SINET: Ethiop. J. Sci., 44(1):13–26, 2021 ISSN: 0379–2897 (PRINT)
 eISSN: 2520–7997

DOI: https://dx.doi.org/10.4314/sinet.v44i1.2

Management of Cabbage Aphid (*Brevicoryne brassicae* L. (Homoptera: Aphididae)) on Ethiopian Mustard (*Brassica carinata* Braun) using Entomopathogenic Fungi and Selected Insecticides

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ABSTRACT: Cabbage aphid (*Brevicoryne brassicae L.*) is an important sucking insect pest of cabbage and other vegetables. It can be controlled using continuous chemical insecticides that cause human health and environmental problems. The present study was, therefore, designed to evaluate antagonistic activities of indigenous entomopathogenic fungi together with selective insecticides within the context of integrated pest management (IPM). To this effect, four strains of entomopathogenic fungi from *Beauveria bassiana* and *Metarhizium anisopliae* were tested for their antagonistic activities against the cabbage aphid. The result showed that microbial inoculums of 1×10^7 and 1×10^8 conidia mL^{-1} showed high mortality (66.7-100%) of aphids after 6 days of incubation under laboratory condition. Among them, BEI1 (*B. bassiana*) isolate was the most virulent strain on adult aphids and nymphs showing 67 to 100% and 39-72% mortality after 4^{th}-5^{th} days of treatments. The data also indicated that the fungal isolates were compatible to the three insecticides with 70% - 91% conidial germination by M. anisopliae and 68%-98% conidial germination by *B. bassiana* after 24 hours of treatment of which Karate was the most compatible insecticide to the isolates. The single treatment with the entomopathogens gave a substantial percentage mortality of insect pests after 11 days compared to (80-100%) mortality obtained from a combined treatment with half recommended dose of Karate in seven days, which was similar to the treatment with full dose of the insecticide control. The treatments were slightly more effective on adults than they were on aphid larvae. It is interesting to note that the combination of BEI1 + Ka induced 100% mortality of adult aphids after seven days of incubation compared to the 11 day incubation required to kill the nymphs. Given that the full dose of karate killed the adult aphids in five days, the 100% mortality of the half dose of karate with BEI1 within seven days was a good indication that the IPM could reduce pollution problem. Thus, *B. bassiana* and *M. anisopliae* with Karate could be further tested in the field to realize their potential as bioinsecticides for integrated pest management to control mustard aphids.

Key words/ phrases: Compatibility; Germination; Mortality; Virulence

INTRODUCTION

The Brassica (*Brassicaceae*) family includes cruciferous vegetables like cabbage, broccoli, cauliflower, and mustards. It also includes the oil seed crops rapeseed and canola (Burel *et al*., 2000). A huge number of insect pests are known to attack brassicas (Sibanda *et al*., 2000) and reduce growth and crop yield of brassicas (Kanrar *et al*., 2002). The cabbage aphid (*Brevicoryne brassicae* L.) is one of the important insect pests of brassicas that can cause direct damage by piercing and sucking sap from the plant and expose them to secondary infection by fungal, bacterial and viral pathogens (Munthali and Tshegofatso, 2014).

The advent of chemical insecticides in the mid twentieth century created the concept that insect crop pests could be eliminated that led to the development of more than 32 insecticides to control them (Jaronski, 2010). However, continuous application of chemical insecticides accumulates high levels of toxic residues in the environment leading to consumer's health risk, contamination of the environment, destruction of non-target soil organisms (Aziz *et al.*, 2013). In addition, extensive and indiscriminate uses of chemical insecticides have resulted in the selection of resistant pests. The phytotoxicity, secondary

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pest outbreaks, and pesticide resistance as a result of the continuous use of chemicals necessitate different options to reduce chemicals and produce organic vegetable products with no or little chemical residues (Hale and Elliott, 2003). Thus, an integrated approach of insect pest management (IPM) through biological control, chemical control and cultural practices is well recommended (Ren *et al*., 2010).

Biological control has been given attention for pest management that focuses on the reduction of pest populations using natural enemies or beneficial species. However, effective biological control often requires a good understanding of the biology of the pest and its natural enemies (Michaud, *et al*., 2008). Although different microbial groups are implicated with biological control agents' insect pests, the most important ones are the entomopathogenic fungi (EMF). These fungi comprise a diverse group of over 90 genera, with approximately 750 species, reported from different insects (Rai *et al*., 2014). They possess the special features such as effectiveness to decimate insect pests (high virulence), their host specificity to the target pest and are ecologically non-disruptive that are required for implementation of IPM system (Loc *et al*., 2010). Some of the entomopathogenic fungi (*Beauveria bassiana* and *Metarhizium anisopliae*) have a very broad insect host range capable of producing high concentration of aerial conidia to control insect pest of crops (Yonas Chekol *et al*., 2017 a & b).

Ethiopian mustard (*Brassica carinata A Braun*) is a major horticultural crop believed to have originated from the Ethiopian highlands (Nigussie Alemayehu and Becker, 2002). Mustard production is mainly constrained by different insect pests **(**Ujjan and Shahzad, 2012). *Brevicoryne brassicae* (L.) (Cabbage aphid) is one of the most important insects that attack brassica leafy vegetables Munthali and Tshegofatso, 2014). Therefore, this study was initiated to design the effect of selected local entomopathogenic fungi alone, or together with some insecticides, on adult and nymphal stages of cabbage Aphid (*Brevicoryne brassicae* L.**)** attacking Ethiopian Mustard (*Brassica carinata* A. Braun**)** under laboratory and greenhouse conditions.

