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Full Length Research Paper

The *in vitro* efficacy of a Zimbabwean isolate of *Zoophthora radicans* Brefeld (Batko) in the control of Lepidoptera larvae infesting *Brassica* sp.

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The use of microbes in pest control is an important aspect of integrated pest management (IPM). A Zimbabwean isolate of the fungal entomopathogen *Zoophthora radicans* was tested for efficacy in the control of *Brassica* infesting lepidopteran larvae. *Z. radicans* caused 98.68% mortality on small (1st - 2nd instar) diamondback moth (*Plutella xylostella* L.) larvae and 21.34% mortality on large (3rd - 4th instar) diamondback moth (DBM) larvae 6 days after treatment. *Z. radicans* was not effective against larvae of *Helicoverpa armigera*, webworm (*Hellula undalis*), cabbage moth (*Crocidolomia* sp.) and cabbage looper (*Trichoplusia ni*). There was no significant difference (p > 0.05) in adult *Cotesia plutellae* emergence from cocoons treated with *Z. radicans* and cocoons sprayed with water. There was 95% emergence of *C. plutellae* adults from cocoons treated with Dimethoate 40 EC. No adults of *C. plutellae* emerged from cocoons treated with Carbaryl 75 WP, Malathion 25 WP and Malathion 50 EC. The study showed that *Z. radicans* was effective against early instar DBM larvae and did not have adverse impacts on the emergence of *C. plutellae* adults from cocoons and *Diaeretiella rapae* adults from aphid mummies.

Key words: Zimbabwe, *Zoophthora radicans*, efficacy, *in vitro*, Lepidoptera, *Brassica* spp.

INTRODUCTION

Fungal and bacterial pathogens have been successfully used for pest management with the bacterium *Bacillus thuringiensis* (*Bt*) being widely commercialized due to ease of production and effectiveness against numerous insect pests (Tanada and Kaya, 1993). The entomopathogen *Zoophthora radicans* (Brefeld) Batko belongs to the fungal division Eumycota, subdivision Zygomycotina, class Zygomycetes, order Entomophthorales (Tanada and Kaya, 1993). The fungus has not been widely exploited in the control of diamondback moth [(*Plutella xylostella* L.) (DBM)] primarily because it is susceptible to dry conditions and hence efficacy may be low under hot tropical conditions. Nonetheless, the fungus is an important regulating agent in insect populations and can spread rapidly through a population causing extensive mortality, particularly when the host insect population is high (Shepard et al., 1999; Sarfraz et al., 2005).

Zoophthora sp. infects DBM larvae, pupae and adults. It forms an extensive mat of hyphae which grows out from the host. Numerous white spores are formed on, and ejected from this mat (Shepard et al., 1999). The fungus can cause dramatic epizootics in *P. xylostella* populations, especially in moist upland areas of the tropics where infection can approach 100% in the field. *Zoophthora* sp. can also infect other lepidopteran species and cabbage aphids (Shepard et al., 1999; Walter et al., 2003). The large amount of fungal spores produced during natural epizootics in brassica fields represents a valuable resource as large quantities of diseased

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cadavers can be harvested from the field and stored for future use. This is more pertinent for a fungus such as *Zoophthora* sp. which is difficult and expensive to grow on artificial media.

The potential of various entomopathogens as biological control agents has been tested for DBM management, but few studies have demonstrated their efficacy under field conditions (Kim et al., 2002; Sarfraz et al., 2005). The study was a preliminary assessment of the efficacy of *Zoophthora* sp. in the control of several *Brassica* infesting Lepidoptera larvae and its impact on *Diaeretiella rapae* parasitoid emergence from aphid mummies and *Cotesia plutellae* parasitoid emergence from cocoons.

MATERIALS AND METHODS

Maintenance of DBM and *C. plutellae* parasitoid cultures

A densely planted stand of covo (*Brassica oleracea* var. *acephala*) was used to maintain a field culture of DBM at Africa University farm (18°53'70.3''S: 32°36'27.9''E, at 1,131 meters above sea level) from where larvae or pupae were collected when required for laboratory experiments.

In the laboratory, DBM larvae were reared according to the protocol developed by the Asian Vegetable Research and Development Centre (AVRDC, 1997) on a natural diet of covo (*Brassica oleracea* var. *acephala*) at a temperature of 24°C ± 2°C. For *C. plutellae*, cocoons were collected from the same covo crop at Africa University farm and placed in Perspex® vials and held at a room temperature of 24°C ± 2°C, and 16:8 (L:D) hour photoperiod. Adults were fed on 20% sugar/water solution in a polythene gauze cage and were used when required in laboratory experiments.

