species of saxaul plant, *Haloxylon persicum* Bunge ex Boiss. & Buhse (zard-tagh in Persian) and *Haloxylon ammodendron* (C.A. Mey.) Bunge (siah-tagh in Persian). The locust *D. albidula* is a monophagus pest and feeds on saxaul plants (*Haloxylon* spp.) and causes severe damage in different growth periods (Adeli and Abaei, 1989; Moniri, 1998). During the last two decades, most outbreaks of *D. albidula* have likely developed due to of the expansion of cultivated *Haloxylon* trees. To limit damage to crops, treatment is required virtually every year, usually with large amounts of broad-spectrum chemical pesticides that pollute the environment and present health and safety issues, as well as exacerbating locust problems due to the loss of natural enemies (Moniri *et al*., 2005).

[1]Agricultural and Natural Resources Research Center of Qom, Qom, Iran.
[2]Shahed University, Faculty of Agricultural Sciences, Postal code: 3319118651 Tehran, Iran.
[3]Islamic Azad University, Department of Plant Protection, Khorramabad Branch, Khorramabad, Iran,
[*]Corresponding author (msco_1381@yahoo.com).
[4]Iranian Research Institute of Plant Protection, Tehran, Iran.
[5]Research Institute of Forest and Rangelands, Tehran, Iran.
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In recent years, some studies have focused on developing fungal insecticides to control locusts and grasshoppers (Prior, 1992; Moore and Prior, 1993). Four to five hundred species of fungi have pathogenic effects on insects. There are entomopathogenic species among all five sub-divisions of fungi (Mastigomycotina, Zygomycotina, Ascomycotina, Basidiomycotina, and Deuteromycotina) (Burges and Hussey, 1971; Whitten and Oakeshott, 1991; Starnes *et al*., 1993). Fungi penetrate the insect cuticle. The host can be infected by direct treatment and by transportation of inoculums from treated insects to untreated ones (Lacey *et al*., 1999; Quesada-Moraga *et al*., 2004). After attaching itself to an insect host, the conidium penetrates the cuticle with the help of pressure of the germ tube and enzymatic degradation (Starnes *et al*., 1993). As well, the infection can occur through the respiratory system (Burges and Hussey, 1971). The genus *Metarhizium* (Deuteromycotina: Hyphomycetes) includes three species: *M. anisopliae* (Metschn.) Sorokin, *M. flavoviride* (W. Gams & Rozsypal) and *M. album* (Petch). Unlike *M. anisopliae*, which affects a large number of insect orders, the other two species have a more restricted host range (Rombach *et al.,* 1986; 1987). One of the most promising biological agents for controlling locusts and grasshoppers is the Acridid-specific fungal pathogen *Metarhizium anisopliae* var. *acridum* (formerly *Metarhizium flavoviride*) (Ascomycota: Hypocreales) (Langewald *et al*., 1997; Driver *et al*., 2000; Hunter *et al*., 2001; Peng *et al*., 2008; Bischoff *et al*., 2009; USDA, 2010). Depending on insect species and size, *M. anisopliae* results in host death between 3 and 4 d after infection (Whitten and Oakeshott, 1991; Starnes *et al*., 1993). A variety of this species, *M. anisopliae* var. *acridum*, under the commercial name Green Muscle, has been developed to control locusts and grasshoppers (Thomas *et al*., 2000). Using oil-based formulations has improved the virulence of conidia and resulted in very promising acridid control (Symmon, 1992; Bateman, 1997).

According to the authors' knowledge, reports about *D. albidula* are limited and there have been no investigations regarding the effect of mycoinsecticide on the species. In this study, we investigated the effect of various concentrations of *M. anisopliae* on different nymphal stages of *D. albidula* under laboratory condition over two years.

MATERIALS AND METHODS

Insect rearing

The initial population of the locust *D. albidula* was collected from desert areas in Qom province, Iran. Saxaul bushes (*H. ammodendron*) were used for feeding and egglaying. Insect stock was maintained at 23 ± 2 °C and 35 ± 5% relative humidity (RH) under a 16:8 (L:D) cycle in a growth chamber.