MATERIAL AND METHODS

Cultivation of entomopathogenic fungi

Pure cultures of the entomopathogenic fungi (*Beauveria bassiana* and *Metarhizium anisopliae*) were obtained from the Mycology Laboratory, Department of Microbial, Cellular and Molecular Biology, Addis Ababa University. The fungal isolates were *Beauveria* (BEI1, BEI2, MEI1 & *Metarhizium* (MEI2), and reactivated on Potato Dextrose Agar (PDA) slants containing chloramphenicol (25 mg/L).

Determination of spore concentration of entomopathogenic fungal isolates

The test entomopathogens were grown on PDA for 3-4 weeks from which 10 and 30g of conidia were harvested in 100 and 300 ml distilled water (H_2O), respectively and supplemented with 0.01 % Triton X-100 in sterile 500 ml Erlenmeyer flask. The conidia suspensions were filtered through sterile cheesecloth and their concentrations were adjusted to $1x10^7$ spores/ml and 1×10^8 conidia/ml using Neubauer Haemocytometer under a compound microscope (400x magnifications) and the subsequent doses were prepared for laboratory and greenhouse bioassay experiments.

Conidia viability tests of entomopathogenic fungal isolates

Conidial viability was assessed according to Inglis *et al*. (2012) by spread-plating 0.1 ml of spore suspension on Potato-Dextrose Agar (PDA) plates with three replications. The plates were sealed with parafilm membrane and incubated, at 25°C under complete darkness for 18 hrs and treated with Lactophenol cotton blue stain to terminate germination and to easily count and observe the germinating spores and the germination rate of the first 200 conidia counted at 400 magnifications. Conidia were considered germinated when the

germ tubes were at least twice as long as the conidia.

In vitro compatibility of entomopathogenic fungal isolates with insecticides

The insecticides were selected based on their frequent use by vegetable grower farmers and recommended by the producing companies as described in Table 1. The test concentrations for all insecticides were according to the field rate (FR), 0.75 FR and 0.5× FR (FR-50%) recommended by the manufacturer's instruction (Dow Agro Sciences, 2008; Syngenta E.A.LTD, Syngenta Crop protection AG). The concentration and volume of insecticides tested for each product was calculated based on the spray volume rate of 100 L ha^{-1} recommended for field compatibility tests (Gurulingappa *et al*., 2011).

The effects of insecticides on conidial germination were tested on conidia extracted from thirty-days-old PDA culture suspended with sterile distilled water containing 0.01% Triton x-100 and adjusted to 1x 10^8 conidia/ml. Each suspension was added to 9 ml of each of the 3 test insecticides sufficient to make a final volume of 10 mL per concentration (FR, 0.75 FR and 0.5 FR) in three replications using sterile water as control. Then, 100µl of each suspension was transferred to PDA and incubated at 25°C for 24 hrs. The germination was assessed as before and the percent of spore germination was calculated using formula given below:

$$\text{Percent spore germination} = \frac{\text{Number of spores germinated}}{\text{Total number of spores examined}} \times 100$$

Bioassay of entomopathogenic fungi on cabbage Aphids

The efficacy test of each entomopathogenic fungus on nymphs and adult aphids was undertaken on fresh leaves of *Brassica carinata* that were surface sterilized with 5% sodium hypochlorite for 30 seconds and then rinsed with sterilized distilled water. The leaves were placed on Petri plate lined with Whatman filter papers moistened with distilled water. Nymphs and adult aphids were brought to the laboratory with their host plant leaves for test. Every three days, the old leaves were replaced with fresh leaves using the same procedure.

The experiment was run with CRD (Completely Randomized Design) with five treatment, (four treatment with two conidial concentrations (1×10^5 and 1×10^8conidia/ml) from each entomopathogen (BEI1,BEI2, MEI1 and MEI2 and 0.01 per cent X-T 100 solution served as control). In two separate experiments, 10 ml of each spore suspensions of *B. bassiana* isolate BEI1 and BEI2 and *M. anisopliae* isolate MEI1 and MEI2 were sprayed directly on 6 adults and nymphs placed on each Petri plate in three replicates. A total of 108 adult and nymph aphids were used in each treatment including the controls. The number of live, dead adults and nymphs and aphids were assessed after 24, 48, 72, 96, 120 and 144 hours of application following the method of Kivett (2015).

Table 1. Insecticides specification and information written on package of the product used for experimental trails.

Tread name	Formula	Chemical group	Common name	Concentration of active ingredient	Recommended Dose
Actara, Syngenta	25WDG	Neonicotinoid	Thiamethoxam	250 g/kg WG	20 g/ha
Karate	5EC	Pyrethroid	L-cyhalothrin	50 g/l	50 ml/ha
Radiant®	12Sc	Spinosyn	Spinetoram	120g/l	100 ml/ha

Formulation types: Emulsifiable concentrate (EC), suspension concentrate (SC), water dispersible granules (WDG), The recommended rate of application for each insecticide was 100 L ha^{-1} of water suspension for Brassica

Bioassay of entomopathogenic fungi on cabbage Aphids under greenhouse condition

Experimental design and Treatments

Ten treatments with three replicates were laid out in Completely Randomized Block Design (CRBD) under the greenhouse conditions. The treatments comprised; four treatments with entomopathogens alone (BEI1, BEI2, MEI1 and MEI2), four treatments entomopathogens combined with half of recommended Karate field rate, one treatment (Positive control karate with recommended field rate and the control with 0.01 triton X100 suspension without biocontrol agent (Table 2).