Field sampling for diseased larvae

During field scouting, records of fungal infection on DBM larvae on cabbage (*Brassica oleracea* var. *capitata*) were taken at Africa University farm. Diseased larvae were taken to the laboratory in plastic Perspex® cups and stored in a freezer at 0°C. For purposes of fungus identification, diseased larvae were viewed under a stereo-microscope to determine the growth characteristics of the fungus. Fungal spores were mounted on glass slides and viewed under a compound microscope to determine spore morphology.

Preparation of *Z. radicans* fungal spore suspension

Field collected DBM cadavers were air-dried at about 27°C. To prepare a fungal spore suspension, dried cadavers were gently macerated in a mortar using a pestle. The resultant coarse powder was mixed with sterile distilled water to form a liquid suspension. The suspension was strained through three layers of cheesecloth to remove large particles. The concentration of fungal spores was measured using a haemocytometer and was adjusted to about 1.0 X 10^6 conidia/ml. This spore concentration had been determined from other studies (Hua and Feng, 2005) to be optimum for DBM infection in laboratory studies.

Preparation of pesticide stock solutions

Pesticide stock solutions were prepared at the recommended field rates for controlling diamondback moth larvae on *Brassica* spp. Dimethoate 40 EC was used at the rate of 7.5 ml/10 litres of water, Carbaryl 85% WP at 20 g/10 litres, Malathion 25% WP at 60 g/10 litres and Malathion 50 EC at 25 ml/10 litres. Sterile distilled water was used as the control. Diamondback moth larvae and pupae, *H. armigera* larvae, cabbage looper larvae (*T. ni*), webworm larvae (*Hellula undalis*), cabbage moth larvae (*Crocidolomia* sp.) and aphid (*Brevicoryne brassicae*) colonies for the bioassays were collected from the Africa University farm and Weirmouth farm (18°59'19.9''S: 32°34'92.2''E, at 1,028 meters above sea level) in December 2008.

Bioassays

The leaf residue bioassay procedure described by Kim et al. (2002) was used. Covo leaf discs (7 cm diameter) were dipped in sterile distilled water, Carbaryl, Dimethoate, Malathion 25% WP and Malathion 50% EC stock solutions for 10 s in each concentration, held vertically using a pair of forceps to allow excess solution to drip off, and placed on a tray in a laminar hood to dry for 2 h. After the surfaces of the leaf disks were dried, each leaf disc was placed in a glass Petri dish (9 cm diameter) on top of two layers of moistened Whatman® filter paper.

In each Petri dish, individual larvae of small DBM, large DBM, cabbage looper (*T. ni*), *H. armigera*, webworm (*H. undalis*) and cabbage moth (*Crocidolomia* sp.) were placed on the leaf discs, using a clean camel hair brush. There were three replicates per treatment with ten larvae of each species per replicate. Larval mortality was recorded every 24 h for up to 6 days. The leaf area consumed by each larva was measured at the end of the experiment, using grid paper and cabbage looper equivalence (CLE) values were derived by dividing the leaf area consumed by non-parasitized test larvae (X) divided by the leaf area consumed by non-parasitized cabbage looper larvae (Greene, 1972). Covo leaf discs of 7 cm diameter with an average of twenty (20) aphids per disc were placed in Petri dishes and treated with the prepared stock solutions as described for the lepidopteran larvae.

In order to determine adult emergence of *D. rapae* from aphid mummies, DBM adults and *C. plutellae* from pesticide treated cocoons, a hand held sprayer with a hollow cone nozzle was used to spray the pesticide solutions and the spore suspension onto cocoons laid on Whatman® filter paper in glass Petri dishes. Ten cocoons were placed in each Petri dish with a total of twenty replicates per insect species.

Data analysis

All data were analyzed using Minitab® Version 15 (Minitab, 2006). Normality was tested using the Anderson-Darling test. The percentage mortality data for each pesticide was first transformed to arcsine values and subjected to one-way ANOVA. Data on leaf area consumed by larvae were analyzed for treatment effects, using ANOVA. Treatment means were compared using Tukey's honest significant difference (HSD) at the 5% probability level.