Bioassay tests

The miscible flowable gasoline formulation of *M. anisopliae* var. *acridum* isolate IMI 330189 with 400 g (2×10^{13}) viable conidia per liter was used in the experiments. A spray method was used in the toxicity assays. Different conidial (Green Muscle®) concentrations (10^6, 10^7, 10^8, 10^9, 10^{10}, and 10^{13} spore mL^{-1}) were prepared in sterile distilled gasoline. As a control, gasoline was used. Two-year-old saxaul bushes were cultured in plastic pots (15 cm in diameter and 25 cm height) and 10 1-d-old nymphs were placed on each bush. Ten milliliters of each concentration (fungus diluted in gasoline) and control group were sprayed on bushes. The mortality was recorded after 15 d from treatments. The experiments were repeated three times. This method was performed for various nymphal instars (2nd, 3rd, 4th, and 5th).

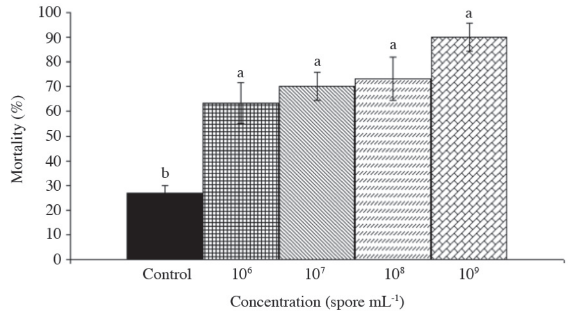
Data analysis

The data obtained were submitted to a one-way ANOVA ($P < 0.05$) after checking for normality. Means were compared by Tukey's Studentized Range Test, admitting significant differences at $P < 0.05$. SAS software was used for all analyses (SAS Institute, 1997).

RESULTS

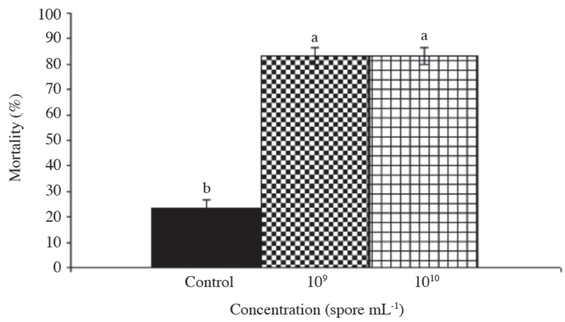
Insecticidal effect of *M. anisopliae* on *D. albidula* in 2005

Figures 1-3 show the effect of various concentrations of *M. anisopliae* var. *acridum* on the 3rd (Figure 1) 4th (Figure 2) and 5th (Figure 3) nymphal instars of *D. albidula* in the first year (2005). Figure 1 reports that various concentrations (10^6, 10^7, 10^8, and 10^9) significantly affected the 3rd nymphal instars of *D. albidula* compared to control. In addition, the effect of concentrations was not different (df = 5, 12, F = 10.47, P = 0.0004). Concentrations 10^9 and 10^{10} had a significant effect on the 4th nymphal instars of *D. albidula* (Figure 2). There were no differences among these concentrations (df = 2, 6, F = 108, P < 0.0001). Comparison of toxicity of different concentrations of *M. anisopliae* var. *acridum* on the 5th



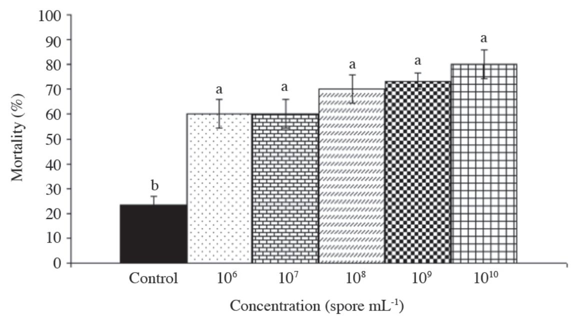
The same letters indicate no significant difference.

Figure 1. Toxicity of various concentrations of *Metarhizium anisopliae* var. *acridum* on the 3rd nymphal instar of *Dericorys albidula* (Orth.: Dericorythidae) (2005).



