

This article was downloaded by: [University of Pretoria]

On: 03 June 2012, At: 23:50

Publisher: Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



International Journal of Pest Management

Publication details, including instructions for authors and subscription information:
<http://www.tandfonline.com/loi/ttprm20>

Compatibility of *Metarhizium anisopliae* isolate ICIZE 69 with agrochemicals used in French bean production

S. Niassy^{a,b}, N.K. Maniania^a, S. Subramanian^a, M.L. Gitonga^b, R. Maranga^b, A.B. Obonyo^a & S. Ekesi^a

^a International Centre of Insect Physiology and Ecology (icipe), P.O. Box 30772, Nairobi, Kenya

^b Jomo Kenyatta University of Agriculture and Technology (JKUAT), P.O. Box 62000, Nairobi, Kenya

Available online: 25 Apr 2012

To cite this article: S. Niassy, N.K. Maniania, S. Subramanian, M.L. Gitonga, R. Maranga, A.B. Obonyo & S. Ekesi (2012): Compatibility of *Metarhizium anisopliae* isolate ICIZE 69 with agrochemicals used in French bean production, *International Journal of Pest Management*, 58:2, 131-137

To link to this article: <http://dx.doi.org/10.1080/09670874.2012.669078>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.tandfonline.com/page/terms-and-conditions>

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae, and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand, or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Compatibility of *Metarhizium anisopliae* isolate ICIPE 69 with agrochemicals used in French bean production

S. Niassy^{a,b}, N.K. Maniania^{a*}, S. Subramanian^a, M.L. Gitonga^b, R. Maranga^b, A.B. Obonyo^a and S. Ekesi^a

^aInternational Centre of Insect Physiology and Ecology (icipe), P.O. Box 30772, Nairobi, Kenya; ^bJomo Kenyatta University of Agriculture and Technology (JKUAT), P.O. Box 62000, Nairobi, Kenya

(Received 20 February 2011; final version received 25 January 2012)

The compatibility of the *Metarhizium anisopliae* (Metschnikoff) Sorokin isolate ICIPE 69, which is being developed as a biopesticide for the control of *Frankliniella occidentalis* Pergande, was assessed under laboratory conditions with 12 agrochemicals including 5 insecticides (thiamethoxam, L-cyhalothrin, imidacloprid, chlorpyrifos, diazinon), 1 botanical insecticide (azadirachtin), 2 acaricides (abamectin, spiromesifen), and 3 fungicides (carbendazim, copper hydroxide, probineb) used in French bean production. The insecticides abamectin and imidacloprid were highly compatible with *M. anisopliae*; thiamethoxam was compatible, whereas azadirachtin and L-cyhalothrin were toxic to the fungus, adversely affecting vegetative growth and sporulation. The acaricide spiromesifen was moderately toxic, while the fungicides carbendazim, probineb, and copper-hydroxide were very toxic to the fungus. The combination of the *M. anisopliae* isolate ICIPE 69 with imidacloprid or thiamethoxam did not result in any synergistic or antagonistic effects to larvae of *F. occidentalis*. However, the combination of lower concentrations of the fungus with thiamethoxam resulted in a shorter lethal time (LT₅₀) compared with individual treatments. Our results suggest that application of the fungus with agrochemicals has to be assessed carefully prior to any field intervention.

Keywords: compatibility; entomopathogenic fungus; French bean; *Metarhizium anisopliae*; pesticides; pest management; vegetables

1. Introduction

French bean, *Phaseolus vulgaris* L. (Fabaceae), is one of the most important vegetables exported from East Africa. In Kenya, it accounts for over 60% of all export crops (Nderitu et al. 2007). French bean is attacked by a wide range of insect pests including aphids, beetles and thrips (Koutsika-Sotiriou and Traka-Mavrona 2008). Among the latter, the western flower thrips (WFT), *Frankliniella occidentalis* (Pergande), is considered to be the most important insect pest of French bean in East Africa, causing considerable damage to the crop during the bean pod production phase. Losses of 40–60% on farms and 20% at the collecting points have been reported (Nderitu et al. 2007). In addition to insect pests, French bean is also vulnerable to many diseases caused by bacteria, fungi and viruses (Koutsika-Sotiriou and Traka-Mavrona 2008). Presently, the option commonly resorted in controlling insect pests and microbial pathogens is to apply synthetic chemical insecticides, acaricides, fungicides and, to some extent, botanical insecticides. However, WFT is believed to have developed resistance to all the major classes of chemical insecticides (Jensen 2004; Nderitu et al. 2010).