Table 2. The experimental treatments of entomopathogenic fungi (1×10^8 conidia/ml) and karate on nymph and adult Aphids for 11 days under greenhouse conditions.

Treatment No	Treatments	Test Aphid insect
T1	BEI1	Adult and Nymph
T2	BEI2	
T3	MEI1	
T4	MEI2	
T5	BEI1 + 0.5FR Karate	
T6	BEI2 + 0.5FR Karate	
T7	MEI1+ 0.5FR Karate	
T8	MEI2+ 0.5FR Karate	
T9	FR Karate (+ve control)	
T10	X-T 100 solution (-ve control	

NB: - BEI1= Beauveria isolate-1, BEI2= Beauveria isolate-2, MEI1 Metarhizium isolate-1, MEI2 Metarhizium isolate-2 and 0.5FR = half field rate.

Fig.1. Cultivation of Ethiopian mustard (*Brassica carinata* A. Braun) under greenhouse experiment.

Efficacy test of entomopathogenic fungi under greenhouse conditions

Seeds of *Brassica carinata* were collected from Addis Ababa local market. Five seeds were planted on plastic pots with (26x18 x 24) cm height, width and diameter containing a soil mixture of loam and sand (2:1). The seedlings were subsequently thinned to two plants per pot after fifteen days of planting, watered three times a week, and kept in the greenhouse outside the cage until they reached sufficient height (40 cm tall) (Fig.1).

There were three benches (blocks) each measuring 130, 150 and 50 cm (L, H, W). The two blocks each divided into 4 plots, where 4 treatment combinations and the third one was divided in to two plots with 2 treatment combinations and 10 unit plots altogether. Each replication consisted of a pot with two plants. Pots (40 cm diameter) which were placed inside large cages, at 25 ±2°C, while the water was supplied to the plants three times a week through the zip hole at the front of cages (Fig.2). Each plant was artificially infested with adult and nymphs of aphids (inoculums) from rearing Ethiopian mustard plants, and 30 healthy adults and nymphs' aphids were infested per each mustard seedling. Totally, 180 adults and nymphs' aphids were used for each treatment. Then adult and nymphs of aphids were seeded on each plant for five days before the first conidial concentration

application was made (Kivett, 2015). Both treated and non-treated leaves of each plant were counted to determine if aphids migrated away from the original sites.

Then conidial concentration at the inoculum size of 10^8 conidia/ml (100ml/pot was sprayed on the nymphs and adult aphids seeded on each plant in a closed net cage, and aphids sprayed with 0.01 percent X-T 100 solution served as control with 3 replications. The spray was performed during late evening time. To prevent carry over effect among fungal isolates, the potter tower was cleaned with 70% ethanol and sterile distilled water between spraying sessions. Thus, mortality of aphids was recorded separately at 3rd, 5th, 7th, 9th and 11th of days.

Fig. 2. A representative experimental cage loaded with potted *Brassica carinata* seedling.

Bioassay of single and combined inoculation of entomopathogens against nymph and adult Aphids on mustard plants in pot culture

The experiment was run under five treatments against control treatment (Table 3). The formulations of the karate were diluted with tap water based on the spray volume rate of 100 L ha^{-1}(Silva *et al*., 2013). The combination treatments were done in a sequential treatments approach, in which Karate followed by entomopathogenic fungal isolates. The concentrations employed were based on their field recommendations rate (FR) and 0.5×FR with one respective concentration of all the fungal isolates (1×10^8 conidia/ml) prepared for both nymphs and adult stages of aphids (Malekan *et al*., 2012). After two days of inoculation, the leaves were sprayed with the half field recommendations rate of the insecticides sequentially with fungal concentration; while the full field recommendations rate (positive control as standard check) were sprayed alone without fungal concentration from a 20 cm distances.. The fungal suspensions were sprayed on the insect infested mustard one day after exposure to half field recommendations rate of the insecticide karate. Aphids sprayed with 0.01 per cent X-T 100 solution served as a negative control.

To prevent carry over effect among fungal isolates, the potter tower was cleaned as before between spraying sessions. Mortality of aphids was recorded separately, at 3rd, 5th, 7th, 9th and 11th of days. Dead aphids were taken out of the cages and incubated under a high moisture environment with moistened Whatman filter paper and, when the cadavers were subsequently colonized by the fungal isolates, these insects were considered dead from mycosis. Retrieved mycelia were cultured on PDA and were identified. Mortality of insects was analyzed at day 6 and 11 of the laboratory and greenhouse experiments, respectively.

Data analysis

One-way analysis of variance (ANOVA) was conducted on the mortality data to test the level of significance of the difference in response between the treatments and comparison of means. Multiple comparisons were used to determine significant differences between the treatments (Turkey test). Values were computed by using statistical computer program of Statistical Package of Social Sciences (SPSS version 20 software). Percent growth inhibition of each fungal isolate over untreated check was worked out for the tested insecticides and ANOVA was used to analyze germination measurements followed by comparison of means

of total growth using the statistical program SAS version 9.3. Mean separation was calculated using the LSD test value when the F-test was significant at p = 0.05%.