The same letters indicate no significant difference.

Figure 2. Toxicity of various concentrations of *Metarhizium anisopliae* var. *acridum* on the 4th nymphal instar of *Dericorys albidula* (Orth.: Dericorythidae) (2005).



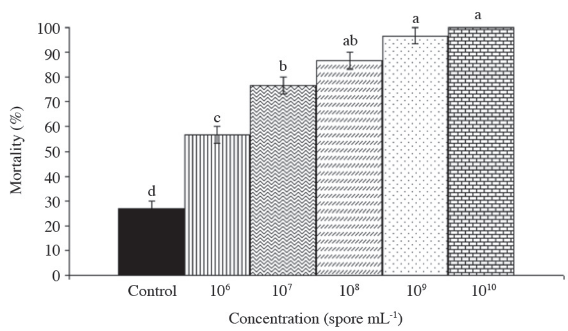
The same letters indicate no significant difference.

Figure 3. Toxicity of various concentrations of *Metarhizium anisopliae* var. *acridum* on the 5th nymphal instar of *Dericorys albidula* (Orth.: Dericorythidae) (2005).

nymphal instars of *D. albidula* indicated that nymph mortality rates at all concentrations (10^6, 10^7, 10^8, 10^9, and 10^{10}) were significantly higher than for the control (Figure 3). The toxicity levels of the various concentrations were different but not significant ($df = 5, 12$, $F = 15.54$, $P < 0.0001$).

Insecticidal effect of *M. anisopliae* on *D. albidula* in 2006

Nymphal mortality of *D. albidula* exposed to *M. anisopliae* var. *acridum* in 2006 is shown in Figures 4-7. Effect of conidial concentrations on the 2nd nymphal instar of locust showed that all concentrations (10^6, 10^7, 10^8, 10^9, and 10^{10}) significantly increased mortality of nymphs. Furthermore, there were differences in the insecticidal ability of various concentrations. Concentration 10^{10} killed 100% of tested insects (Figure 4) ($df = 5, 15$, $F = 238.66$, $P < 0.0001$). Figure 5 shows the mortality trends (to specify the increasing or decreasing trends) of the 3rd nymphal instar of *D. albidula* after treating by *M. anisopliae* var. *acridum*. There was a significant difference between control and all concentrations. As well, the concentration of 10^{10} caused the highest mortality (100%). Other concentrations also showed high toxicity on the 3rd nymphal instar (Figure 5) ($df = 5, 12$, $F = 71.62$, $P < 0.0001$). The efficacy of conidia of *M. anisopliae* var. *acridum* on the 4th instars of locust can be observed in Figure 6. All of the tested concentrations significantly affected the *D. albidula* nymphs. The highest mortality was observed in the concentration of 10^{10} and 10^{13} (Figure 6) ($df = 5, 12$, $F = 76.62$, $P < 0.0001$). *M. anisopliae* var. *acridum* in the various doses caused significant mortality of the 5th nymphal instar of *D. albidula*. As well, there were differences among all concentrations (Figure 7) ($df = 5, 12$, $F = 122.60$, $P < 0.0001$).



The same letters indicate no significant difference.

Figure 4. Toxicity of various concentrations of *Metarhizium anisopliae* var. *acridum* on the 2nd nymphal instar of *Dericorys albidula* (Orth.: Dericorythidae) (2006).

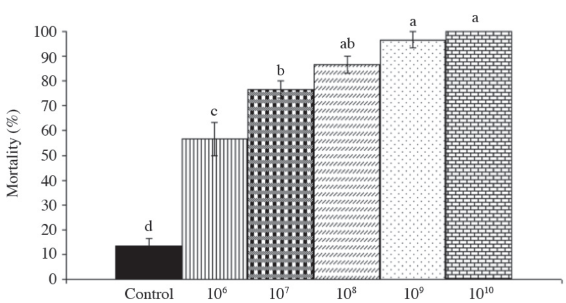


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Figure 5. Toxicity of various concentrations of *Metarhizium anisopliae* var. *acridum* on the 3rd nymphal instar of *Dericorys albidula* (Orth.: Dericorythidae) (2006).

