ENTOMOPATHOGENIC FUNGI A PROMISING BIOCONTROL AGENT FOR *ICERYA SEYCHELLARUM* (HOMOPTERA: MARGARODIDAE) IN EGYPT

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ABSTRACT

Seven fungal genera *Alternaria infectoria*, *Aspergillus alliaceus*, *Beauveria bassiana*, *Cladosporium oxysporum*, *Penicillium sp.*, *Trichoderma sp.*, and *Verticillium lecnii* were isolated from the mealybug *I. seychellarum* cadaver. *A. infectoria* was the most frequently isolated fungus. The virulence fungi *A. infectoria* and *B. bassiana* on *I. seychellarum* were tested. The correlation between the fungal concentrations and the percentage of mortality was positive. The highest mortality percentage was recorded day 12 after application. Results also indicated that, both fungi significantly reduced the number of *I. seychellarum* populations under field conditions. The insecticide, Sumithion achieved a very strong inhibition effect to the radial growth of the two fungi, whereas the acaricides, Vertmic had no effect on the fungi. These results are early steps toward the implementation of *A. infectoria* and *B. bassian* as microbial control agents for use in an IPM program.

INTRODUCTION

Insect resistance, residues pollution, wide toxicity spectrum and the adverse effects of the widespread use of pesticides in pest control warrant for a change in control tactics. In the search for safer and economical methods, entomologists have turned their attention to the possibility of using insect pathogens, as promise potential biological control agents.

Numerous entomopathogenic fungi have shown considerable promise as biocontrol agents. *Cladosporium oxysporum* (Samways and Grech, 1986), *Alternaria infectoria* (Shabana and Ragab, 1997) *Neozygites fumuosa* (Villacarlos, 2000) *Beauveria bassiana* and *Metarhizium anisipoliae* (El-Adawy *et al.*, 2001).

Diseases of mealybug insects have been very little studied. None have been reported specifically infecting *Icera seychallarum*.

Successful microbial control system run through sequence steps, a survey of the biocontrol agent existing naturally in the insects, or in the agriculture soils, selecting the agents that capable to depress pest population, economical studies and finally mass reproduction for the promising agent.

Fungal infections were observed in different developmental stages of mealybug collected from mulberry, guava and mango trees at Ismailia Governorate. The aims of present investigation are to isolate and identify the fungal pathogens and also to determine its efficacy as a biocontrol agent against *I. seychellarum*.

MATERIAL AND METHODS

I. Isolation and Identification

During our primary study conducted in 2003-2004 season at each of Ismailia and North Sinai Governorates, fungal infections were observed in different developmental stages of the mealybug, *I. seychellarum* which collected from guava, mango and mulberry trees.

The method of El-Safty and Boraei (1987) with slight modification was employed to isolate the fungi as follow: The field collected cadavers of *I. seychallrum* were surface sterilized with 1% sodium hypochloride solution for 2 minutes, to remove external contaminations rinsed 3 times in sterile distillated water and arranged on filter papers inside Petri dishes. Dishes were kept at 25°C and 100 % relatve humidity, maintained by saturating the filter papers with water. Cadavers showed external growth of the fungus were considered killed by the fungus. Then placed on freshly prepared potato dextrose agar medium (PDA) which prepared by boiling 200 g of fresh cut potato in 800 ml of distillated water and filtering to obtain potato extract, then added 20 g of D-glucose, 16 g of agar and distilled water to make 1 L of medium, supplemented with streptomycin sulphate 3.7 mg/ml and chloramphenicol 2.5 mg/ml, and autoclaving at 121 °C for 20 min. About 15 ml of media was poured into each of sterilized petri dishe (9 cm. diameter).

To make a pure culture of the fungi, mycelium from the cadaver was transferred to PDA dishes with a sterile loop. Dishes were incubated at 25°C. The obtained cultures were identified at Plant Pathology Institute, ARC, Egypt.

II. Laboratory tests

II.1. Production of Conidia

The cultures of the obtained fungi *Alternaria infectoria* and *Beauveria bassiana* were maintained at 25 ± 1°C, 85 ± 5 % RH on PDA medium supplemented with 0.5 % yeast extract for 12-15 days to induce the growth and sporulation under these conditions.