RESULTS

Characteristics of entomopathogenic fungal species

The isolates BEI1 and BEI2 showed white to cream color, flat powdery and cotton like appearance on the front side of the PDA medium characteristics of *B. bassiana* (Fig.3). Likewise, MEI1 and MEI2 displayed dark herbage green or yellowish green, olivaceous colonies on the front side of PDA after 7 days of incubation similar to the cultural characteristics of *Metarhizium anisopliae* (Fig. 4). The conidia of *B. bassiana* and *M.anisopliae* were small round and varied in width and length between isolates (Fig.5 & 6). The isolates of both species showed variations in conidial length x width (Table 3). The conidial viability of the fungal isolates was in the range of 97-100% without showing significant difference among one another.

Figure 3. Isolates of *Beauveria* on PDA medium grow, at 25°C for 14-21- days.

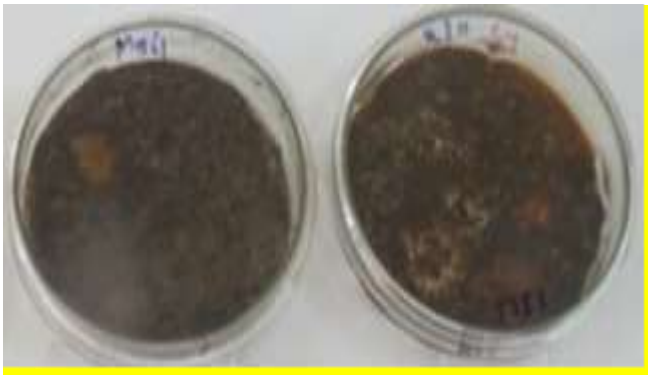

Fig. 4. Isolates of *Metarhizium* on PDA medium grow, at 25°C for 14-21-days.

Table 3. Macroscopic and microscopic characters of *B. bassiana and M. anisopliae* isolates

Isolates	Surface colour	Spore shape	Spore size
BEI1	White	round	6 - 8.5 x 7.5- 9 µm
BEI2	White	round	6.5-7 x 5.5 -8.5 µm
MEI1	Greenish	Cylindrical	7.5-16.5 x 4.5-8.5 µm
MEI2	Greenish	Cylindrical	6.5-15.3 x 5.4-9.5 µm

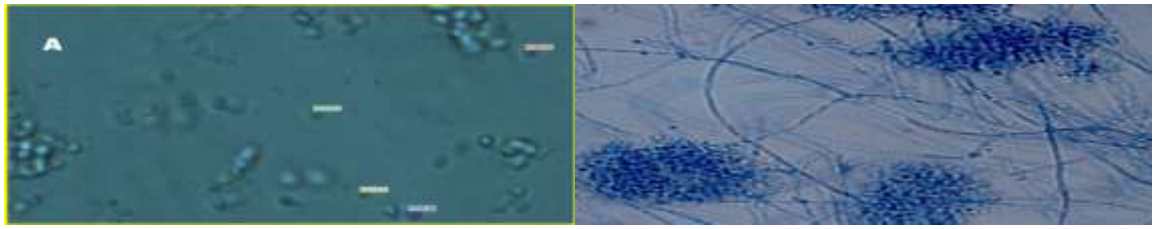

Fig. 5. (A) Spore and (B) Mycelial structures of *Beauveria* isolates observed at 400 X of compound microscope

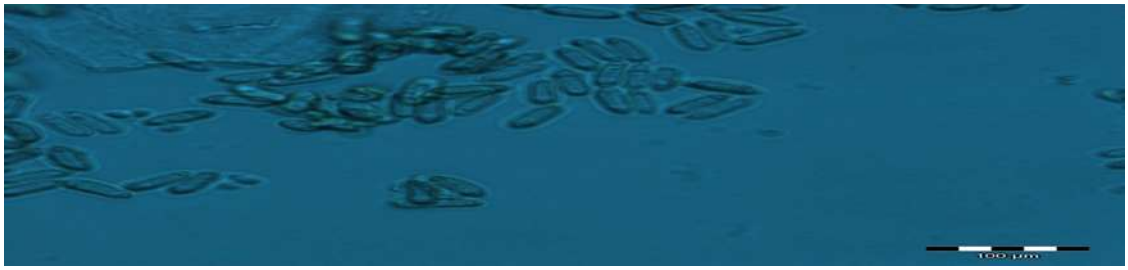

Figure 6. (A) Spore and (B) Mycelial structures of *Metarhizium* isolates observed at 400 X of compound microscope.

Percent mortality of adult aphids treated with B. bassiana and M. anisopliae under laboratory condition

The *Metarhizium* and *Beauveria* isolates did not induce substantial death on the fourth day of incubation, except with the treatments of MEI1 and BEI1 at a dose of $1x10^8$ (Table 4). However, they showed a significant increase (at $p<0.008$) in percent mortality of the adult aphids ranging from 50-100% after 5 and 6 days of incubation irrespective of their inoculums size (dose treatment).The data also showed that *Beauveria* isolates were more effective with an average mortality of 78% in less time than 69% mortality with *Metarhizium* isolates on the adult aphids after five days of treatment (Table 4). However, the overall performance of the two groups did not show significant difference after six days of treatment.