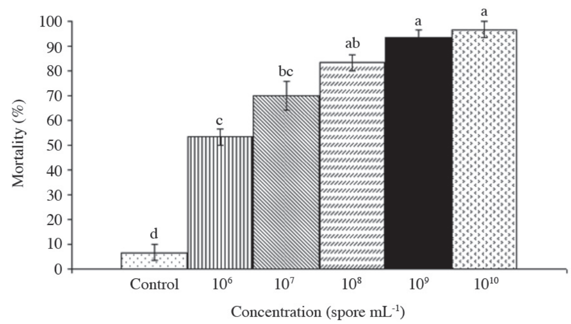

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Figure 6. Toxicity of various concentrations of *Metarhizium anisopliae* var. *acridum* on the 4th nymphal instar of *Dericorys albidula* (Orth.: Dericorythidae) (2006).

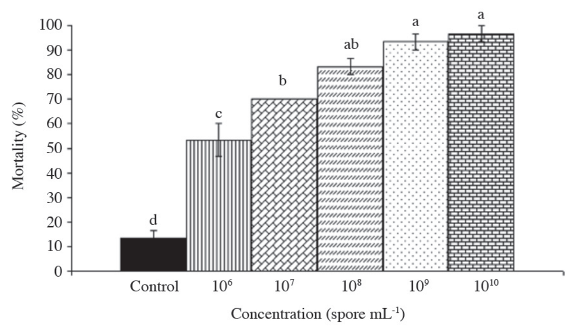

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Figure 7. Toxicity of various concentrations of *Metarhizium anisopliae* var. *acridum* on the 5th nymphal instar of *Dericorys albidula* (Orth.: Dericorythidae) (2006).

DISCUSSION

In this study, the effect of various concentrations of conidia of *M. anisopliae* var. *acridum* on different nymphal instars of *D. albidula* was assayed under laboratory conditions over two years. This was the first study of the efficacy of a fungal insecticide on *D. albidula*. The results indicate that *M. anisopliae* var. *acridum* affects the 2nd, 3rd, 4th, and 5th nymphal instars of this important pest of saxaul plants. In our study, the susceptibility of *D. albidula* nymphs was assessed over 2 yr using similar concentrations. The susceptibility of nymphs was greater in the second year (2006) than in the first year (2005). Unlike our study, Peng *et al*. (2008) researched the effect of *M. anisopliae* var. *acridum* against oriental migratory locusts, *Locusta* *migratoria manilensis* (Meyen) over two years (2002 and 2003) and reported that mortality rates caused by this fungus on the aforementioned locust was relatively similar in both years. In this study, the conidia of *M. anisopliae* were diluted in gasoline and results of this method were evaluated well. Using petroleum products had been tested previously. de Faria *et al*. (2002) showed that conidia of *M. anisopliae* var. *acridum* in a mixture of soybean oil and kerosene had a good effect on the locust, *Rhammatocerus schistocercoides* Rehn. Because saxaul plants are not used for food, using petroleum products can be a suitable method of control. In the current study, mortality was recorded after 15 d. Similar to our results Lomer *et al*. (1997) stated that *M. anisopliae* killed over 90% of nymphs after 15 d. These similar results indicate that 15 d is an adequate period for the fungus to take effect. Lomer et al. found that a dose 10^{10} of *M. anisopliae* var. *acridum* killed 100% of second instar nymphs of *D. albidula*. Magalhaes *et al*. (2000) stated that a 2×10^{13} concentration of *M. anisopliae* var. *acridum* caused 88% mortality on the 2nd nymphal instar of *R. schistocercoides*. As well, Alves *et al*. (1999) observed that *M. anisopliae* caused 79-90% mortality of short-horned locust in Africa, Brazil, and Australia. Kassa *et al*. (2004) examined the effect of Green muscle on *Locusta migratoria* (R. & F.) and reported that this compound can be effective on this pest.