The conidial suspensions used as inocula were obtained from 2-week old cultures. The cultures were harvested by scraping off the contents from the surface of each petri dish with a sterile scalpel and susppending in sterile distilled water with 0.01% Tween 80% in a blender for 10 seconds. The fungi suspensions were filtered through a sterile piece of cheesecloth.

The spore concentrations were determined using a Neubauer hemocytometer and subsequant appropriate dilutions were made. The viabilty of the conidia was verified before carrying out the bioassys. The fungi conidia were considered viable if the germination tube was evident and produced >95% germination.

II.2. Bioassay tests

Pathogencity of the two isolated fungi to *I. seychallarum* were assessed. Leaves of ficus seedlings were dipped into one of each fungal suspension. Then placed in a sterile petri dish lined with sterile filter paper moistened with 2ml of one of the following fungal suspensions; $2.3X10^6$, $2.3X10^5$, $2.3X10^4$, $2.3X10^3$/ml for *A. infectoria*, and $3.2X10^7$, $3.2X10^6$, $3.2X10^5$ $3.2X10^4$/ml for *B. bassiana*.

Twenty-one adults and fifty-five nymphs healthy looking of *I. seychallarum* were released on the leaves in each dish. Detached leaves dipped in sterile distilled water and placed on sterile filter papers sprinkled with 2ml of sterile distilled water in sterile petri dishes were used as control. Four replicate were used for each treatment. All petri dishes were wrapped with two layers of parafilim.

The number of dead insects were recorded each 48 h after inoculation untill 12 days. Dead insects were removed from dishes and incubated on media PDA in petri dishes for 3-5 days or until diagnosis based on fungal sporulation could be made to confirm that the deaths were caused by infection by the fungi.

The mean lethal time (LT_{50}) was assessed for the fungi and the methodology used was similar to that for the pathogenecity test. The mortality data were analyzed by probit analysis to estimate regression parameters to determine LT_{50}.

III. Field trail

Four fungal concentrations of the obtained fungi $2.3X10^6$, $2.3X10^5$, $2.3X10^4$, $2.3X10^3$/ml for *A. infectoria*, and $3.2X10^7$, $3.2X10^6$, $3.2X10^5$ $3.2X10^4$/ml for *B. bassiana*. Four branches were served as replicates. Heavily infested mulberry branches with the *I. seychellarum* were sprayed with one of the tested fungal concentration by the aid of hand sprayer. Four untreated branches were employed as control. Untreated branches were sprayed with sterile distilled water only. A pre-count was taken just before spraying at each replicate.

Treated branches were examined by a stereomicroscope and number of insects was recorded before treatment and after 7, 14, 21, and 28 days of application.

In order to confirm that the insect mortality was due to infection with the fungi, re-isolation trail was conducted. The same aforementioned steps of isolation and identification were followed.

Reduction percentage was calculated by using Henderson and Tilton (1955) formula.

IV. Side effect of certain pesticides on the isolated fungi

The susceptibility of the two isolatd fungi *A. infectoria* and *B. bassiana* to certain registered pesticides (Table 1), were determined in the laboratory by adding the pesticide to PDA, the growth medium of the fungi as described by Pachamuthu *et al.*, (1999). Just the growth of the fungi in flask become sufficiently cooled, recommended concentrations of each pesticide were added to 100 ml of media. The flasks were then hand–shaken and rolled on the clean bench to ensure the uniform mixing of pesticides with the media. Approximately 15 ml of the media amended with insecticides was poured into each petri dish and allowed to solidify at room temperature under the table top horizontal laminar flow.

Approximately 1cm. in-diameter of each of the isolated fungi was placed in the centre of each Petri dish. Then Petri dish sealed with cellotape and incubated. Untreated media was used as a check. Four replicates of each treatment and check were incubated in the dark at 25 ±2°C. The linear growth of each culture was measured after growth of each fungus completed in the control. The averages diameter of colonies (cm) and the percentage of growth rate of each fungus to the corresponding control were recorded.