Percentage mortality of nymph aphids treated with B. bassiana and M. anisopliae isolates at different concentrations

The treatment of the nymphal aphids with 10^7 and 10^8 conidial concentrations of the entomopathogens showed variations in percentage mortality ranging from 55.6% up to 100% after 5-6 days of incubation except MEI2 ($P=0.0001$) (Table 5). The three isolates showed better, but not significant mortality, of the nymphs between the two dose treatments. The *Beauveria* isolates (BEI, and BE2) showed better performance (100% mortality) than the *Metarhizium* isolates (83% mortality with the highest dose (10^8) and longest (six days) treatment. Generally, the mortality pattern against adult and nymphal aphids showed very slow mortality until day 4 with a substantial increase in nymph mortality between 4-6 days.

Table 4. Dose dependent percentage mortality of adult aphids treated with different entomo-pathogenic fungal isolates after 3-6 days of incubation time

Isolates	Treatment	Day3	Day4	Day5	Day6
	Conidia ml-1	M (%)	M (%)	M (%)	M (%)
BEI1	10^7	16.7^{ab}	33.3^{ab}	72.2^{ab}	94.5^{a}
	10^8	38.9^{a}	66.7^{a}	100 ^{a}	-
BEI2	10^7	11.1^{ab}	27.8^{ab}	61.3^{ab}	83.3^{a}
	10^8	16.7±^{ab}	44.5^{ab}	77.8^{ab}	94.5^{a}
MEI1	10^7	22.2^{ab}	38.9^{ab}	61.1^{ab}	77.8^{a}
	10^8	27.8^{ab}	50.0^{ab}	83.4^{ab}	94.5^{a}
MEI2	10^7	5.6^{bc}	16.7^{bc}	55.6^{bc}	83.3^{a}
	10^8	16.7 ^{ab}	44.5^{ab}	77.0^{ab}	94.5^{a}
Control	None	Nd	Nd	Nd	5.6^{b}
P value		0.008	0.001	0.001	0.001

Adult aphids BEI1 (108=66.7-100; BE12 (45--78); Nd=not detected
Means followed by the same letter with in a column are not significantly different (Tukey's HSD test, p< 0.05%), Legend: M (%) = Percentage mortality

Table.5. Dose dependent percentage mortality of nymph aphids treated with different entomopathogenic fungal isolates upon 3-6 days of incubation

Isolates	Treatment	Day3	Day4	Day5	Day6
	Conidia ml-1	M (%)	M (%)	M (%) ± SE	M (%) ± SE
BEI1	10^7	11.1^{ab}	38.9^{a}	77.8^{a}	88.9^{ab}
	10^8	14.7^{ab}	39.2±^{a}	72.2^{ab}	100.0 ^{a}
BEI2	10^7	16.8^{ab}	27.8^{ab}	55.6^{ab}	88.9^{ab}
	10^8	13.5^{ab}	38.9^{a}	72.2^{ab}	100.0^{a}
MEI1	10^7	16.7^{ab}	27.8^{ab}	55.6^{ab}	72.2^{ab}
	10^8	27.8^{a}	38.9^{a}	66.7^{ab}	82.3^{ab}
MEI2	10^7	Nd	5.6^{bc}	36.9^{bc}	66.7^{ab}
	10^8	5.6^{bc}	22.2^{ab}	38.9^{bc}	83.3^{ab}
Control	None	Nd^{bc}	Nd	Nd	Nd
P value		0.031	0.009	0.0001	0.0001

Means followed by the same letter with in a column are not significantly different (Tukey's HSD test, p< 0.05%), Legend - M (%) = Percentage mortality; Nd= not detected

Compatibility test of insecticides with entomopathogenic fungal isolates

All the insecticides showed different level of inhibition on conidial germination of *B. bassiana* and *M. anisopliae* isolates to the recommended doses of the three insecticides FR, 0.5× FR and 0.75× FR) after 24 hours of incubation (Table 6). The isolates showed resistance to the insecticides ranging from 70% -86% viability treated with Karate; 71%-92% viability with Actara; and 81%-98% with Radiant (Table 6).

The overall mean viability of the different groups (*Beauveria* and *Metarhizium* isolates) treated with different concentrations of the recommended insecticides showed 79-91% with (*Beauveria*) and 78-86% with *Metarhizium*) without showing significant difference between the groups. However, *Beauveria* isolates showed slightly higher (92%) germination potential (resistance) than the *Metarhizium* isolates with mean germination of 86%. This indicates that all isolates were relatively resistant to radiant at full dose, but showed variations with dose dependent treatment of the other two insecticides. Among the isolates, BEI2 displayed the highest resistance to all insecticides; followed by MEI1 and MEI2 resistant to full dose of Actra and radiant; and Karte and Radiant, respectively. Isolate BEI1 was the most sensitive with reduced viability when treated with full dose of the insecticides, except Radiant.

Table.6. Effect of insecticides (compatibility test) at different concentration on conidial germination of entomopathogens tested on PDA and incubated at 25°C for 24 hrs.