In recent years, entomopathogenic fungi have been developed as microbial insecticide alternatives

to synthetic chemical insecticides for the control of many insect pests including thrips (Butt and Brownbridge 1997; Chandler et al. 2008). *Metarhizium anisopliae* var. *anisopliae* (Metschnikoff) Sorokin (Hypocreales: Clavicipitaceae) isolate ICIPE 69 is among the fungal pathogens currently under development for the control of WFT and other thrips species (*Megalurothrips sjostedti* Trybom and *Thrips tabaci* Lindeman) of importance in vegetable and field crops in East Africa (Ekesi et al. 1998, 2000; Maniania et al. 2002, 2003). Since French bean is also a host to various arthropod pests and fungal diseases, which require application of synthetic chemical pesticides to control them, their judicious use and compatibility with other control agents such as microbial insecticides is of paramount importance in the context of integrated pest management (Ekesi and Maniania 2000; Irigaray et al. 2003; Da Silva and Neves 2005; Maniania et al. 2008). Here, we investigate the effects of 12 agrochemicals commonly used in French bean production on *M. anisopliae* isolate ICIPE 69 in terms of vegetative growth, conidia production, mycelial mass and virulence against second-instar larvae of *F. occidentalis*.

*Corresponding author. Email: nmaniania@icipe.org

1. Materials and methods

1.1. Agrochemicals

Twelve agrochemicals commonly used in French bean production were selected to assess their compatibility with *M. anisopliae*. They included five insecticides, two acaricides, three fungicides and one botanical pesticide (Table 1).

1.2. Fungus

Metarhizium anisopliae var. *anisopliae* isolate ICIPE 69 was obtained from the Arthropod Germplasm Centre of the International Centre of Insect Physiology and Ecology (*icipe*). It was cultured on Sabouraud Dextrose Agar (SDA) in 9-cm Petri dishes and incubated at $25 \pm 2^\circ\text{C}$ in complete darkness. Conidia were harvested by scraping the surface using a spatula. Conidia were suspended in 10 ml sterile distilled water containing 0.05% Triton X-100 in universal bottles containing glass beads. Conidial suspensions were vortexed for 5 min to produce a homogeneous suspension. Spore concentrations were determined using a haemocytometer.

1.3. Vegetative growth

An aliquot of 100 μl of conidial suspension titrated at 1×10^6 conidia ml^{-1} was spread-plated onto Potato Dextrose Agar (PDA) medium and incubated at 25°C . Each chemical was mixed with 300 ml of PDA medium (previously autoclaved and cooled at 50°C), according to the recommended concentration on the prescription,

before pouring the solution onto sterilized Petri dishes. After 48 h post-inoculation, 0.4 cm diameter plugs were removed using a cork borer and inserted on the chemical-treated PDA plates from which an equivalent 0.4 cm diameter plug had been removed. Orthogonal lines were drawn on the plates to monitor the growth of the mycelial plug. Data for vegetative growth were recorded at 5, 11 and 19 d after insertion of the plugs. No chemical was added in the control treatments, and all treatments were repeated six times with four replicates each time. Plates showing contamination were discarded.

1.4. Mycelial mass

Petri dishes used for vegetative growth were kept up to 21 d after insertion of the plug and the mycelial mat was harvested using a spatula and immediately weighed and then placed in an oven at 50°C for 30 min to assess the dry weight. Six replicates were used in this experiment.

1.5. Conidia production

Fresh plates of PDA, previously mixed with each chemical, were inoculated with 48 h-old *M. anisopliae* plugs as described earlier. The mycelial mat was harvested 3 weeks after inoculation and suspended in 10 ml sterile distilled water containing 0.05% Triton X-100 in universal bottles containing glass beads, then vortexed for 1 min to obtain homogeneous suspension. Conidia were quantified using a Neubauer counting chamber.

Table 1. List of main agrochemicals used in used in French bean production in Kenya.

Chemicals	Trade name	Active ingredient (a.i.)	Formulation	Purpose of utilization	Group
Insecticides	Actara, Syngenta	Thiamethoxam	250 g/kg WDG	Citrus thrips	Neonicotinoid
	Duduthrin (Karate)	L-cyhalothrin	EC 1.75 g/l	Lepidoptera, Hemiptera, Diptera, Coleoptera	Pyrethroid
	Confidor, Bayer Crop	Imidacloprid	SC 200	Hemiptera, Siphonaptera	Neonicotinoid
	Dursban, Dow AgroSciences	Chlorpyrifos	EC 480 g/l	Hymenoptera, Hemiptera, Lepidoptera, Siphonaptera, Acari	Organophosphate
	Murphy, Murphy Chemicals	Diazinon	EC 30–60 ml/20 L	Hemiptera, Lepidoptera, Acari, Thysanoptera	Organophosphate
Botanical insecticides	Achook	Azadirachtin	EC 0.15%	Nematicide/insecticide	Limonoid
	Neemrock	Azadirachtin	EC 0.03%	Vegetable pests (diamondback moth)	Limonoid
Acaricides	Dynamec, Syngenta	Abamectin	EC 1.8	Acari, Thysanoptera, Hemiptera	Naturally derived insecticide/acaricide
	Oberon, Bayer Crop	Spiromesifen	SC 240 g a.i./L	Acari, Thysanoptera, Hemiptera	Keto-enol
Fungicides	Goldazim, Collin Campbell	Carbendazim	SC 500	Fungi	Benzimidazole carbamate
	Kocide, Dupont	Copper hydroxide		Bacteria and fungi	Copper fungicide
	Milraz WP76, Dow AgroSciences	Propineb + Cymoxamil	700 g/kg, 60 g/kg	Fungi	Dithiocarbamate + ethyl urea

EC = emulsifiable concentrate, SC = suspension concentrate, WDG = water dispersible granule.

