Joint action of an entomopathogenic fungus (*Metarhizium anisopliae*) with synthetic insecticides against the scarab beetle, *Anomala cuprea* (Coleoptera: Scarabaeidae) larvae

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(Received 7 May 1997; Accepted 15 August 1997)

Abstract

An entomopathogenic fungus, *Metarhizium anisopliae*, has been recognized as a safe control agent against scarab larvae and many other insects. However, in many cases, *M. anisopliae* is not sufficiently effective when used alone. To enhance its effectiveness, we attempted to use *M. anisopliae* together with a small amount of synthetic insecticides against 1st stadium larvae of *Anomala cuprea*. Our results indicated adequate effectiveness for practical use under the laboratory conditions. However, under field conditions, control effects were unstable against grubs. We speculated that the joint action between *M. anisopliae* and synthetic insecticides resulted from easy penetration of this fungus under conditions of disturbance or decline of the grub defense system caused by the existence of a small quantity of synthetic insecticides.

Key words: Joint action, *Metarhizium anisopliae*, *Anomala cuprea*, synthetic insecticide, IGR

INTRODUCTION

Scarab larvae are major root feeding pests of agriculture and forestry. In Japan, *Anomala cuprea* is a particularly serious pest in these areas, causing damage to various cultivated plants such as turf of golf courses, forest tree nurseries and many other field crops. Until now, this insect has been controlled by application of synthetic insecticide at the larval stage. However, adequate results are not usually obtained, because it is difficult for sufficient quantities of the active ingredients of insecticides to penetrate to the bodies of the grubs inhabiting the deep soil. Recently, to overcome this difficulty, excessive amounts of insecticides have been applied to the soil, resulting in many environmental problems. Hence, many new strategies are being investigated to obtain effective and safe control of scarab larvae. Among these, the effectiveness of microbial control agents such as entomopathogenic nematodes (Kushida, 1987), and entomogenous fungi (Shimazu, 1993) have been investigated. *Metarhizium anisopliae* is an entomopathogenic fungus, with known insecticidal activity against scarab grubs. However, the effects of such biological control agents are, in general, easily influenced by many environ-

mental factors such as soil temperature and rainfall, as well as antagonistic organisms, and the effects are made unstable. Therefore, we aimed at obtaining synergistic effects by a mixed application using entomopathogenic fungi and synthetic insecticides to obtain both stable control effects and environmental safety.

As a general concept, the term “joint application” is used as a total effect of two or more types of insecticides applied together or simultaneously. At present, this joint action has been investigated between such biological control agents as *Bacillus popilliae* and *Rickettsiella melolonthae* (Hurpin and Robert, 1968), *Steinernema carpocapsae* and *Aphelenchus avenae* (Ishibashi and Choi, 1991), *Bacillus popilliae* and *Heterorhabditis bacteriophora* (Thurston et al., 1993, 1994).

As mentioned above, although insecticides can barely reach the bodies of grubs living under the soil and have only little effect on the grubs, this effect may put cause some stress to the scarab grubs. We considered that such a slight effect of insecticides might serve as an inhibitor of the grub defense system. Hence, in this study, we anticipated an excellent control effect of *M. anisopliae* by cooperative treatment with very low quantities of insecticides.

Also, we considered that insect growth regulator (IGR), similar to synthetic insecticides, would be effective as a synergistic agent of *M. anisopliae*. The cuticle of insects has a very important role in the insect's defense system against penetration of entomopathogenic fungi, and IGRs have been used as inhibitors of biosynthesis of insect cuticle. When *M. anisopliae* is treated with IGRs, conidia can easily penetrate the body of grubs, because IGR weakens the cuticle defense system of the grubs.

In this study, we first tried to investigate the tolerance of *M. anisopliae* towards insecticides under laboratory conditions. Next, we estimated the synergistic effects of simultaneous application of *M. anisopliae* and some insecticides on scarab larvae.

MATERIALS AND METHODS

The fungus *M. anisopliae* (strain ACMA-5) used for all experiments was isolated from 3rd stadium larva of *A. cuprea*. This fungus was cultured on Sabouraud dextrose agar with 1% yeast extract (SDAY) at 25°C for 2 weeks. Formed conidia were washed from plates with Tween 40 containing sterilized distilled water. The concentration of the conidial suspension was adjusted with a hemocytometer under a phase contrast microscope.

The scarab beetle, *A. cuprea*, was reared for successive generations under laboratory conditions at Shizuoka University. Eggs were placed in a plastic container filled with leaf-mould for hatching. Three or 4 d-old 1st stadium larvae of *A. cuprea* were used for all experiments.