Fungal isolates	Pesticide concentration	Karte	Actara	Radiant	Mean
		G (%)	G (%)	G (%)	
BEI1	0.5FR	86^{bc}	92^{abc}	98^{abc}	92
	0.75FR	77^{cde}	88^{bcd}	94^{abcd}	86
	FR	68e	74^{ef}	89^{bcdef}	77
BEI2	0.5FR	84^{cd}	84^{abc}	92^{abcde}	87
	0.75FR	77^{cde}	79^{def}	86^{cdef}	81
	FR	79^{cde}	77^{def}	85^{def}	80
Mean		**79%**	**82%**	**91%**	84%
MEI1	0.5FR	85^{bc}	87^{bcd}	91^{abcde}	88
	0.75FR	85^{bc}	82^{cde}	85^{def}	84
	FR	75^{cde}	79^{def}	81^{f}	78
MEI2	0.5FR	78^{cde}	84^{cde}	87^{cdef}	83
	0.75FR	73de	78^{cde}	85^{ef}	79
	FR	70^{e}	71^{f}	84^{def}	75
Mean		**78%**	**80%**	**86%**	
Control	None	100^{a}	100^{a}	100^{a}	
LSD		11.1	9.5	10.2	
		937	975	1057	

All the means within a column followed by the same letter are not significantly different at (p=0.05). Legend -FR, 0.75FR and 0.5FR implies field rate, three- forth field rate and half field rates and LSD- Least significant difference, G (%) - Percentage germination, FR= Field Rate

Experiments under Greenhouse Condition

The effect of single and combined effects of entomopathogenic fungal isolates with insecticides on nymph stages of potted plants

Percentage mortality of the nymph aphids treated with the antagonistic fungi alone and selected insecticides showed significant (P=0.001) difference among the treatments (Table7). The data showed that the single treatment with entomopathogenic fungi isolates did not show significant reduction of the nymph stage aphids until the 9th day of treatment (Table 7). Thereafter, most of the isolates induced percentage mortality ranging from 50% to 90% within 9 and 11 days. The most effective isolate BEI1 inflicted more than 90% death on the larvae followed by BEI1 and MEI1 with 88% and 84% mortality respectively.

All the dual treatments of the insecticides and the entomopathogens (IPM) effectively reduced the larval aphid pests beginning from five days of treatment with a substantial mortality rate of 50-60% (Table 7). There was a drastic increase in mortality of 80-95% in seven days, compared to the 100% mortality treated with full dose chemical treatment with Radiant at the same incubation time. This indicates that the IPM treatment with larval stage of entomopathogens was slightly but not significantly lower than the full dose of recommended karate concentration upon the same treatment time. The combined treatment with *Beauveria* isolates showed a rapid death within a relatively short time (7 days) compared to the longer time (9-11 days) required by *Metarhizium* isolates.

The effect of single and combined inoculation of entomopathogenic fungal isolates with insecticide on adult stages of aphids on potted plants

The antagonistic effect of fungal isolates on adult aphids alone or in combination showed variation in their effectiveness to kill the adult aphis (Table 8). In general, single inoculation of all the isolates showed a substantial mortality of 43-81% after seven days of incubation (Table 8). After 7 days of exposure, the IPM treatments showed mortality rates ranging from 91.6% with treatment MEI2 +0.5FRka to 100% mortality with BEI1+ 0.5FRka (Table 8) comparable with standard chemical (Karate) treatment FR (100). In general, the pattern showed combined treatment (IPM treatment) reduced the time required to kill both the adult and the nymph aphids, and the entomopathogens were equally effective after a prolonged treatment time.

Table. 7. The combined and single efficacy tests of 4 isolates with inoculums dose of 1× 10^8conidia/ml and insecticides at 50% field recommended doses against nymphs of aphids pests on Ethiopian mustered in a pot experiment.

Isolate	Treatment	Day 3	Day 5	Day 7	Day 9	Day 11
	Conidia ml^{-1}	M (%)	M (%)	M (%)	M (%) ± SE	M (%)
BEI1	10^8	10^{c}	23.3^{d}	41^{d}	59.4^{b}	93.9^{a}
BEI2	10^8	6.7^{c}	18.9^{d}	35^{d}	62.8^{b}	88.2^{bc}
MEI1	10^8	5.6^{c}	14^{d}	27^{de}	50^{b}	84.4^{cd}
MEI2	10^8	1^{cd}	7^{de}	16.7^{ef}	33.9^{bc}	72.8^{e}
BEI1 + Ka	10^8+0.5FR Ka	30^{b}	64^{b}	95^{a}	93.5^{a}	100^{a}
BEI2 + Ka	10^8 + 0.5FR Ka	27^{b}	60.6^{b}	91.8^{ab}	95.2^{a}	100^{a}
MEI1 + Ka	10^8 + 0.5FR Ka	25^{b}	56.7^{b}	86.7^{ab}	97.2^{a}	100^{a}
MEI2 + Ka	10^8 + 0.5FR Ka	21^{b}	50.8^{bc}	80.5^{bc}	97.5^{a}	100^{a}
+ve control	FRKa	45^{a}	92^{a}	100^{a}	-	-
-ve control	Water	Nd	Nd	Nd	Nd	Nd
P value		0.0001	0.0001	0.0001	0.0001	0.0001

Means followed by the same letter with in a column are not significantly different (Tukey's HSD test, p< 0.05%), Legend- M (%) = Percentage mortality, FR Ka = Field rate karate

Generally, it is interesting to note that the combination of BEI1 + Ka induced 100% mortality of adult aphids after seven days incubation compared with eleven days of incubation required to kill all the nymphal aphids (Tables 6 and 7). Given that the full dose of karate killed the adult aphids in five days, the 100% mortality of the half dose of karate with BEI1 within seven days was a good indication that the IPM controls the aphids without excess use of the insecticide that could reduce pollution problem. The same pattern of killing the aphid larva was recorded in 7 and 11 days of the treatments respectively (Fig 7 and 8).