1.6. Effects of combining imidacloprid and thiamethoxam with *M. anisopliae* ICIPE 69 on the susceptibility of second-instar larva of *F. occidentalis*

Three concentrations of imidacloprid and thiamethoxam (10%, 20%, and 50% of the recommended concentrations) were combined with three doses of the *M. anisopliae* (1×10^6 , 1×10^7 , and 1×10^8 conidia ml^{-1}). Recommended doses (100%) of imidacloprid (0.5 ml/l) and thiamethoxam (0.2 g/l) were included as a check. Treatments consisted of soaking French bean pods in various suspensions for 10 seconds. Pods were then transferred to paper towel and allowed to dry for 5–10 min. French bean pods were surface-sterilized in 3% sodium hypochlorite and rinsed thrice in sterile distilled water before use. Treated pods were later transferred individually into 10-ml glass tubes containing paper towel to allow insect pupation as well as absorption of the excess moisture from the pod. Twenty, second-instar WFT larvae were introduced into the tube containing treated French bean pods. Test-insects were maintained at 25°C and 70% humidity for 8 d. The tube was closed using a lid with a hole of 1 cm diameter covered by a thrips-proof mesh to allow for ventilation. Mortality was recorded daily for 8 days and the experiment was repeated four times.

1.7. Data analysis

All data were analysed using SAS software (SAS Institute 2003). Data of vegetative growth, mycelial weight and conidia production were arcsine-transformed for normalization and subjected to analysis of variance (ANOVA), and post-ANOVA comparisons of means were made using the Student–Newman–Keuls test. Compatibility (T) was calculated according to Alves's formula (Alves et al. 1998):

$$T = [(20 \times \text{VG}) + (80 \times S)]/100,$$

whereby the values for vegetative growth (VG) and spore production (S) are expressed as percentages in relation to the control, and T takes values from 0 to 30 = very toxic, 31 to 45 = toxic, 46 to 60 = moderately toxic, 60 to 90 = compatible, and >90 = highly compatible.

Correlations between vegetative growth, conidia production, mycelial mass and mycelial dry mass were tested using Pearson's correlation, and a Principal Component Analysis (PCA) was used to confirm the distribution of the chemical effect on the fungus. Percentage mortality (at 7 d post-treatment) was adjusted for natural mortality in controls using (Abbott 1925) formula before analysis and data were then subjected to ANOVA followed by the Student–Newman–Keuls test. The lethal treatment LT_{50} values were determined for each replicate using probit analysis and compared among themselves using ANOVA followed by the Student–Newman–Keuls test.

2. Results

2.1. Vegetative growth

At the recommended dose of each agrochemical, the VG of *M. anisopliae* varied significantly between the treatments at 5 d ($F_{12, 282} = 97$, $P < 0.001$), 11 d ($F_{12, 277} = 195$, $P < 0.001$), and 19 d ($F_{12, 283} = 252$, $P < 0.001$) post-inoculation. With the exception to 19 d post-inoculation, where VG in the control and the imidacloprid treatment were similar, the VG in the control was significantly higher than in the other treatments (Table 2). No VG was recorded with carbendazim.

Table 2. Effect of 12 selected agrochemicals on vegetative growth of *Metarhizium anisopliae* ICIPE 69 at 5, 11 and 19 d after inoculation at 25°C.^a

Treatments	Vegetative growth (mm)		
	Days after inoculation		
	5	11	19
Control	8.6 ± 2.3a	17.4 ± 0.3a	29.2 ± 0.3a
Abamectin	6.7 ± 0.2b	12.5 ± 0.3cd	22.7 ± 0.9c
Azadirachtin 0.03%	5.6 ± 0.2c	11.2 ± 0.7ed	18.9 ± 1.1d
Azadirachtin 15%	6.8 ± 0.2b	13.7 ± 0.2bc	21.4 ± 0.3c
Carbendazim	0.0 ± 0.0f	0.0 ± 0.0h	0.0 ± 0.0i
Chloropyrifos	2.3 ± 0.2e	5.1 ± 0.3gf	11.2 ± 0.3g
Copper Hydroxide	2.8 ± 0.2e	5.8 ± 0.4f	11.1 ± 0.4g
Diazinon	2.3 ± 0.2e	4.3 ± 0.3g	9.2 ± 0.5h
Imidacloprid	6.6 ± 0.3b	14.3 ± 0.4b	29.3 ± 0.4a
L-Cyhalothrin	5.7 ± 0.3b	10.8 ± 0.3e	16.5 ± 0.3e
Probineb	3.8 ± 0.3d	12.4 ± 0.3cd	13.1 ± 0.5f
Spiromesifen	5.4 ± 0.2c	12.4 ± 0.3cd	21.6 ± 0.8c
Thiamethoxam	5.3 ± 0.2c	12.7 ± 0.4cd	25.7 ± 0.5b
	$F_{12, 282} = 97$; $P < 0.001$	$F_{12, 277} = 195$; $P < 0.001$	$F_{12, 283} = 252$; $P < 0.001$

^aMeans in columns followed by the same letters are not significantly different by the Student–Newman–Keuls test.