Insecticides used in this study were as follows: fenthion (MPP), fenitrothion (MEP), dichlorvos (DDVP), diazinon, and chlorpyrifos as organophosphates; cypermethrin and permethrin as pyrethroids; methomyl as carbamate; and teflubenzuron, chlorfluazuron, flufenoxuron, tebfenozide as insect growth regulators (IGR). All compounds were commercially available as emulsifiable concentrate or floable formulation.

Tolerance of ACMA-5 to insecticides. In this experiment, SDAY agar plates were treated with the given concentrations of insecticides and contained 300 ppm streptomycin. From a colony of ACMA-5, a mycelial mat of 5 mm in diameter was inoculated onto each SDAY plate. The

plates were incubated for 2 weeks and the diameter of the colonies was measured. The degree of tolerance is indicated as the proportion of colony diameter remaining compared to the control which was treated with 300 ppm streptomycin only.

Effect of insecticides in soil. Sterilized soil and distilled water were mixed (w/w 2 : 1) and put into a plastic petri dish (9 cm long as diameter, 1.5 cm deep). One milliliter of conidial suspension of ACMA-5 (1.0×10^7 conidia/ml) and the given concentrations of insecticides were inoculated simultaneously into each dish. These were incubated at 25°C for 4 weeks. After the incubation, the soil was diluted with distilled water, and this soil suspension was inoculated with the selection medium *Metarhizium anisopliae* (Yagimura, 1986). A single application of conidial suspension without insecticides was used as the control, against which the formed colony numbers were compared. Each application was done in 5 replicates.

Joint action of ACMA-5 and insecticides against *A. cuprea* larvae. Thirty grams of sterilized soil and distilled water were mixed (w/w 2 : 1) and put into each plastic petri dish. One milliliter of conidial suspension of ACMA-5 (1.0×10^7 conidia/ml) and insecticides was added at the same time. One 1st stadium larva of *A. cuprea* was released into each petri dish. Additionally, 1 ml of conidial suspension of ACMA-5 (2.0×10^7 conidia/ml) and IGR was added at that time. Thirty larvae were used for each application. Slices of carrot roots were added into the petri dish as food for the larvae. After incubation at 25°C for 21 d, the numbers of dead larvae were counted, and the symptoms were used to determine whether the cause of death of each larva was the result of ACMA-5 infection or the effect of the insecticide. For instance, the cuticle of larvae killed by IGR look blistered or peeled, and larvae killed by ACMA-5 are covered with white hyphae or grayish green conidia.

Field pot test of joint action. Japanese lawn grass, *Zoysia japonica* Steud., was planted in 1/5,000 a Wagner pots which were filled with sand and soil, and were grown until the roots were well developed. Ten individuals of 5 or 6 d old 1st stadium *A. cuprea* larvae were released

into each pot and allowed to settle for one day. Ten milliliters of a conidial suspension of ACMA-5 (1.0×10^7 conidia/ml) and 10 ml of insecticides diluted to the given concentrations (fenthion, fenitrothion) were applied to the pots at the same time. Pot tests were conducted for 2 weeks from the 1st to the 15th of August. Then, all the soil in the pots was removed and the numbers of surviving and dead larvae in the soil were counted. The main factors contributing to death of the larvae were determined by the symptoms.

RESULTS

Tolerance of ACMA-5 for insecticides

Tolerance of ACMA-5 for synthetic insecticides is shown in Table 1. ACMA-5 indicated a high tolerance for some insecticides such as methomyl (3+ at 1,000 ppm). ACMA-5 had medium tolerance (2+ at 1,000 ppm) for fenthion, permethrin and diazinon. Tolerance for fenitrothion (+ at 1,000 ppm) was lower than that for fenthion. ACMA-5 showed the highest tolerance for tebufenozide among all the IGRs (+ at 4,000 ppm) tested. On the teflubenzuron and chlorfluazuron plates, ACMA-5 showed slow growth and did not show high tolerance as it did for tebufenozide. Tolerance for flufenoxuron was lowest among all insecticides used. Conse-

quently, flufenoxuron did not appear to be suitable for treatment with ACMA-5 at high concentrations.

Effect of insecticides in soil

Table 2 shows the effects of insecticides against ACMA-5 in the soil. ACMA-5 had comparatively high survivability in soil treated with fenthion and fenitrothion. Ten number of colony-forming units was not stable in the teflubenzuron, chlorfluazuron and flufenoxuron applications. The degree of these three IGRs was 2+ at 1,000 μg . On the other hand, tebufenozide was 3+ at 1,000 μg .