Table. 8. The combined and singular efficacy of 4 isolates of fungi at 1×10^{8}conidia/ml and insecticides at 50% field recommended doses against adult of aphids on potted Ethiopian mustered plants

Isolate	Treatment	Day 3	Day 5	Day 7	Day 9	Day 11
	Conidia ml^{-1}	M (%)	M (%)	M (%)	M (%)	M (%)
BEI1	10^8	11^c	29^d	49^b	81^b	97.5^a
BEI2	10^8	9^{cd}	23^d	41^b	69^c	92.2^b
MEI1	10^8	7.8^{cd}	19^{ed}	33^{bc}	57.8^d	85^c
MEI2	10^8	2.8^{cd}	10^{ef}	22^d	43^e	80^d
BEI1 + Ka	10^8+0.5FR Ka	36^b	74^b	100^a	-	-
BEI2 + Ka	10^8 + 0.5FR Ka	35^b	71^b	97.8^a	100^a	-
MEI1 + Ka	10^8 + 0.5FR Ka	29^b	64^{bc}	93.9^{ab}	100^a	-
MEI2 + Ka	10^8 + 0.5FR Ka	28^b	62.8^{bc}	91.6^{ab}	99.9^a	-
+ve control	FR Ka	52.8^a	100^a	-	-	-
-ve control	Water	Nd	Nd	Nd	Nd	Nd
P value		0.0001	0.0001	0.0001	0.0001	0.0001

Means followed by the same letter within a column are not significantly different (Tukey's HSD test, p< 0.05%), Legend- M (%) = Percentage mortality FRKa = Field rate karate; Nd=not detected

Figure 7. (A) Host plant treated with fungal only after 11 day (B) Host plant treated with combined application of fungi and insecticide (C) Host plant treated with insecticide only.

Fig.8. (A and B) untreated negative control mustard performance at 11 days after inoculation (C) Untreated negative control mustard performance at 20 days after inoculation

DISCUSSION

The present study showed the potential of entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae*, as biocontrol agents against adult and nymphal stages of aphids of Ethiopian cabbage. Amy and Tobin (2007) also observed that *bassiana* and *M. anisopliae* are widely distributed in tropical environments and well known for their potential as biological control agents against insect pests of agricultural crops.

The effectiveness of entomopathogens on aphid pests differs depending upon the strains, site of isolation, incubation time and dose, and the target pest (Ujjan and Shahzad, 2012; Eidy *et al*., 2016; Anna *et al*., 2020). Although the local isolates, in general, achieved mortality rate of 94.5% after 6 days of inoculation ($p< 0.001$), BEI1 displayed up to 67% and 100% mortality within fewer days (after 4 and 5 days) of incubation indicating its better performance than the other isolates (Table 4). Its performance was also higher than the mortality of (90%) recorded from *B. bassiana* isolate BB-01 on wheat aphid (*Schizaphis graminum*), and *(*100%) on brassica aphid after 6 and 7 days of treatment, respectively reported by Asi *et al*. (2009).

Based on the pattern of inhibition of the aphids, *B. bassiana* achieved mean mortality rate of 78% compared to 69% mortality induced by the *M. anisopliae* isolates after five days of incubation. Saranya *et al.* (2010) also showed that *B. bassiana* isolates from India displayed 87-97% mortality against 52-81% mortality rates of adult cowpea aphids treated with best isolates of *M. anisopliae* after 6 days of treatment. Although the local *M. anisopliae* isolates were slow in their effectiveness compared to the *B. bassiana* isolates, their effectiveness on mortality of cabbage aphids in 6 days (71-83%) was much better than the mortality rate of 20-62% of the same type of aphids inoculated with $1x10^7$ conidia ml^{-1} within 6 days (Asi *et al*., 2009).

On the contrary, the local isolates were not as effective as the isolates of *B. bassiana* PDRL 1187 and *M. anisopliae* PDRL526 from Pakistan with mortality rate of 88.2% and 72.6% on mustard aphids inoculated with 1 $x10^7$within 3 days of exposure under laboratory conditions, respectively (Ujjan and Shahzad, 2012). Another isolate, *Beauveria* from Iran effectively controlled (75-100% mortality) rose adult aphids with treatments of 10^4-10^8 spores /ml within 4-5 days of exposure (Eidy *et al*., 2016).

The effectiveness of the local isolates on the aphid nymph was slightly lower than the adults ones (Table 5). The *B. bassiana* isolates inflicted substantial percentage mortality (72-78%) compared with *M. anisopliae* isolates with the mortality rate (39-66%) on larval stage compared to 61-100% with *B. bassiana* and 56-83% with *M anisopliae* on adult aphids after five days of incubation. Although there was a steady increase in larval mortality in 6 days, the data showed a significant difference between the *B. bassiana* and *M anisopliae* isolates unlike that of their effect on the adult aphids (Table 4 and Table 5). The mortality of the aphids recorded in this study was much better than the 39% and 80 % mortality recorded with a bio-control agent *B. bassiana* CKB-048 at a concentration of 2×10^8 after 3 and 7 days of treatment, respectively (Maketon *et al*., 2013).

The data, in general, showed that the adults were significantly more sensitive than the nymph at all concentrations. Consequently, maximum percent mortality of 100% was recorded after 5 days for adult whereas; the same mortality rate with nymphs was recorded after 6 days of treatment at a concentration of 1×10^8 ($P < 0.001$)(Table, 6). Similarly, Murerwa *et al.* (2014) showed higher susceptibility of five to seven days old aphids (41%) than immature (20%) day old nymphs' to *M. anisopliae* isolate ICIPE 51 at a concentration of 1×10^7 after 6 days of treatment.