2.2. Conidia production

The conidia production varied significantly among the treatments ($F_{12, 277} = 19.1, P < 0.0001$). There was no significant difference between the control and among each of the abamectin, imidacloprid and thiamethoxam treatments. There was significant difference between the control and the other treatments. Carbendazim caused the most deleterious effect and was significantly different from the other treatments (Table 3).

2.3. Mycelial mass

Mycelial mass was higher in the control and L-cyhalothrin treatments than in the other treatments ($F = 109.0, P < 0.0001$) (Table 3). *Metarhizium anisopliae* in association with L-cyhalothrin produced the highest dry weight (284.4 mg), followed by the control (147.2 mg) (Table 3).

2.4. Classification of toxicity of agrochemicals against *M. anisopliae* isolate ICIPE 69 according to Alves's model

According to Alves's model, the *M. anisopliae* ICIPE 69 was highly compatible with abamectin, imidacloprid and compatible with thiamethoxam. Spiromesifen showed a moderately toxic effect, while azadirachtin and L-cyhalothrin were toxic to the fungus (Table 4). Chlorpyrifos, carbendazim, diazinon, copper hydroxide and probineb were very toxic to the fungus. There was a strong correlation between VG and conidia production (Pearson's $r = 0.9; P = 0.0002$), and between the mycelia mass and the mycelia dry mass (Pearson's $r = 0.9; P < 0.0001$); whereas there was no strong correlation between the mycelial mass and conidia production (Pearson's $r = 0.4; P = 0.15$) (Table 5). Principal Component a Analysis (PCA) showed that imidacloprid, abamectin and thiamethoxam can be grouped as chemicals with no effects on VG

and conidia production of the *M. anisopliae* (Figure 1). On the other hand, azadirachtin and the fungicides, copper hydroxide and propineb, affected the VG and conidia production of the *M. anisopliae*. The fungicide carbendazim had the most deleterious effect

Table 4. Compatibility of 12 selected agrochemicals with *Metarhizium anisopliae* ICIPE 69, according to Alves' model (Alves et al. 1998).

Chemicals	VG	SP	T	Classification
Abamectin	77.5	102.3	97.4	Highly compatible
Azadirachtin 0.03%	64.7	33.6	39.9	Toxic
Azadirachtin 15%	73.2	21.1	31.5	Toxic
Carbendazim	0	0	0	Very toxic
Chlorpyrifos	38.1	4.2	11.0	Very toxic
Copper hydroxide	38.0	8.5	14.4	Very toxic
Diazinon	31.5	1.0	7.1	Very toxic
Imidacloprid	100.2	101.2	101.0	Highly compatible
L-Cyhalothrine	56.5	25.5	31.7	Toxic
Probineb	44.9	8.5	15.8	Very toxic
Spiromesifen	73.8	38.7	45.7	Moderately toxic
Thiamethoxam	87.8	70.9	74.2	Compatible

VG = vegetative growth; SP = spore production; T = compatibility value.

Table 5: A Pearson Correlation ($r; P$) between 4 variables: vegetative growth, conidia production, mycelial mass and dry mass.

	Mycelial mass	Mycelial dry mass	Conidia production
Vegetative growth	0.5; 0.07	0.3; 0.4	0.9; 0.0002
Mycelial mass		0.9; <0.0001	0.4; 0.15
Mycelial dry mass			0.2; 0.6

When P is greater than $|r|$, we reject the null hypothesis (H_0) and conclude that there is a strong correlation between variables.

Table 3. Conidia production and mycelial mass (mg), of ICIPE 69 exposed to recommended doses of 12 agrochemicals on Sabouraud Dextrose Agar for 3 weeks after plug insertion.

Chemicals	Conidia production $\times 10^8$	Mycelial mass	Mycelial dry mass
Control	14.0 \pm 0.8a	338.2 \pm 16.9a	147.2 \pm 10.7b
Abamectin	14.3 \pm 1.3a	90.8 \pm 0.9cd	34.0 \pm 1.9c
Azadirachtin 0.03%	4.7 \pm 0.8b	142.3 \pm 4.5b	64.0 \pm 7.3c
Azadirachtin 0.15%	2.9 \pm 0.8b	100.7 \pm 13.0cd	34.1 \pm 3.1c
Carbendazim	0.0 \pm 0.0c	0.0 \pm 0.0f	0.0 \pm 0.0c
Chlorpyrifos	0.6 \pm 0.1b	74.4 \pm 8.0ed	28.6 \pm 4.0c
Copper hydroxide	1.2 \pm 0.1b	70.8 \pm 4.0ed	54.7 \pm 4.6c
Diazinon	0.1 \pm 0.0b	98.2 \pm 7.7cd	28.6 \pm 4.0c
Imidacloprid	14.1 \pm 3.9a	118.0 \pm 52.0bc	54.6 \pm 2.9c
L-Cyhalothrin	3.6 \pm 0.3b	318.0 \pm 13.0a	284.4 \pm 75.0a
Probineb	1.2 \pm 0.1b	52.4 \pm 6.0e	46.1 \pm 16.1c
Spiromesifen	5.4 \pm 1.1b	73.3 \pm 2.0ed	34.4 \pm 1.4c
Thiamethoxam	9.9 \pm 1.0a	125.0 \pm 4.0bc	47.0 \pm 0.5c
	$F_{12, 282} = 19.1; P < 0.0001$	$F_{12, 277} = 109.0; P < 0.0001$	$F_{12, 283} = 10.7; P < 0.0001$

Means in columns followed by the same letters are not significantly different by the Student–Newman–Keuls test.

among all the agrochemicals tested. Spiromesifen, which was moderately toxic in Alves model, centred between the compatible and the toxic chemicals (Figure 1).