RESULTS

Small DBM larvae (1^{st} - 2^{nd} instars) were more susceptible to infection by *Zoophthora* sp. when compared to the large DBM larvae (3^{rd} - 4^{th} instars). A mortality rate of 98.68% for small larvae was recorded in comparison to a mortality rate of 21.34% for large DBM larvae (Table 1). The fungus had no effect on larvae of *H. armigera* and

Table 1. Percentage mortality of lepidopteran larvae 6 days after biocide treatment.

Insecticide	% Mortality (±SE) n=30			
	Small DBM	Large DBM	Cabbage looper	*Helicoverpa armigera*
H_2O (control)	0.0±0.0 c	0.0±0.0 c	0.0±0.0 a	0.0±0.0 a
Zoophthora	98.68±0.03 b	21.34±0.07 b	0.0±0.0 a	0.0±0.0 a
Malathion 25	100.0 a	100.0 a	100.0 b	100.0 b
Malathion 50	100.0 a	100.0 a	100.0 b	100.0 b
Dimethoate	0.0±0.0 c	0.0±0.0 c	100.0 b	100.0 b
Carbaryl	0.0±0.0 c	0.0±0.0 c	100.0 b	100.0 b

Means in a column followed by the same letter are not significantly different (Tukey's HSD, α = 0.05).

Table 2. Mean leaf area of covo (*Brassica oleracea* var. *acephala*) consumed by lepidopteran larvae 6 days after biocide treatment.

Insecticide	Mean leaf area consumed (cm^2±SE) n=30			
	Small DBM	Large DBM	Cabbage looper	*Helicoverpa armigera*
H_2O (control)	2.91±0.0 e	2.28±0.34 c	26.23±0.73 a	26.60±0.12 a
Zoophthora	0.89±0.03 c	1.90±0.0 c	26.77±0.81 a	26.47±0.12 a
Malathion 25	0.0±0.0 a	0.0±0.0 a	0.0±0.0 b	0.0±0.0 b
Malathion 50	0.0±0.0 a	0.0±0.0 a	0.0±0.0 b	0.0±0.0 b
Dimethoate	2.25±0.02 d	1.75±0.04 c	0.0±0.0 b	0.0±0.0 b
Carbaryl	0.48±0.04 b	0.82±0.02 b	0.0±0.0 b	0.0±0.0 b

Means in a column followed by the same letter are not significantly different (Tukey's HSD, α=0.05).

and cabbage looper (Table 1). The insecticides Malathion 25 WP and Malathion 50 EC resulted in 100% mortality of DBM, cabbage lopper and *H. armigera* larvae (Table 1). Dimethoate and Carbaryl were not effective against small and large DBM larvae, but both insecticides were effective against *H. armigera* larvae and cabbage looper larvae (Table 1).

Small DBM larvae treated with *Z. radicans* consumed a significantly lower ($p < 0.05$) leaf area of covo when compared to the control. There were no significant differences in the leaf area consumed by large DBM larvae, cabbage looper larvae and *H. armigera* larvae on leaf discs treated with *Z. radicans* and the control (Table 2). On leaf discs treated with Malathion 25 WP and Malathion 50 EC, larvae of all species died without consuming any leaf area (Table 2). Small DBM larvae and large DBM larvae continued to feed on leaf discs treated with Dimethoate and Carbaryl insecticides (Table 2). Larvae of cabbage looper and *H. armigera* on leaf discs treated with Dimethoate and Carbaryl died without consuming any leaf area (Table 2).

Z. radicans had no adverse impact on the time to adult emergence of the aphid parasitoid from aphid mummies, *C. plutellae* adults from DBM cocoons and DBM adults from DBM pupae (Table 3). Also, *Z. radicans* did not have a significant impact on the overall percentage emergence of the aphid parasitoids from aphid mummies, *C. plutellae* adults from cocoons and DBM adults from DBM pupae (Table 4) as these were not significantly different ($p > 0.05$) from the control.

Non-parasitized early instar larvae of the cabbage looper consumed a significantly higher ($p < 0.05$) leaf area of 27.59 cm^2 compared to the other macro-lepidoptera such as cabbage webworm, cabbage moth and *H. armigera* which consumed 13.71, 13.07 and 14.47 cm^2 respectively (Table 5). Non-parasitized neonate DBM larvae consumed an average leaf area of 2.16 cm^2 (Table 5). There was a significantly higher ($p < 0.05$) leaf area consumed by non-parasitized cabbage moth larvae compared to parasitized larvae. There was also a significantly higher ($p < 0.05$) leaf area consumed by non-parasitized *H. armigera* larvae compared to parasitized *H. armigera* larvae (Table 5). There was no significant difference in the leaf area consumed by parasitized larvae of cabbage moth, *H. armigera* and DBM (Table 5). The cabbage looper equivalence (CLE) values were 0.50 for webworm, 0.48 for cabbage moth, 0.53 for *H. armigera* and 0.08 for DBM (Table 5).