TABLE (I)

The tested pesticides, their trade name, active ingredients contents and applied concentration.

Pesticides	Trade name	Active ingredient	Chemical name	Recommended concentration g or ml/100 liter water
Acaricides	Ortus	Fenpyroximate (5% SC)	*tert*-butyl(*E*)-4-[(1,3-dimethyl-5 phenoxypyrazol-4yl) methylenaminooxymethyl] benzoate	50 ml
	Vertemic	Abamectin (1.8 % EC)		40 ml
Fungicides	Copper Oxychloride	Copper oxychloride	copper chloride oxide hydrate	250 gm
	Kocid 101	77% WP		
	Sulfur	Sulfur		250 gm
	Topsin M	Thiofanat methyl (70% WP)	dimethyl [1,2-phenylenebis(iminocarbonothioyl)]bis[carbamate]	100 gm
Insecticides	Actellic	Pirmifos methyl (50% EC)	*O*-[2-(diethylamino)-6-methyl-4-pyrimidinyl] *O.O*-dimethyl phosphorothioate	375
	Cidial L	Phenthoate (50% EC)		150
	KZ oil	Mineral oil (95% EC)		1500
	Sumthion	Fenitrothion (50% EC)	O,O-dimethyl O-(3-methyl-nitrophenyl) phosphorothioate	375

RESULTS AND DISSCUSION

I. Isolated fungi from *I. seychellarum* cadavers

Data in Table (2) showed the presence of seven fungal genera infected the margarodid mealybug *I. seychellarum*. These fungi were identified as *Alternaria infectoria*, *Aspergillus alliaceus*, *Beauveria bassiana*, *Cladosporium oxysporum*, *Penicillium* sp., *Trichoderma* sp., and *Verticillium lecnii*. The occurrence of these fungi varied in each of the two surveyed Governorates, Ismalia and North Sinai. It is obvious that, *A. infectoria* was the most frequently isolated microorganisms (39.7.4%) followed by *Cladosporium oxysporum* (19.4), *B. bassiana* (16.7%), *Verticillium lecnii*

(6.9%), *Trichoderma* sp. (4.4%), *Penicillium* sp. (4.0%), *Aspergillus alliaceus* (3.6%) in Ismailia Governorate, whereas in North Sinai , *Alternaria infectoria*, *Aspergillus* sp., *Beauveria bassiana*, *Cladosporium oxysporum* were found. *Aspergillus* sp. was the most frequency, representing 33.4% from the total samples, followed by *A. infectoria* 29.0, *C. oxysporum* 20.8 and *B. bassiana* 16.8%.

In most cases, the fungus of sooty mold is saprophytic fungi, however, unidentified species in these genus have been reported to be entomopathogenic to the mealybug, *Planococcus citri* (Hemiptera, Pseudococcidae) (Samway, 1983).

The fungus *A. infectoria* is widespread in temperate region and recorded as the anamorph and/or teleomorph on bromegrass, *Bromus* L. (on dead culms), oatgrass, *Danthonia* DC (causing leaf rot), lupin, *Lupinus* L. (on twigs), bluegrass, *Poa* L and cattail weed, *Typha* L. (Simmons, 1986). The teleomorph is a plant pathogen to some of mentioned plants.

These fungi considered the first record on the Seychelles fluted mealybug, *I. seychellarum*. The two fungi *A. Infectoria*, and *B. bassaina* were selected to examine its virulence on *I. seychellarum*.

II. Pathogencity of *A. infectoria* and *B. bassiana* to *I. seychellarum*

Data in Tables (3 & 4) showed the effect of each of the tested fungi take the same trend. Using the isolated fungus *A. infectoria* at the four concentrations of $2.3X10^6$, $2.3X10^5$, $2.3X10^4$and $2.3X10^3$conidia/ml, at 2, 4, 6, 8, 10 and 12 days after treatment on adult female of *I. seychellarum* causing moratility rates ranged from 30.0 to 9.6, 37.6 to 12.9, 71.4 to 19, 81.0 to 28.6, 81.0 to 35.6 and 90.5 to 38.1 %, respectively. The mortalities on *I. seycheyllarum* nymphs ranged from 52.7 to 16.4 67.3 to 27.3, 74.5 to 30.9 81.8 to 36.4, 85.5 to 83.2 and 92.7 to 45.5 %, respectively.