2.5. Effects of imidacloprid and thiamethoxam on the virulence of *M. anisopliae* ICIPE 69 to second-instar larvae of *F. occidentalis*

The mortality caused by imidacloprid alone varied between 44.3% (10% of recommended concentration) and 94% (recommended concentration) and the mortality caused by thiamethoxam ranged between 33% (10% recommended concentration) and 92% (recommended concentration) (Table 6).

M. anisopliae applied alone caused mortalities of 34, 54 and 76% at the concentrations of 1×10^6 , 1×10^7 and 1×10^8 conidia ml^{-1} , respectively, at 8 d post inoculation (Table 6). At 1×10^6 conidia ml^{-1} , the combination with imidacloprid and thiamethoxam at 10, 20 and 50% was different from the fungus alone. The combination of different doses of *M. anisopliae* with different concentrations of the two chemicals did not affect the virulence of the fungus (Table 6). However, the combination of thiamethoxam (10%) with *M. anisopliae* at 1×10^6 conidia ml^{-1} resulted in a shorter LT_{50} of 6 d as compared to single treatments of thiamethoxam (13 d) and *M. anisopliae* at 1×10^6 conidia ml^{-1} (11 d) (Table 7).

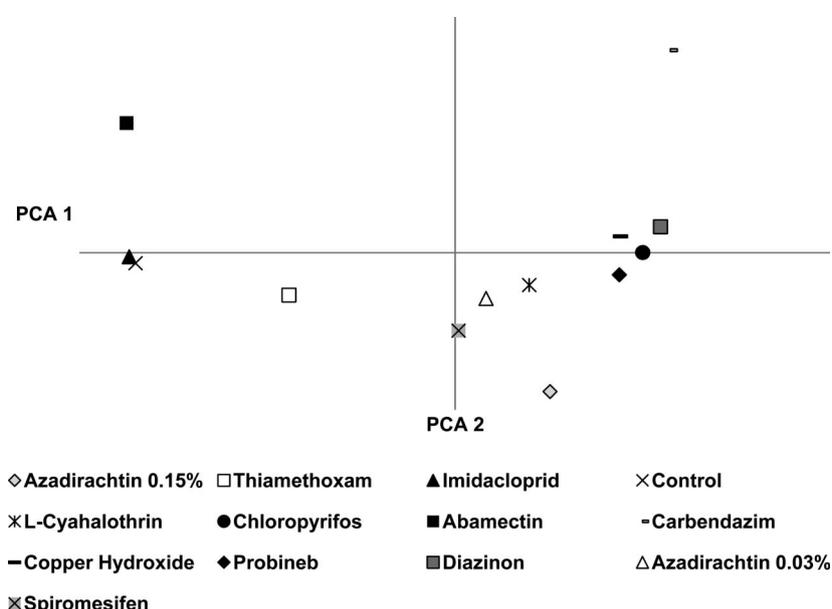


Figure 1. Principal Component Analysis: Effects of 12 agrochemicals on the vegetative growth, spore production and mycelial mass of *M. anisopliae* ICIPE 69 on Potato Dextrose Agar at 25°C.

Table 6. Mean mortality ($\bar{X} \pm \text{SE}$) of second-instar larvae of *Frankliniella occidentalis* treated with *Metarhizium anisopliae* ICIPE 69 in combination with different rates of imidacloprid and thiamethoxam.

Chemicals		Conidial concentrations (ml^{-1})			
		0	10^6	10^7	10^8
Imidacloprid	0% RC	–	$33.5 \pm 4.6\text{b}$	$54.0 \pm 2.1\text{a}$	$76.2 \pm 2.1\text{a}$
	10% RC	$44.3 \pm 10.9\text{b}$	$69.6 \pm 11.3\text{a}$	$66.5 \pm 11.9\text{a}$	$77.0 \pm 11.7\text{a}$
	20% RC	$74.0 \pm 14.5\text{ab}$	$74.8 \pm 11.3\text{a}$	$78.5 \pm 8.6\text{a}$	$82.2 \pm 6.4\text{a}$
	50% RC	$86.3 \pm 10.8\text{a}$	$75.0 \pm 1.8\text{a}$	$88.5 \pm 9.6\text{a}$	$89.0 \pm 4.8\text{a}$
	100% RC	$94.0 \pm 3.5\text{a}$	–	–	–
		$F_{3,12} = 4.94, P = 0.03$	$F_{3,12} = 5.63, P = 0.01$	$F_{3,12} = 2.86, P = 0.08$	$F_{3,12} = 0.68, P = 0.58$
Thiamethoxam	10% RC	$33.3 \pm 9.2\text{c}$	$59.2 \pm 8.5\text{a}$	$65.0 \pm 9.0\text{a}$	$82.2 \pm 10.5\text{a}$
	20% RC	$55.7 \pm 13.9\text{bc}$	$64.5 \pm 6.6\text{a}$	$74.2 \pm 5.9\text{a}$	$91.0 \pm 6.0\text{a}$
	50% RC	$73.0 \pm 6.8\text{ab}$	$76.0 \pm 10.5\text{a}$	$75.2 \pm 4.2\text{a}$	$91.8 \pm 4.8\text{a}$
	100% RC	$92.2 \pm 6.0\text{a}$	–	–	–
			$F_{3,12} = 8.17, P = 0.006$	$F_{3,12} = 5.18, P = 0.016$	$F_{3,12} = 2.86, P = 0.08$