The compatibility studies between the entomopathogens and different chemicals are important tools in selection of the appropriate combination of bio-insecticides and chemicals within the context of integrated pest management (IPM). In this study the *Beauveria (*BEI1) and *Metarhizium (*MEI2*)* isolates showed significant reduction in viability with Actara, (RR) treatment compared to the lower concentrations and the control (74 and 71%) respectively. In contrast, Neves *et al.* (2001) reported that non-significant reduction compared to control with 86.6% and 94.6 % germination, respectively.

Thus, almost all fungal isolates showed similar pattern of tolerance (mean germination of 88%, 81% and 78% with radiant, Actra and Karte, respectively. The *Beauveria* isolates showed a slightly higher resistance to the insecticides with BEI2 showing the highest resistance of the other

isolates; whereas *Metarhizium anisopliae* (MEI2) was relatively sensitive to all insecticides (Table 8 and 9). Similarly, Oliveira *et al.* (2003) showed that *B. bassiana* isolates were compatible with the insecticide Thiamethoxam at half and field recommended doses with conidial viability from 62% -93%. The compatibility of *M. anisopliae* with thiamethoxam and lambda-cyhalothrin was similar with the work of Silva *et al*. (2013) at FR concentration that showed a compatibility of *M. anisopliae* (Strain CG 168) with thiamethoxam (81.8% germination) and lambda-cyhalothrin (86.3% germination). Generally, the fungal isolates showed conidial germination of 68%-98% after treated with the insecticides Karate (lambda-cyhalothrin), Actara (Thiamethoxam) and Radiant at half the recommended rate indicating that these insecticides would be suitable for use in combination with *B. bassiana* and *M. anisopliae* for insect pest control as part of an integrated pest management strategy (IPM) for cabbage.

In this study, *B. bassiana* isolates were more virulent than those of *M. anisopliae* isolates in killing both nymph and adult aphids under greenhouse conditions (Table8 and 9). *B. bassiana* (BEI1) was the most virulent strain showing mortality rates of 97.5% and 93.9% after 11days of treatment for adult and nymph aphids, respectively. Under the circumstances, the local fungal isolates were less effective in terms of time to kill the aphids compared to the 7 days treatment (67%-100% mortality) with the same inoculums of ($1x10^8$ spores/ml) by *B. bassiana* isolates recorded elsewhere (Ujjan and Shahzad, 2012; Selvaraj and Kaushik, 2014).

Likewise, *B. bassiana* induced 30.7 to 48.3% mortality rates with $1X10^8$ spores/ml concentration on green peach aphid adults after 7 days of inoculation under the greenhouse conditions. The data on the mortality of 49.2% after 7 days treatment with *B. bassiana* (BEI1) showed same pattern of mortality recorded with similar entomopathogens by Al-alawi and Obeidat (2014). It is clearly indicated that the BEI1 and MEI2 showed aphid mortality rates of 77.8% and 55.6% under laboratory condition while they showed mortality rates of 30% and 18%) under *in vivo*, within 5 days of treatment, respectively. These findings were in conformity with earlier reports (Kumar *et al.,* 2012) who indicated that the virulence of entomopathogenic fungal isolates decreased during *in vivo* bioassays against insect pests.

In this study, the combined applications of fungal isolates with a lower dosage of karate resulted in a significantly higher mortality levels compared to sole applications of fungal isolates. During the first two days, high mortality was registered from the FR chemical alone due to fast killing. But later, after the 7^{th} - 11^{th} day post treatment similar results were observed from the combination and chemical alone. The karate combined with EPF (BEI1) gave the highest mortality on both adult and nymph aphids. Faraji *et al*. (2016) indicated the basic approaches of chemical and entomopathogen compatibility may weaken and make the insect pest vulnerable to disease or mortality.

Generally, the combined application of the insecticide karate with highly efficacious entomopathogenic fungi (BEI1) in a sequential way proved to be promising indicating synergistic action of the two components in IPM. The result was more or less in conformity with Niassy *et al*. (2012) who used different insecticides Actara (Thiamethoxam) and Imidacloprid with *Metarhizium anisopliae* ICIPE inflicting 69, 91 and 82.2 % mortality, respectively in a sequential manner by using them against flower thrips (*Frankliniella occidentalis*).

CONCLUSION

The data showed the mortality of infected aphids with fungal isolates under *in vitro* increased with increasing concentration of conidial suspensions and exposure time. The different treatments showed substantial increase in insect mortality after five days of incubation inducing the maximum death in 9-11 days. The compatibility test showed dose dependant conidial germination rates of 77% - 98% with *Beauveria* isolates and 75% - 91% with *Metarhizium* isolates. The insecticide, Karate in combination with all fungal isolates in sequence gave higher mortality than the fungal isolates alone against nymph and adult aphids.

RECOMMENDATION

Based on the result, *Beauveria bassiana* (BEI1) and *Metarhizium anisopliae* (MEI1) isolates could be

further tested as microbial insecticides alone and combined with half recommended dose of insecticides in the field trials to fully realize their potential as bioinsecticides for integrated pest management to control mustard aphids and obtain quality products (organic farming). Additional investigations are recommended for more effective EPF against different aphids controlling the pest with a relatively short time, and tolerance to higher dose of different insecticides.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Offices of the Vice President of Research and Technology Transfer, Addis Ababa University for financial support and the Department of Microbial, Cellular and Molecular Biology, AAU for accessing its laboratory facilities to the research work that were provided to us to complete this study.

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