On using the isolated fungus *B. bassiana* at four levels of spore concentrations of $3.2X10^7$, $3.2X10^6$, $3.2X10^5$ and $3.2X10^4$ conidia/ml, at 2, 4, 6, 8, 10 and 12 days after treatment to adult female of *I. seychellarum* causing mortality rates ranged from 28.6 to 14.3, 36.7, 52.4 to 34.4, 52.4 to 38.1, 61.9 to 38.1 and 71.4 to 42.9 %, respectively. In case of *I. seychellarum* nymphs, it ranged from 34.5 to 19.6, 49.1 to 29.1, 56.4 to 34.5 61.8 to 38.2, 63.6 to 41.9 and 86.4 to 44.2, respectively.

Mortalities among untreated adults of *I. seychellarum* were 0.0, 0.0, 0.0, 3.5, 3.5, 3.5 and 3.5, respectively, while nymphs of *I. seychellarum* were 0.0, 0.0, 2.3, 3.9, 3.9 and 3.9 %, respectively.

TABLE (II)

Frequency of the occurrence of isolated fungi from I. seychellarum in Ismailia and North Sinai Governorates.

Isolated microorganisms	Ismailia governorate																		North Sinai Governorate		
	Guava						Mango						Mulberry								
	El-Tall	Fayed	Ismailia	Quantara Gharb	Quantara Shark	Mean	El-Tall	Fayed	Ismailia	Quantara Gharb	Quantara Shark	Mean	El-Tall	Fayed	Ismailia	Quantara Gharb	Quantara Shark	Mean	General average	Guava	Mulberry	General average
Alternaria infectoria	49.4	64.7	35.7	10.0	67.4	**45.4**	25.4	8.1	34.1	44.4	64.6	**35.3**	33.3	46.5	3.4	34.1	73.7	**38.2**	**39.7**	29.4	28.6	**29.0**
Aspergillus alliaceus	0.0	0.0	0.0	0.0	0.0	**0.0**	0.0	0.0	0.0	0.0	0.0	**0.0**	0.0	0.0	55.7	0.0	0.0	**11.1**	**3.7**	36.8	30.1	**33.4**
Beauveria bassiana	12.3	11.8	28.6	0.0	11.2	**12.8**	16.9	35.5	5.7	11.1	20.2	**17.9**	16.7	23.3	31.8	4.8	5.3	**16.4**	**15.7**	14.7	18.8	**16.8**
Cladosporium roseum	13.6	0.0	35.7	0.0	21.3	**14.1**	0.0	24.2	56.8	0.0	15.2	**19.2**	50.0	30.2	9.1	14.5	21.1	**25.0**	**19.4**	19.1	22.6	**20.8**

% Frequency = No. of colonies for the microorganism /Total no. of the microorganisms X 100

TABLE (II) continued

Isolated microorganisms	Ismailia governorate																	North Sinai Governorate		
	Guava						Mango						Mulberry								
	El-Tall	Fayed	Ismailia	Quantara Gharb	Quantara Shark	Mean	El-Tall	Fayed	Ismailia	Quantara Gharb	Quantara Shark	Mean	El-Tall	Fayed	Ismailia	Quantara Gharb	Quantara Shark	Mean	General average	Guava	Mulberry	General average
Penicillium sp.	0.0	0.0	0.0	0.0	0.0	**0.0**	0.0	0.0	0.0	27.8	0.0	**5.6**	0.0	0.0	0.0	32.4	0.0	**6.5**	**4.0**	0.0	0.0	**0.0**
Trichoderma sp.	18.5	0.0	0.0	0.0	0.0	**3.7**	32.2	0.0	0.0	11.1	0.0	**8.7**	0.0	0.0	0.0	4.8	0.0	**1.0**	**4.4**	0.0	0.0	**0.0**
Verticillium lecnii	6.2	23.5	0.0	0.0	0.0	**5.9**	25.4	30.3	3.4	5.6	0.0	**12.9**	0.0	0.0	0.0	9.7	0.0	**1.9**	**6.9**	0.0	0.0	**0.0**

% Frequency = No. of colonies for the microorganism /Total no. of the microorganisms X 100

Data presented in the Tables (3 & 4), show that there is a positive correlation between the concentration of the fungal spore concentrations and the percentage of mortality. The mortality increased significantly after inoculation; it then continued to increase gradually, the highest percentage mortality was obtained after 12 days of exposure.