RC = Recommended Dose of the chemical on French bean crop per litre of water for imidacloprid and thiamethoxam, respectively, 0.5 ml/L and 0.2 g/L. Within a column means followed by the same letters are not significantly different by the Student–Newman–Keuls test.

Table 7. Median tethal time LT_{50} (days \pm SE) of second-instar larvae of *Frankliniella occidentalis* treated with *Metarhizium anisopliae* ICIPE 69 in combination with different concentrations of imidacloprid and thiamethoxam.^a

Chemicals	Rates	Conidial concentrations			
		0	10 ⁶	10 ⁷	10 ⁸
Imidacloprid	0% RC	–	11.0 \pm 0.1aA	6.5 \pm 0.4aB	4.0 \pm 0.6aB
	10% RC	8.4 \pm 1.0aA	5.0 \pm 0.5bA	5.9 \pm 1.0bA	5.0 \pm 1.1aA
	20% RC	4.3 \pm 0.7bA	4.8 \pm 1.0bA	3.7 \pm 0.6bA	3.8 \pm 0.2aA
	50% RC	3.7 \pm 0.3bA	4.0 \pm 0.1bA	3.3 \pm 0.2bA	3.3 \pm 0.3aA
	100% RC	2.3 \pm 0.1b	–	–	–
Thiamethoxam	10% RC	12.5 \pm 5.4aA	6.2 \pm 1bAB	5.8 \pm 0.9abAB	3.5 \pm 0.5aB
	20% RC	6.4 \pm 1.4aA	5.1 \pm 0.2bA	4.3 \pm 0.6bA	3.4 \pm 0.5aA
	50% RC	4.4 \pm 0.2aA	4.8 \pm 0.9bA	4.3 \pm 0.2bA	3.1 \pm 0.4aA
	100% RC	2.6 \pm 0.4a	–	–	–

RC = Recommended dose.

^aMeans within the same row with the same capital letters are not significantly different by the Student–Newman–Keuls test.

3. Discussion

A wide range of agrochemicals, including synthetic chemical pesticides and botanicals, is applied to control pests in French bean production. The agrochemicals commonly used to control arthropod pests and diseases in French bean showed various effects on the entomopathogenic fungus *M. anisopliae* isolate ICIPE 69. For instance, azadirachtin, the three fungicides, L-cyhalothrin and chlorpyrifos were toxic to the fungus. Chlorpyrifos has already been reported to negatively affect *M. anisopliae*, probably due the presence of chlorus (Li and Holdom 1995). On the other hand, a combination of chlorpyrifos with sublethal doses of *M. anisopliae* has been reported to have synergistic effects on German cockroach, *Blattella germanica* (L.) (Diptoptera: Blattellidae) (Pachamuthu and Kamble 2000). Both synergistic and antagonistic effects of azadirachtin have been reported for several isolates of *Beauveria bassiana* (Balsamo) (Mohan et al. 2007). Depieri et al. (2005) and Rachappa et al. (2007) reported the inhibition of entomopathogenic fungi by azadirachtin. Although high mycelial mass and dry mass was produced with L-cyhalothrin, it was classified as toxic to *M. anisopliae* ICIPE 69. The negative effect of L-cyhalothrin on entomopathogenic fungi has been previously reported (Olajire and Oluyemisi 2009). The deleterious effects of carbendazim on *M. anisopliae* mycelia growth and conidia production observed in our study are similar to the findings of Moorhouse et al. (1992) and Rachappa et al. (2007) on the same fungus. The acaricides showed variable effects on the *M. anisopliae* ICIPE 69 as reported earlier by Shi et al. (2005). For instance, abamectin was highly compatible, while spiromesifen was moderately toxic. Abamectin has also been reported to be compatible with entomopathogenic fungi (Tamai et al. 2002). Imidacloprid and thiamethoxam did not have deleterious effects on VG and conidia production. Compatibility of the two neonicotinoids with entomopathogenic fungi has already been reported by many workers (Filho et al. 2001;

Neves et al. 2001; Wenzel et al. 2004). Although the combination of these two chemicals with the fungus did not affect the virulence of the fungus, no synergism was observed, except in association with the fungus at the concentration of 1×10^6 conidia ml^{-1} and thiamethoxam at the dose of 10%, resulting in a short LT_{50} . Dara and Hountondji (2001) also reported the lack of synergism between imidacloprid and *Hirsutella thompsonii* Fisher against the cassava green mite *Mononychellus tanajoa* Bondar. However, many studies have shown that imidacloprid significantly increases insect pest susceptibility when combined with *M. anisopliae* (Ramakrishnan et al. 1999; Shah et al. 2001; Ansari et al. 2007; Santos et al. 2007).