CONCLUSION

In conclusion, the results of this study indicate that fungal insecticide *M. anisopliae* var. *acridum* diluted in gasoline was highly effective in causing mortality of 2nd, 3rd, 4th, and 5th nymphal instars of locust *D. albidula* in 2005 and 2006. As well, the current report shows that mortality of all stages in the second year (2006) was higher than in the first (2005).

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Ensayos de laboratorio de *Metarhizium anisopliae* var. *acridum* (Green muscle®) contra la langosta de saxaul, *Dericorys albidula* Serville (Orthoptera: Dericorythidae). La langosta del saxaul, *Dericorys albidula* Serville (Orthoptera: Dericorythidae), es la principal plaga de plantas de saxaul de las provincias Qom, Irán. Durante 2005-2006, diferentes instars ninfales de *D. albidula* se asperjaron con *Metarhizium anisopliae* var. *acridum* (Green Muscle®). La formulación de gasolina de *M. anisopliae* var. *acridum* aislamiento IMI 330189 se aplicó en diferentes concentraciones conidiales (10^6, 10^7, 10^8, 10^9, 10^{10} and 10^{13} esporas mL^{-1}) preparadas en gasolina destilada estéril. Los resultados demostraron que varias concentraciones afectaron significativamente los instars ninfales de *D. albidula* comparado con el control. Además el efecto de concentraciones no fue diferente en 2005 pero sí significativamente en 2006. La concentración 10^{10} mató 100% de insectos 15 d después del tratamiento. La comparación de los resultados de estos años mostró que la susceptibilidad de ninfas en el segundo año (2006) fue mayor que en el primer año (2005). En conclusión, los resultados de este estudio indicaron que el insecticida fúngico *M. anisopliae* var. *acridum* diluido en gasolina tuvo buena eficacia sobre los instars ninfales de langosta *D. albidula* en los años 2005 y 2006.

Palabras clave: Plantas de Saxaul, insecticida fúngico, estados ninfales, formulación de gasolina.

LITERATURE CITED

Adeli, A., and M. Abaei. 1989. Study of harmful pests of saxaul plants of Iran. Annual report of research project. Environmental Research Studies Center, University of Tehran, Tehran, Iran.

Alves, R.T., R.P. Bateman, C. Prior, and S.R. Leather. 1999. Effects of simulated solar radation on conidial germination of *Metarhizium anisopliae* indifferent formulation. Crop Protection 17:675-679.

Bateman, R.P. 1997. Methods of application of microbial pesticide formulations for the control of locusts and grasshoppers. Memoires Entomology Society Canada 171:69-81.

Bischoff, J.F., S.A. Rehner, and R.A. Humber. 2009. A multilocus phylogeny of the *Metarhizium anisopliae* lineage. Mycologia 101:512-530.

Burges, H.D., and H.W. Hussey. 1971. Microbial control of insects and mites. Academic Press, London, UK.

de Faria, M.R., B.P. Magalhães, R.T. Alves, F.G.V. Schmidt, J.B.T. da Silva, and H. Frazão. 2002. Effect of two dosages of *Metarhizium anisopliae* var. *acridum* against *Rhammatocerus schistocercoides* Rehn. Pesquisa Agropecuaria Brasileira 37:1531-1539.

Driver, F., R.J. Milner, and J.H.W. Trueman. 2000. A taxonomic revision of Metarhizium based on a phylogenetic analysis of ribosomal DNA sequence data. Mycological Research 104:115-131.

Hunter, D.M., R.J. Milner, and P.A. Spurgin. 2001. Aerial treatment of the Australian plague locust, *Chortoicetes terinifera* (Orthoptera: Acrididae) with *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes). Bulletin of Entomological Research 91:93-99.

Kassa, A., D. Stephan, S. Vidal, and G. Zimmermann. 2004. Laboratory and field evaluation of different formulations of *Metarhizium anisopliae* var. *acridum* submerged spores and aerial conidia for the control of locusts and grasshoppers. BioControl 49:63-81.

Lacey, L.A., A.A. Kirk, L. Millar, G. Mercadier, and C. Vidal. 1999. Ovicidal and larvicidal activity of conidia and blastospores of *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) against *Bemisia argentifolii* (Homoptera: Aleyrodidae) with a description of a bioassay system allowing prolonged survival of control insects. Biocontrol Science Technology 9:9-18.