TABLE (III)

Effect of two fungi *A. infectoria* and *B. bassiana* against adults of *I. seychellarum*.

Fungus	Conc. (Spores/ml)	% of cumulative mortality					
		Days after treatment					
		2	4	6	8	10	12
A. infectoria	2.3×10^6	30.0a	37.6a	71.4a	81.0a	81.0a	90.5a
	2.3×10^5	27.1a	30.5a	57.1b	61.9b	71.4b	76.2b
	2.3×10^4	14.3b	14.3b	34.3c	35.7c	57.1c	61.9c
	2.3×10^3	9.5b	12.9b	19.0d	28.6c	35.2d	38.1d
L.S.D.		8.53	9.01	11.63	8.17	8.69	11.43
B. bassiana	3.2×10^7	28.6a	36.7a	52.4a	52.4a	61.9a	71.4a
	3.2×10^6	23.8ab	33.3a	47.6a	47.6a	55.0a	63.8ab
	3.2×10^5	20.1ab	28.6a	42.9ab	42.9a	52.3a	58.1b
	3.2×10^4	14.3b	25.2a	34.3b	38.1a	38.1b	42.9c
L.S.D.		9.68	14.17	10.43	13.93	9.94	11.83

The current results is contrary to that of Fransen *et al.*, (1987) who found that the efficacy of the fungus *Aschersonia aleyroids* in controlling the green house whitefly *Trialeurodes vaporariorum* (Homoptera, Aleyrodidae) decreased with the larval instars developed and the fungus was unable to kill the adults of this pest. According to Pachamuthu *et al.*, (1999), the virulence of any fungal isolate to cause mortality in insect is directly related to spore concentration.

The obtained results in Table (5) present the mean lethal time (LT_{50}) for the two evaluated fungi were calculated. LT_{50} values of *A. infectoria* insignificantly shorter than with those produced by *B. bassiana* for both nymphs and adult of *I. seychellarum*. The LT_{50} values for *A. infectoria*, at four concentrations were ranged

from 4.52 to 17.41 days for adults and form 2.62 to 19.58 days for nymphs. Whereas the LT_{50} values for *B. bassiana* ranged from 7.26 to 19.28 days and from 4.88 to 21.12 days for adult and nymphs of *I. seychellarum*, respectively.

Probit analysis of data at 95% confidence limits of LT_{50}s showed significant differences in the susceptibility of nymphs and those of adults of *I. seychellarum* to both tested fungi (Table 5).

Higher slopes values indicate a more rapid rise in mortality associated with a given increase in concentration and are correlated with more narrow fiducial limits around LT_{50}s estimates (Burges and Thompson, 1971). A correlation between an increase in conidial concentration and a decrease in LT_{50}s estimate of both entomopathogenic fungi has also been observed in the case of other insect species by other authors. Vandenberg (1996) reported decreasing LT_{50}s with increasing dose of *B. bassiana* and *Paecilomyces fumosoroseus* against Russian wheat aphid *Diuraphis noxia*.

TABLE (IV)

Effect of two fungi *A. infectoria* and *B. bassiana* against nymphs of *I. seychellarum*.