Our results show that some of the synthetic chemical pesticides and botanical insecticides used to control insects and diseases in French bean have negative effects on *M. anisopliae* ICIPE 69. Therefore, it is inadvisable to apply them at the same time as *M. anisopliae* ICIPE 69 which is meant for the control of WFT. On the other hand, imidacloprid and thiamethoxam can be recommended in French bean production in combination with the fungus. Field studies to evaluate the compatibility of these agrochemicals with the fungal isolate, applied either as combinations or incorporated singly with the isolate, should generate additional information on how *M. anisopliae* ICIPE 69 can be successfully incorporated in integrated pest management systems together with the insecticides.

Acknowledgements

We are grateful to Miss Catherine Adongo for technical support. This study was funded by the German Academic Exchange Services through the African Regional Postgraduate Programme in Insect Science (ARPPIS) of *icpe* and from the BMZ Thrips IPM Project.

References

Abbott WS. 1925. A method of computing the effectiveness of an insecticide. *J Econ Entomol.* 18:265–267.

- Alves SB, Monino A Jr, Almeida JEM. 1998. Controle Microbiano De Insetos. São Paulo, Fealq. In: Alves, S.B. (ed.), Produtos fitossanitários e entomopatogênicos; p. 289–370.
- Ansari MA, Shah FA, Whittaker M, Prasad M, Butt TM. 2007. Control of western flower thrips (*Frankliniella occidentalis*) pupae with *Metarhizium anisopliae* in peat and peat alternative growing media. *Biol Control*. 40:293–297.
- Butt TM, Brownbridge M. 1997. Fungal pathogens of thrips. In: Lewis T, editor. *Thrips as crop pests*. Wallingford (UK): CAB International. p. 399–433.
- Chandler D, Davidson G, Grant WP, Greaves J, Tatchell GM. 2008. Microbial biopesticides for integrated crop management: an assessment of environmental and regulatory sustainability. *Trends Food Sci Technol*. 19:275–283.
- Da Silva RZ, Neves PMOJ. 2005. Techniques and parameters used in compatibility tests between *Beauveria bassiana* (Bals) Vuill and in vitro phytosanitary products. *Pest Manage Sci*. 61:667–674.
- Dara SK, Hountondji FCC. 2001. Effects of formulated imidacloprid on two mite pathogens, *Neozygites floridana* (Zygomycotina: Zygomycetes) and *Hirsutiella thompsonii* (Deuteromycotina: Hyphomycetes). *Insect Sci Appl*. 21(2):133–138.
- Depieri RA, Martinez SS, Menezes AOJ. 2005. Compatibility of the fungus *Beauveria Bassiana* (Bals.) Vuill. (Deuteromycetes) with extracts of neem seeds and leaves and the emulsible oil. *Neotrop Entomol*. 34(4):601–606.
- Ekesi S, Maniania NK, Lwande W. 2000. Susceptibility of the legume flower thrips to *Metarhizium anisopliae* on different varieties of cowpea. *Biocontrol*. 45:79–95.
- Ekesi S, Maniania NK, Onu I, Lohr B. 1998. Pathogenicity of entomopathogenic fungi (Hyphomycetes) to the legumes flower thrips, *Megalurothrips sjostedti* (Thysanoptera: Thripidae). *J Appl Entomol*. 122:629–634.
- Ekesi S, Maniania NK. 2000. In: Updhayay R, editor. *Metarhizium anisopliae*: An effective biological control agent for the management of thrips in horti- and floriculture in Africa. *Advances in microbial control of insects pests*. New York: Kluwer Academic / Plenum publishers. p. 164–180.
- Filho AB, Almeida JEM, Lamas C. 2001. Effect of thiamethoxam on entomopathogenic microorganisms. *Neotrop Entomol*. 30(2):437–447.
- Irigaray FJ, Marco-Manceb V, Ignacio P. 2003. The entomopathogenic fungus *Beauveria bassiana* and its compatibility with triflumuron: effects on the twospotted spider mite *Tetranychus urticae*. *Biol Control*. 26:168–173.
- Jensen ES. 2004. Insecticide resistance in the western flower thrips, *Frankliniella occidentalis*. *Integr Pest Manage Rev*. 5(2):131–146.
- Koutsika-Sotiriou M, Traka-Mavrona E. 2008. Snap Bean. In: Prohens J, Nuez F, editors. *Vegetables II: Fabaceae, Liliaceae, Solanaceae and Umbelliferae*. New York (USA): Springer. p. 27–83.