DISCUSSION

Entomopathogenic fungi may be an alternative source of insect control agents (Kim et al., 2002; Sarfraz et al., 2005). The current study showed that small DBM larvae (1^{st} - 2^{nd} instars) were more susceptible to infection by *Z.*

Table 3. Impact of biocides on time to emergence from pupae for adults of *Diaeretiella rapae, Cotesia plutellae* and DBM.

Insecticide	Mean days to emergence (±SE)		
	Aphid mummies	*Cotesia* cocoons	DBM pupae
	n=182	n=193	n=187
H_2O (control)	5.2 ± 0.13 a	5.3 ± 0.21 a	4.6 ± 0.16 a
Zoophthora radicans	5.4 ± 0.16 a	5.5 ± 0.17 a	4.9 ± 0.18 a
Malathion 25 WP	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b
Malathion 50 EC	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b
Carbaryl 85 WP	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b
Dimethoate 40 EC	0.0 ± 0.0 b	0.0 ± 0.0 b	5.7 ± 0.26 a

Means in a column followed by the same letter are not significantly different (Tukey's HSD, α = 0.05).

Table 4. Percentage emergence of adults of *Diaeretiella rapae, Cotesia plutellae* and DBM 6 days after biocide treatment of pupae.

Insecticide	% Emergence from cocoons (± SE)		
	Aphid mummies	*Cotesia* cocoons	DBM pupae
	n=182	n=193	n=187
H_2O (control)	99.6±0.14 a	98.7±0.32 a	97.0±0.35 a
Zoophthora radicans	98.3±0.37 a	98.0±0.29 a	98.3±0.19 a
Malathion 25 WP	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b
Malathion 50 EC	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b
Carbaryl 85 WP	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b
Dimethoate 40 EC	0.0±0.0 b	0.0±0.0 b	95.6±0.31 a

Means in a column followed by the same letter are not significantly different (Tukey's HSD, α=0.05).

Table 5. Mean leaf area consumed by non-parasitized neonate larvae and parasitized early instar larvae on untreated covo (*Brassica oleracea* var. *acephala*) leaf discs**.**

Pest species	Mean leaf area consumed (cm^2±SE)		
	Non-parasitized larvae	Parasitized larvae[1]	Cabbage looper
	n=30	n=15	Equivalence[2]
Hellula undalis	13.71 ± 0.49 a	-	0.5
Trichoplusia ni	27.59 ± 1.44 b	-	1.0
Crocidolomia sp.	13.07 ± 0.16 a A	1.36±0.22 a B	0.48
Helicoverpa armigera	14.47±1.75 a A	0.87±0.11 a B	0.53
Plutella xylostella	2.16±0.24 c A	0.88±0.19 a B	0.08

Means in a column followed by the same lowercase letter are not significantly different (Tukey's HSD, α=0.05).
Means in a row followed by the same uppercase letter are not significantly different (Tukey's HSD, α = 0.05).
[1] = No parasitized larvae were available for *Hellula undalis* and *Trichoplusia ni*.
[2] = The Cabbage Looper Equivalence (CLE) was derived as: Leaf area consumed by test species larva (X) divided by the leaf area consumed by the cabbage looper larva.

radicans when compared to the large DBM larvae (3rd - 4th instars). The differences in larval mortality rates could be attributable to the physical changes in the larval cuticle which hardens as the larva enters the last instar (Tanada and Kaya, 1993). The fungus had no effect on larvae of *H. armigera* and cabbage looper (*T. ni*) and this is consistent with reports by other workers (Walter et al., 2003; Shah and Pell, 2003) who noted that although *Z. radicans* is considered a species complex, strains isolated from one host species do not usually cross-infect other host species.

Small DBM larvae treated with *Z. radicans* consumed a significantly lower leaf area of covo when compared to the control. There were no significant differences in the

leaf area consumed by large DBM larvae, cabbage looper larvae and *H. armigera* larvae on leaf discs treated with *Z. radicans* compared to the control. This observation is because *Z. radicans* acts slowly against larger targets as there is need for a larger spore load to enable the fungus to invade the host and get established (Tanada and Kaya, 1993; Shah and Pell, 2003).