Fungus	Conc. (Spores/ml)	% of cumulative mortality					
		Days after treatment					
		2	4	6	8	10	12
A. infectoria	2.3×10^6	52.7a	67.3a	74.5a	81.8a	85.5a	92.7a
	2.3×10^5	40.0b	52.7b	61.8b	63.6b	70.9b	78.2b
	2.3×10^4	25.5c	32.7c	40.0c	45.5c	52.7c	56.7c
	2.3×10^3	16.4c	27.3c	30.9c	36.4c	38.2d	45.5d
L.S.D.		11.13	9.21	10.53	10.35	12.66	10.26
B. bassiana	3.2×10^7	34.5a	49.1a	56.4a	61.8a	63.6a	86.4a
	3.2×10^6	25.5b	41.8ab	43.6b	56.4ab	58.2ab	65.5b
	3.2×10^5	22.1b	38.2b	40.0b	49.1bc	54.5b	60.0b
	3.2×10^4	19.6b	29.1c	34.5b	38.2c	41.8c	44.2c
L.S.D.		8.45	8.54	12.6	11.03	7.80	10.81

TABLE (V)

Regression of the mortality rates on days for series of spore concentrations used in assays of two isolated fungi *A. infectoria* and *B. bassiana* against *I. seychellarum*.

Fungus	Conc. (Spores/ml)	Adults		Nymphs	
		LT50 (days) (95% Fudicial limits)	Slope ± SE	LT50 (days) (95% Fudicial limits)	Slope ± SE
A. infectoria	2.6×10^6	4.52 (3.12 – 5.69)	2.74 ± 0.59	2.62 (1.49 – 2.92)	1.64 ± 0.31
	2.6×10^5	6.28 (4.88 – 7.86)	2.18± 0.59	4.11 (3.28 – 4.85)	1.31 ± 0.30
	2.6×10^4	10.31(8.74 – 13.84)	3.28 ± 0.85	11.2 (9.72 – 13.55)	1.24 ± 0.33
	2.6×10^3	17.41 (14.44 – 24.80)	2.59 ± 1.42	19.58(14.77– 25.21)	1.25 ± 0.38
B. bassiana	3.2×10^7	7.26 (5.94 – 9.14)	1.62 ± 0.55	4.88 (2.2 – 7.32)	1.63 ± 0.31
	3.2×10^6	9.56 (7.88 – 12.72)	1.52 ± 0.58	7.67 (6.58 – 9.18)	1.44 ± 0.32
	3.2×10^5	11.24 (9.54 – 14.36)	1.63 ± 0.63	9.54 (8.39 – 13.81)	1.41 ± 0.33
	3.2×10^4	19.28 (13.85 – 28.11)	1.42± 0.71	12.21(10.62–14.75)	1.46 ± 0.36

III. Field application

The obtained data in Table (6) showed that both tested fungi affected the population of *I. seychellarum*, based on means reduction percentage of the mealybug population. the results revealed that using four spore concentrations ($2.3X10^6$, $2.3X10^5$, $2.3X10^4$ and $2.3X10^3$ conidia/ml) of *A. infectoria* causing in general mean reductions, 64.0, 50.9, 48.2, and 33.5%. Whereas, applying *B. bassiana* at four concentrations of ($3.2X10^7$, $3.2X10^6$, $3.2X10^5$ and 3.2×10^4 conidia/ml) caused 72.5, 64.7, 47.7, and 36.4% reductions. No mortalities were observed among untreated groups in all cases.

It is to be noted that, *B. bassiana* was effective and resulted in higher percentage of mortalities than that observed in case of the *A. infectoria* applications. Lower rates of the two used fungi tended to give less control of the *I. seychellarum* than higher concentrations and it gave significantly inferior and inadequate plant protection. Results indicated that, both fungi significantly reduced the number of *I. seychellarum* populations under field conditions.

No disease symptoms appeared on the inoculated leaves or twigs of guava, mango, and mulberry until 21 days after inoculation. Therefore, *A. infectoria* appears to be safe on one of the most common host plants of the target insect. However, exhaustive host-range studies need to be conducted to ensure that this biocontrol agent, if used on mealybug insects, will damage desirable insects or plants.

TABLE (VI)

Efficacy of two isolated fungi of each *A. infectoria* and *B. bassiana* under field conditions against mealybug, *I. seychellarum* infested mulberry trees.