Langewald, J., C. Kooyman, O.K.D. Kpindou, C.J. Lomer, A.O. Dahmoud, and H.O. Mohammed. 1997. Field treatment of Desert Locust (*Schistocerca gregaria* Forskal) hoppers in the field in Mauritania with an oil formulation of the entomopathogenic fungus *Metarhizium flavoviride*. Biocontrol Science and Technology 7:603-611.

Lomer, C.J., C. Prior, and C. Kooyman. 1997. Development of *Metarhizium* spp. for the control of grasshoppers and locusts. Memoirs of the Entomological Society of Canada 171:265-286.

Magalhaes, B.P., Q.M. Leco, M.R. Faria, F.G.V. Schmidt, and W.D. Guerra. 2000. Field trial with the entomopathogenic fungus *Metarhizium anisopliae* var. *acridum* against bands of the grass

hopper *Rhammatocerus Schistocercoides* in Brazil. Biocontrol Science and Technology 10:427-441.
Moniri, V.R. 1998. Comparison of effect of chemical, microbial and hormonal compositions against *Dericorys albidula* in Isfahan province of Iran. p. 68. Final report of research project, Research Institute of Forest and Rangelands, Tehran, Iran.
Moniri, V.R., R. Omid, E. Farashiani, E. Azizkhani, and A. Salahi. 2005. Determination of best time to control of nymphs of the saxual locust, *Dericorys albidula* using hormonal insecticides (IGR). Journal of Research Institute of Forest and Rangelands of Iran 3:57-68.
Moore, D., and C. Prior. 1993. The potential of mycoinsecticides. Biocontrol News and Information 14:31-40.
Peng, G., Z. Wang, Y. Yin, D. Zeng, and Y. Xia. 2008. Field trials of *Metarhizium anisopliae* var. *acridum* (Ascomycota: Hypocreales) against oriental migratory locusts, *Locusta migratoria* manilensis (Meyen) in Northern China. Crop Protection 27:1244-1250.
Prior, C. 1992. Discovery and characterisation of fungal pathogens for locust and grasshopper control. p. 159-180. *In* Lomer, C.J., and C. Prior (eds.) Biological control of locusts and grasshoppers. CAB International, Wallingford, UK.
Quesada-Moraga, E., R. Santos-Quiros, P. Valverde-Garcia, and C. Santiago-Alvarez. 2004. Virulence, horizontal transmission, and sublethal reproductive effects of *Metarhizium anisopliae* (anamorphic fungi) on the German cockroach (Blattodea: Blattellidae). Journal of Invertebrate Pathology 87:51-58.
Rombach, M.C., R.A. Humber, and H.C. Evans. 1987. *Metarhizium album*, a fungal pathogen of leaf hoppers plant hoppers of rice. Transactions of the British Mycological Society 88:451-459.
Rombach, M.C., R.A. Humber, and D.W. Roberts. 1986. *Metarhizium flavoviride* var. *minus* var. nov., a pathogen of plant and leaf hoppers on rice in the Philippines and Solomon Islands. Mycotaxon 27:87-92.
SAS Institute. 1997. SAS/STAT. Guide for personal computers. Ver. 6.12. SAS Institute, Cary, North Carolina, USA.
Starnes, R.L., C.L. Liu, and P.G. Marrone. 1993. History, use and future of microbial insecticides. American Entomologist 39:83-91.
Symmon, P. 1992. Strategies to combat the desert locust. Crop Protection 11:25-28.
Thomas, M.B., J. Klass, and S. Blanford. 2000. The year of the locust. Pest Outlook 11:192-195.
USDA. 2010. Field study using *Metarhizium acridum*, a mycoinsecticide for control of grasshoppers. Environmental Assessment December 2010. USDA, Plant Protection and Quarantine Animal and Plant Health Inspection Service, Riverdale, Maryland, USA.
Whitten, M.J., and J.G. Oakeshott. 1991. Opportunities for modern biotechnology in control of insect pests and weeds, with special reference to developing countries. FAO Plant Protection Bulletin 39:155-181.