ACMA-5 does not have very high tolerance against flufenoxuron on the SDAY plates; nevertheless the results indicate that ACMA-5 conidia was able to survive in the soil fairly well.

Joint action of ACMA-5 and insecticides on *A. cuprea* larvae

Table 3 indicates the joint control effects with ACMA-5 and 6 kinds of insecticides against 1st stadium larvae of *A. cuprea*. Twenty one days after treatment, mortality of joint application with the fungus and insecticides was higher than that using insecticides alone. A single treatment of ACMA-5 resulted in only 40% mortality at 21 d after the treatment (1.0×10^7 conidia/ml).

Table 1. Tolerance of ACMA-5 for several insecticides and IGRs on SDAY medium

Insecticide	Concentration of ingredients (ppm)														
	30	50	60	70	100	125	250	350	375	500	550	1,000	1,500	4,000	4,500
Fenitrothion						2+	2+			+		+		+	
Fenthion						3+	3+			2+		2+	+		+
Dichlorvos						3+	3+			2+					
Diazinon						2+	2+			2+		2+			+
Chlorpyrifos						2+	2+			2+		2+			+
Trichlorfon						3+	3+			3+		2+			2+
Cypermethrin						3+	2+			2+		2+			
Permethrin						3+	2+			2+		2+			
Methomyl						3+	3+			3+		3+			
Flufenoxuron	2+		+	-											
Teflubenzuron	3+				2+	2+	2+			+	-				
Chlorfluazuron	3+				2+			+	-						
Tebufenozide						3+	3+			3+		2+		+	

Degree of tolerance of ACMA-5 for the insecticides was indicated with four classes as follows: 3+, hyphal growth was the same as the control; 2+, hyphal growth was less than control; +, hyphal growth was slight; -, hyphal growth was not observed.

Table 2. Tolerance of ACMA-5 for several insecticides in the soil (4 weeks after treatment)

Insecticide	Dose ($\mu\text{g}/\text{dish}$)					
	100	250	500	1,000	2,000	4,000
Fenitrothion				3+	2+	2+
Fenthion				3+	3+	3+
Teflubenzuron			3+	2+		
Chlorfluazuron		3+	2+	2+		
Flufenoxuron	3+	2+		2+		
Tebfenozide			3+	3+		

Degree of tolerance of ACMA-5 for the insecticides was indicated with four classes as follows: 3+, hyphal growth was the same as the control; 2+, hyphal growth was less than control; +, hyphal growth was slight; -, hyphal growth was not observed.

Table 3. Joint action of ACMA-5 and several insecticides against 1st stadium larvae of *A. cuprea* (21 days after treatment)

Dose of insecticide (μg)	Mortality of 21 days after treatment (%)			
	Insecticide single		Insecticide+ACMA-5	
	2.5	5	2.5	5
Fenitrothion	20.0 ab	13.3 ab	43.3 bc	53.3 cd
Fenthion	33.3 bc	76.7 de	83.3 de	90.0 de
Dichlorvos	40.0 bc	70.0 de	80.0 de	93.3 e
Diazinon	43.3 bc	96.7 e	66.7 cd	96.7 e
Chlorpyrifos	16.7 ab	60.0 cd	66.7 cd	60.0 cd
Permethrin	6.7 a	3.3 a	43.3 bc	50.0 cd
ACMA-5 single	40.0 bc			
Control	0.0 a			

Number of larvae at each application as 30. ACMA-5 was applied at a concentration of 1.0×10^7 conidia/ml. Numbers within columns followed by the same letter are not significantly different ($p < 0.05$ by χ^2 -test).

A single application of fenthion resulted in 77% and 33% mortality at 5.0 and 2.5 μg respectively. On the contrary, joint application of fenthion and ACMA-5 resulted in 90% and 83% at 5.0 and 2.5 μg respectively. These increases were considered to have been caused by joint action. A statistically significant difference ($p < 0.05$) was obtained at a dose of 2.5 μg . At lower doses, the mortality from fenitrothion was not as high at either a single application or joint application with ACMA-5 fungus.

Both single and joint applications of permethrin resulted in low mortality during 21 d. Hence, permethrin was not indicated to have

a clear joint action with ACMA-5.