An insect infected by an entomophthorous fungus generally does not display any obvious signs and symptoms in early stages of infection (Hua and Feng, 2005). Only after the infection has spread within the body does the insect become sluggish or display a nervous restlessness and in many cases it stops feeding. The period from infection to death of an insect may be as short as 3 days to as long as 12 days depending on the insect species and size, with most deaths occurring at 5 to 8 days (Tanada and Kaya, 1993; Shah and Pell, 2003; Hua and Feng, 2005).

Z. radicans did not have adverse effects on the emergence of adult aphid parasitoids from mummies, *C. plutellae* adults from cocoons and DBM adults from DBM pupae. The lack of activity of the fungus on the pupal stages can be attributed to the protective effect of the cocoon as well as unsuitable environmental conditions, particularly humidity and temperature which are important in infection and sporulation of *Z. radicans* (Tanada and Kaya, 1993).

The establishment of baseline susceptibility of pest species to insecticides provides vital information required to assess the development of resistance over time and space (Akol et al., 2002). Both small DBM larvae and large DBM larvae continued to feed on leaf discs treated with Dimethoate and Carbaryl, but both insecticides were effective against *H. armigera* larvae and cabbage looper larvae. It is not clear as to whether the ineffectiveness of Dimethoate and Carbaryl against DBM larvae was a result of resistance or because the insecticides had no effect on DBM physiology. Dimethoate suppressed the emergence of *C. plutellae* adults from cocoons as well as the cabbage aphid parasitoid from mummies**.**

Outbreaks of *Z. radicans* invariably follow periods of prolonged soft rains. Fungal epizootics depend upon the presence of suitable weather conditions and high host density (Kfir, 2003). Optimum temperatures for development, pathogenicity and survival for *Z. radicans* fall between 20 and 30°C. High humidity (> 90% RH) is required for spore germination and sporulation outside the host and such conditions are usually met during the peak of the rainy season in tropical areas (Shah and Pell, 2003). Field collections of the fungus were recorded in December 2007 and December 2008 only on cabbage at the peak of the rainy season. It is probable that because cabbage has a “closed” canopy, moist humid conditions inside the canopy micro-environment are conducive for fungal proliferation.

C. plutellae is a koinobiont endoparasitoid which lays eggs within a host that continues to grow and be mobile (Wharton, 1993). This was demonstrated in the bioassays where DBM larvae parasitized by *C. plutellae* continued to feed although the leaf area consumed was significantly less than the leaf area consumed by non-parasitized DBM larvae. In the leaf feeding bioassays there was a significant difference in the leaf area consumed by non-parasitized cabbage moth larvae compared to parasitized larvae. There was also a significant difference in the leaf area consumed by non-parasitized *H. armigera* larvae compared to parasitized *H. armigera* larvae. The fact that there was no significant difference in the leaf area consumed by parasitized larvae of cabbage moth, parasitized larvae of *H. armigera* and parasitized DBM larvae could be an indication that the three lepidopteran species are parasitized at the same larval body size. Diamondback moth larvae fed on a leaf area corresponding to a Cabbage Looper Equivalence (CLE) of 0.08. This is consistent with the 0.1 Cabbage Looper Equivalent (CLE) threshold established by Greene (1972) and used by Smith (2003) and Khan et al. (2004) in South Carolina as the economic threshold (ET) for DBM on collard greens.

The study showed that *Z. radicans* was effective against early instar DBM larvae. It was also shown that *Z. radicans* did not have adverse impacts on the emergence of *C. plutellae* adults from cocoons and *D. rapae* adults from mummies. This is an important consideration in an integrated pest management program where both parasitoids and entomopathogens may be used simultaneously.

Despite the encouraging laboratory results, there are still many hurdles in the full scale use of *Z. radicans* in open fields. The mass production of *Z. radicans* propagules is an expensive process that requires heavy financial investment (Shah and Pell, 2003; Hua and Feng, 2005). For the time being, the promise of using *Z. radicans* lies in natural epizootics that occur following periods of prolonged soft rain as observed by Kfir (2003). Perhaps there is need for brassica growers to ensure that fungicides used to control brassica diseases are not detrimental to epizootic spread of *Z. radicans* in DBM populations.

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