Fungus	Conc. (spores/ml)	No. of alive insects / branche and % reduction					% Mean reduction
		pre treatment	After one week	After two week	After three week	After four week	
A. infectoria	2.6×10^6	7.5	3.1 65.4	1.7 66.3	3.1 56.4	2.3 67.7	64.0
	2.6×10^5	8.2	4.8 51.3	3.8 31.8	3.3 58.1	2.9 62.6	50.9
	2.6×10^4	7.8	5.3 43.3	3.3 37.7	3.6 51.3	2.9 60.3	48.2
	2.3×10^3	5.0	4.8 19.6	1.8 48.0	2.8 42.0	3.6 24.5	33.5
B. bassiana	3.2×10^7	9.7	3.7 68.1	1.7 74.0	2.1 77.2	2.7 70.7	72.5
	3.2×10^6	10.3	4.8 67.6	2.4 65.5	2.8 71.4	3.6 63.3	64.7
	3.2×10^5	5.5	3.1 53.0	2.1 43.3	2.6 50.2	2.9 44.1	47.7
	3.2×10^4	7.0	5.6 33.0	2.9 38.5	3.7 44.3	4.7 29.7	36.4
Control		8.2	9.8	5.5	7.8	7.8	

V. Side effect of certain pesticides on two isolated fungi

Certain pesticides commonly used in the areas under consideration are tested to clarify their effect on the growth of the fungus *A. infectoria* and *B. bassiana*.

Not as expected, data in Table (7) showed that the insecticide, Sumithion achieved a very strong inhibition effect to the radial growth of the fungus *A. infectoria*, (97.7%), followed by the fungicide, Kocid 101 caused suprior reduction (95.8%) and Cupper oxychlore (68.6%) reductions. The insecticides, Actellic,

Cidial, KZ oil have moderate reduction, causing 56.5%, 53.9%, and 53.7% growth reduction of *A. infectoria*, respectively. The tested acaricide, Ortus showed lowest effect (5.3%), whereas the acaricide, Vertemic, and the fungicides Sulfur and topsin had no effect on the fungus growth.

TABLE (VII)

Effect of certain pesticides on the growth of the two isolated fungi *A. infectoria* and *B. bassaina*.

Pesticides	Trade name	Fungus species			
		A. infectoria		*B. bassiana*	
		Mean of radial growth cm	% reduction of the control	Mean of radial growth cm	% reduction of the control
Acaricides	Ortus	8.52	5.3	9.00	0.0
	Vertemic	9.00	0.0	9.00	0.0
Fungicides	Cupper Oxychlor	2.83	68.6	0.25	97.2
	Kocid 101	0.38	95.8	2.73	69.7
	Sulfur	9.00	0.0	6.97	22.6
	Topsin	9.00	0.0	3.12	65.3
Insecticides	Actellic	3.90	56.7	4.00	55.6
	Cidial	4.15	53.9	6.07	32.6
	KZ oil	4.17	53.7	4.60	48.9
	Sumthion	0.21	97.7	0.25	97.2
Control		9.00	0.0	9.00	0.0
L.S.D		0.52		0.58	

As for the fungus *B. bassiana*, the highest effect is manifested by each of the fungicide, Cupper oxychlor and the insecticide, Sumthion (97.2 % growth reduction), followed by the fungicide Kocid 101 (69.7 %) and Topsin (65.3%). The other insecticides showed moderate effect Actellic (55.6%), followed by KZ oil (48.9%) and Cidial 32.6% reductions. The fungicide, Sulfur caused low effect (22.6%), whereas the tested acaricides, Vertmic and Ortus had no effect on the fungus.

The results of our respective experiment were largely in agreement with those obtained by El-Adawy *et al.*, (2001). It is obvious that many pesticides belonging to both fungi tested may have caused great damage to the biocontrol agents. The use of pesticides can thus lead to secondary pest problems in the field if the adverse effect of the chemical on beneficial entomopathogenic fungi is either not known or is ignored.

It can be concluded that the surveyed fungi can reduce the population of mealybugs. It is important to avoid use of harmful pesticides to these agents to serve their populations.

Our findings would ultimately provide a baseline decision-making strategy using the fungal pathogens in the integrate control program against the margarodid mealybug *I. seychellarum*.

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