Table 4 shows the mortality of 1st stadium *A. cuprea* larvae following applications of IGR and IGR+ACMA-5. Teflubenzuron resulted in low activity following a single application; on the other hand, it had high activity in combination with ACMA-5 (200 μg). The mortalities from a single application of teflubenzuron and teflubenzuron+ACMA-5 were significantly different. The same tendency was recognized following flufenoxuron applications. Chlorfluazuron did not show joint action in this study. Tebfenozide had no effects on the insecticidal activity of ACMA-5, because a high dose application of tebfenozide (200 μg) showed no significant difference from a single application of ACMA-5.

Figure 1 shows the cumulative mortality of 1st stadium larvae during 21 d after application of flufenoxuron and ACMA-5. Whereas the mortality of larvae following a single ACMA-5 or flufenoxuron application increased gradually as the days passed, the mortality after joint application with both agents rapidly increased about 10 d after treatment, and 87% of larvae were dead by 21 d. In this joint application, ACMA-5 resulted in earlier infection of larvae than did a single application of ACMA-5. Hence, this result also suggested the possibility of a synergistic effect.

Figure 2 indicates the causes of larval death from joint applications of ACMA-5 and flufenoxuron which were estimated by the symptoms of the dead larvae. In this experiment, the application of 200 μg of flufenoxuron and ACMA-5 caused 50% larval mortality by ACMA-5 infection and the other 50% mortality was caused by flufenoxuron. On the other hand, combined application of ACMA-5 and 10 μg of flufenoxuron caused 87% mortality from ACMA-5 infection and 13% from flufenoxuron. Thus, larval mortality caused by flufenoxuron, increased with the dose of flufenoxuron. The results indicated that lower dose of flufenoxuron induced high infection of ACMA-5.

Factors of death at 30 d after joint applications of chlorfluazuron and ACMA-5 are shown in Fig. 3. Larval mortalities showed no significant difference between the single application of chlorfluazuron and the combined application of

Table 4. Joint action of ACMA-5 and several IGRs against 1st stadium *A. cuprea* larvae (21 days after treatment)

Application	Larval mortality (%)				
	Dose of IGR ($\mu\text{g}/\text{dish}$)				
	10	25	50	100	200
Teflubenzuron + ACMA-5	60.0 cd	70.0 cd	66.7 cd	76.7 de	83.3 de
Teflubenzuron	3.3 a	16.7 ab	16.7 ab	26.7 b	20.0 b
Chlorfluazuron + ACMA-5	16.7 b	43.3 bc	63.3 cd	83.3 de	93.3 e
Chlorfluazuron	6.7 ab	6.7 ab	26.7 b	73.3 de	83.3 de
Flufenoxuron + ACMA-5	60.0 cd	66.7 cd	60.0 cd	86.7 de	80.0 de
Flufenoxuron	3.3 a	3.3 a	20.0 b	20.0 b	50.0 cd
Tebfenozido + ACMA-5	63.3 cd	56.7 cd	60.0 cd	46.7 bc	50.0 cd
Tebfenozido	6.7 ab	0.0 a	0.0 a	3.3 a	0.0 a
ACMA-5 single					50.0 cd
Control					0.0 a

Number of larvae at each application was 30. ACMA-5 was applied at a concentration of 2.0×10^7 conidia/ml. Numbers within columns followed by the same letter are not significantly different ($p < 0.05$ by χ^2 -test).

Fig. 1. Cumulative mortality with joint application of flufenoxuron and ACMA-5. ACMA-5 was applied at a concentration of 2.0×10^7 conidia/ml. Flufenoxuron was applied at $100 \mu\text{g}/\text{petri dish}$.

ACMA-5 + chlorfluazuron. Observations of dead larvae revealed no tendency of death factors between ACMA-5 infection and chlorfluazuron activity. Mortality from $25 \mu\text{g}$ application of chlorfluazuron alone was 7%, however, the same dose of ACMA-5 + chlorfluazuron application mortality was 43%. Of this 43% (13 larvae), 70% (9 larvae) were killed by the activity of chlorfluazuron. These results suggested that synergistic effects exist in this combined application.

Figure 4 indicates the larval mortality 30 d after the application of ACMA-5 and chlor-

fluazuron. At $200 \mu\text{g}$ of chlorfluazuron, all larvae died at the 1st stadium, but at $10 \mu\text{g}$, 20% of larvae died at the 1st stadium and the surviving larvae grew into the 2nd stadium 30 d after treatment. Higher doses of chlorfluazuron caused larval death at the 1st stadium.