- Li DP, Holdom DG. 1995. Effects of nutrients on colony formation, growth, and sporulation of *Metarhizium anisopliae* (Deuteromycotina, Hyphomycetes). *J Invert Pathol*. 65:253–260.
- Maniania NK, Bugeme DM, Wekesa VW, Delalibera IJ, Knapp M. 2008. Role of entomopathogenic fungi in the control of *Tetranychus evansi* and *Tetranychus urticae* (Acari: Tetranychidae), pests of horticultural crops. *Exp Appl Acarol*. 46:259–274.
- Maniania NK, Ekesi S, Lohr B, Mwangi F. 2002. Prospect for biological control of the Western Flower Thrips, *Frankliniella occidentalis*, with the entomopathogenic fungus, *Metarhizium anisopliae* on Chrysanthemum. *Mycopathologia*. 155:229–235.
- Maniania NK, Sithanatham S, Ekesi S, Onu I, Ampong-Nyarko K, Baumgärtner J, Löhr B, Matoka CM. 2003. A field trial of the entomopathogenic fungus *Metarhizium anisopliae* for control of onion thrips, *Thrips tabaci*. *Crop Prot*. 22:553–559.
- Mohan MC, Reddy NP, Devi UK, Kongara R, Sharma HC. 2007. Growth and insect assays of *Beauveria bassiana* with neem to test their compatibility and synergism. *Biocontrol Sci Technol*. 17(10):1059–1069.
- Moorhouse ER, Gillespie AT, Sellers EK, Charnley AK. 1992. Influence of fungicides and insecticides on the entomogenous fungus *Metarhizium anisopliae*, a pathogen of the vine weevil, *Otiorhynchus sulcatus*. *Biocontrol Sci Technol*. 2:49–58.
- Nderitu J, Mwangi F, Nyamasyo G, Kasina M. 2010. Utilization of synthetic and botanical insecticides to manage thrips (Thysanoptera: thripidae) on snap beans (Fabaceae) in Kenya. *Int J Sustain Crop Prod*. 5(1):1–4.
- Nderitu JH, Wambua EM, Olubayo F, Kasina JM, Waturu CN. 2007. Management of thrips (Thysanoptera: Thripidae) infestation on French beans (*Phaseolus vulgaris* L.) in Kenya by combination of insecticides and varietal resistance. *J Entomol*. 4(6):469–473.
- Neves PMO, Hirose JE, Tchujo PT, Moin JA. 2001. Compatibility of entomopathogenic fungi with neonicotinoid insecticides. *Neotrop Entomol*. 30(2):63–68.
- Olajire DM, Oluyemisi FB. 2009. In vitro effects of some pesticides on pathogenic fungi associated with legumes. *Aust J Crop Sci*. 3(3):173–177.
- Pachamuthu P, Kambale ST. 2000. In vivo study on combined toxicity of *Metarhizium anisopliae* (Deuteromycotina : Hyphomycetes) strain ESC-1 with sublethal doses of chlorpyrifos, propetamphos, and cyfluthrin against German cockroach (Dictyoptera: Blattellidae). *J Econ Entomol*. 93(1):60–70.
- Rachappa V, Lingappa S, Patil K. 2007. Effect of Agrochemicals on Growth and Sporulation of *Metarhizium anisopliae* (Metschnikoff) Sorokin. *Karnataka J agric Sci*. 20(2):410–413.
- Ramakrishnan R, Suiter DR, Nakatsu CH, Humber RA, Bennett GW. 1999. Imidacloprid-enhanced *Reticulitermes flavipes* (Isoptera Rhinotermitidae) susceptibility to the entomopathogen *Metarhizium anisopliae*. *J Econ Entomol*. 92(11):25–32.
- Santos AV, De Oliveira BL, Samuels RI. 2007. Selection of entomopathogenic fungi for use in combination with sublethal doses of imidacloprid: perspectives for the control of the leaf-cutting ant *Atta sexdens rubropilosa* Forel (Hymenoptera: Formicidae). *Mycopathologia*. 163:233–240.
- SAS Institute 2003. *SAS User's Guide*. Release 9.2. SAS Institute, Cary, NC, USA.
- Shah FA, Ansari MA, Prasad M, Butt TM. 2001. Evaluation of black vine weevil (*Otiorhynchus sulcatus*) control strategies using *Metarhizium anisopliae* with sublethal doses of insecticides in disparate horticultural growing media. *Biol Control*. 40:246–252.
- Shi W-B, Jiang Y, Feng M-G. 2005. Compatibility of ten acaricides with *Beauveria bassiana* and enhancement of fungal infection to *Tetranychus cinnabarinus* (Acari: Tetranychidae) eggs by sublethal application rates of pyridaben. *Appl Entomol Zool*. 40(4):659–666.
- Tamai MA, Alves SB, de Almeida JEM. 2002. Evaluation of entomopathogenic fungi for control of *Tetranychus urticae* Koch (Acari: Tetranychidae). *Arq Inst Biol*. 69(3):77–84.
- Wenzel IM, Batista FA, de Almeida AMB, Mineiro JLC. 2004. Evaluation of the effect of pesticides on the development and pathogenicity of the entomopathogenic fungus *Lecanicillium lecanii*. *Arq Inst Biol*. 71:172–174.