Field pot test of joint action

Table 5 shows the results of the pot test. A single application of ACMA-5 resulted in 11 dead larvae during 15 d. The number of dead larvae from joint application of $100 \mu\text{g}$ of fenthion and ACMA-5 was higher than at 100

Fig. 2. Percentage of larval death factors in the ACMA-5 + flufenoxuron application.

Fig. 3. Percentage of larval death factors in the ACMA-5 + chlorfluazuron application.

µg fenthion alone, and a significant difference was observed between them. However, joint application of 200 µg fenthion and ACMA-5 showed the reverse results, namely, the number of dead larvae from fenthion alone was higher than from the joint application of it and ACMA-5. Mortalities of larvae were unstable under field conditions. Though the experiments were carried out over a relatively short period (15 d), we observed the formation of conidia on the surface of dead larvae with ACMA-5 combined application.

DISCUSSION

When two kinds of agents are used to obtain joint action, sometimes one agent may act as a stress inducer to the target insects and the other agent as the major control agent (Hurpin and Robert, 1968; Ishibashi and Choi, 1991; Thurston et al., 1993, 1994). In our studies, we expected *M. anisopliae* to act as the major control agent.

When *M. anisopliae* is applied with insecticides into the soil, the insecticides may impose stress or pressure on grubs. For example, organophosphates have been known to act as in-

Fig. 4. Percentage of dead *A. cuprea* larvae at each stadium in the ACMA-5+chlorfluazuron application (30 days after treatment).

Table 5. Field pot test of joint action of ACMA-5 and several insecticides against *A. cuprea* larvae (15 days after treatment)

	Dose per pot (µg)	Percentage of mortality	
		Insecticide single	Insecticide+ACMA-5
Fenthion	200	60.0 a	34.0 cd
Fenthion	100	30.0 cd	60.0 a
Fenitrothion	200	46.0 b	66.0 a
Fenitrothion	100	36.0 bc	40.0 bc
ACMA-5 single			22.0 de
Control			12.0 e

Number of larvae at each application is 50. Ten milliliters of ACMA-5 was at a concentration of 1.0×10^7 conidia/ml. Means within columns followed by the same letter are not significantly different ($p < 0.05$ by χ^2 -test).

hibitors of acetylcholine esterase, which would disturb and destroy the grub defense system and immunity inside the body. Also, in the presence of IGRs (teflubenzuron, flufenoxuron) joint action was observed in our experiment. It is supposed that the grubs are easily infected by *M. anisopliae*, because such benzoil urea IGRs serve as inhibitors to biosynthesis of insect chitin. Hence, the scarab larval surface defense system is probably weakened.

Poprawski and Yule (1991) showed that a single application of *M. anisopliae* was sufficiently effective (90% of mortality) against *Phyllophaga anxia* 40 d after application in sandy loam. Also, Shimazu et al. (1993) reported that in the forestry nursery, insecticidal activity of *M. anisopliae* against *A. cuprea* was high,

but infection by *M. anisopliae* occurred slowly. Rath et al. (1995) also showed that *M. anisopliae* was effective against *Adoryphorus couloni* in a pasture field, but infection by *M. anisopliae* was very slow. In our research, joint use of *M. anisopliae* and insecticides caused a faster spread of *M. anisopliae* infection against larvae. In some cases of joint application of ACMA-5, the strain of *M. anisopliae* used in our study, and insecticides, we observed a faster infection of *M. anisopliae* to larvae than was seen for a single application of ACMA-5. This result suggests that insecticides are effective for increasing the susceptibility of *A. cuprea* larvae to *M. anisopliae*.

In our study, sterilized soil was used for bioassay in the laboratory experiments. This soil

condition allows us to conclude that the results of experiments were due to the joint action of *M. anisopliae* and insecticides against 1st stadium *A. cuprea* larvae. However, in the results of our field pot test, the existence of a joint action was uncertain. One explanation may be variability or unstableness of environmental conditions such as soil temperature and antagonistic organisms. Furthermore, in the soil, there are many factors that suppress entomopathogenic fungi, such as antagonistic microorganisms. For instance, *Penicillium urticae* produces a water-soluble inhibitor of *Beauveria bassiana* (Shields et al., 1981). These factors weaken the stability of the insecticidal activity of entomopathogenic fungi under field conditions. Pereira et al. (1993) showed *B. bassiana* were strongly affected by soil antagonism produced around the nest of red fire ants, *Solenopsis invicta*. In the soil, insecticides may serve as inhibitors of these antagonists. When some insecticides that *M. anisopliae* has a high tolerance for, are applied to the soil, *M. anisopliae* will be more dominant than other microorganisms. Thus, such a condition is more suitable for *M. anisopliae* to kill scarab grubs effectively in the soil.

ACMA-5 has a high tolerance against many organophosphates, such as fenthion and fenitrothion, however, it has lower tolerance to IGRs excepts for tebfenozide on the SDAY agar plates. The results indicate that if some quantity of *M. anisopliae* exists in the soil, *M. anisopliae* could survive even at high levels of many insecticides in the field soil. Hence, joint application will create good conditions for the survival of *M. anisopliae* in soil. In our recent experiment, ACMA-5 conidia, were formulated on SDAY agar containing 100 ppm of teflubenzuron, chlorfluazuron and other organophosphates, and had insecticidal activity against 1st stadium larvae of *A. cuprea* (unpublished). In the soil, *M. anisopliae* will suffer little damage from IGRs and organophosphates, especially, even if the concentration of IGRs is extremely high.

This study demonstrates the effectiveness of joint action of *M. anisopliae* and insecticides such as organophosphate or insect growth regu-

lators. However, further fundamental studies are called for. For instance, studies on the interaction between *M. anisopliae* and insecticides in the soil or on the analysis of the defense system inside the body of grubs under joint applications with entomopathogenic fungi and insecticides are desirable.

ACKNOWLEDGEMENTS

We wish to express our sincere thanks to the members of the laboratory of Applied Entomology, Department of Agriculture, Shizuoka University.

REFERENCES

- Hurpin, B. and P. Robert (1968) Experiments on simultaneous infections of the common cockchafer, *Melolontha melolontha*. *J. Invertebr. Pathol.* 11: 203-213.
- Ishibashi, N. and D. R. Choi (1991) Biological control of soil pests by mixed application of entomopathogenic and fungivorous nematodes. *J. Nematol.* 23: 175-181.
- Kushida, T., Y. Mamiya and J. Mitsuhashi (1987) Pathogenicity of newly detected *Steinernema* sp. (Nematode) to scarabaeid larvae injurious to tree seedlings. *Jpn. J. Appl. Entomol. Zool.* 31: 144-149.
- Pereira, R. M., J. R. Stimac and S. B. Alves (1993) Soil antagonism affecting the dose-response of workers of red imported fire ant, *Solenopsis invicta*, to *Beauveria bassiana* conidia. *J. Invertebr. Pathol.* 61: 156-161.
- Poprawski, T. J. and W. N. Yule (1991) Incidence of fungi in natural populations of *Phyllophaga* spp. and susceptibility of *Phyllophaga anxia* (LeConte) (Col., Scarabaeidae) to *Beauveria bassiana* and *Metarhizium anisopliae* (Deuteromycotina). *J. Appl. Ent.* 112: 359-365.
- Rath, A. C., T. B. Koen, G. C. Anderson and D. Worledge (1995) Field evaluation of the entomogenous fungus *Metarhizium anisopliae* (DAT F-001) as a biocontrol agent for the redheaded pasture cockchafer, *Adoryphorus couloni* (Coleoptera: Scarabaeidae). *Aust. J. Agric. Res.* 46: 429-440.
- Shields, M. S., A. J. Lingg and R. C. Heimsch (1981) Identification of *Penicillium urticae* metabolite which inhibits *Beauveria bassiana*. *J. Invertebr. Pathol.* 38: 374-377.
- Shimazu, M., W. Mitsuhashi, H. Hashimoto and T. Ozawa (1993) Persistence of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) as a control agent of *Anomala cuprea* (Coleoptera: Scarabaeidae) in a forest nursery. *Appl. Entomol. Zool.* 28: 103-105.
- Thurston, G. S., H. K. Kaya, T. M. Burlando and R. E. Harrison (1993) Milky disease bacterium as a stressor to increase susceptibility of scarabaeid larvae to an entomopathogenic nematode. *J. Invertebr. Pathol.* 61: 167-172.
- Thurston, G. S., H. K. Kaya and R. Gaugler (1994) Characterizing the enhanced susceptibility of milky disease-infected scarabaeid grubs to entomopathogenic nematodes. *Biol. Control* 4: 67-73.
- Yaginuma, K. and K. Takagi (1986) Improvement of a selective medium for isolation of *Metarhizium anisopliae* (Metchnikoff) Sorokin. *Jpn. J. Appl. Entomol. Zool.* 30: 300-301.