

## Research Article

# Pesticidal Evaluation of Entomopathogenic Fungi and Selected Medicinal Plants against Cabbage Aphid (*Brevicoryne brassicae* L.)

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Received 26 March 2022; Revised 10 June 2022; Accepted 17 June 2022; Published 1 July 2022

Academic Editor: Xinqing Xiao

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Pesticidal agents such as entomopathogenic fungi and medicinal plant extracts can be used as a component of integrated pest management. Biocontrol agents such as fungal isolates can be used as a component of integrated pest management. An evaluation of plant extracts from *Azadirachta indica* and *Justicia schimperiana* and two strains of entomopathogenic fungi were carried out on Ethiopian rape (*Brassica carinata*) against two stages (adult and nymph) of cabbage insect pests (aphids) in the laboratory and greenhouse condition. The efficacies of different treatments were examined, and results were recorded for plant extracts and entomopathogenic fungi on cabbage aphids. A significant difference was observed in the mortality of aphid insect pests recorded at different intervals of days. The adult aphid was reduced gradually from 8.65/plant to 2.77/plant after six days of spraying with *A. indica* indicating the highest efficacy. Moreover, the adult aphids after spraying of entomopathogenic fungi (BEI1) reduced from 11.2/plant to 6.5/plant after six days of spraying at  $1 \times 10^8$  conidia/mL, showing the highest efficacy. The present results suggest the possibility of using a combination of entomopathogenic fungi and plant extracts to manage *Brevicoryne brassicae* (aphids). It revealed that a given combination displayed considerable efficacy to reduce *B. brassicae* (aphids) infestation. From the result, the adult aphids sprayed with *A. indica* + BEI1 reduced from 7.01/plant to 1.74/plant after six days of spraying with the highest efficacy. Generally, maximum percent of mortality was identified in plant extract treatment next to coapplication against adult aphids on sixth day of application. Similarly, conidial suspension of entomopathogenic fungi was found to have high activities for adult aphids. Therefore, based on the present result, products of fungal isolates and plant extracts should be used for further tests against other insect pests.

## 1. Introduction

A rape is a subtropical plant that belongs to the family of Brassicaceae. It includes *Brassica carinata*, *Brassica juncea*, *Brassica oleracea* var. Botrytis, and other crucifers [1]. *B. carinata* is a species of flowering plant in the Brassicaceae family. It is referred to by the English common names Abyssinian cabbage, Abyssinian mustard, African cabbage, Ethiopian kale, Ethiopian mustard, Ethiopian rape, Mustard collard, and Texsel greens. Ethiopian mustard (*B. carinata* L.) is the most important garden crop believed to have

originated from the Ethiopian highlands [2, 3]. It is locally known as “Gomenzer” (“*Yehabesha Gomen*”) in Amharic. It is used as a food and oilseed crop in arid and semiarid areas [4]. It is a drought- and heat-tolerant crop [5].

*Brassica carinata* production is predominantly inhibited by different insect pests [6, 7]. *Brevicoryne brassicae* is among the most important pests of crops all over the world [8]. It can cause direct devastation of the plant and expose them to secondary infection by pathogenic microorganisms such as fungi, bacteria, and viruses [7]. An insect pest is commonly controlled by pesticides.

Pesticides, which are mostly applied to crops for the protection of plants against a range of pests, have been found in crude medicinal plants [9] and pests control agents such as entomopathogenic fungi [10]. Therefore, entomopathogenic fungi [11] and medicinal plants [12] were widely used as biocides for the control of agricultural and domestic pests worldwide. Although diverse microorganisms are associated with pests control agents, the entomopathogenic fungi are the most important microbes that comprise approximately 750 species, reported from different insects [13]. They are relatively ubiquitous worldwide [14]. Entomopathogenic fungi in the division Deuteromycotina, such as *Beauveria bassiana* have been successfully developed as biological control agents [15], and furthermore, medicinal plants contain bioactive organic chemicals in the form of metabolites [16] against a number of different fields and store pests. Both entomopathogenic fungi and plant material can be used in a traditional system of medicine as a component of integrated pest management [17].

Currently, *B. brassicae* (aphid) is one of the most important insect pests that attack brassica leafy vegetables [7]. The insecticidal activities of secondary metabolites of *B. bassiana* (entomopathogenic fungi) against many pests [18, 19] and the use of medicinal plants in both crude and prepared forms [9] have been described in several reports. Although entomopathogenic fungi (*B. bassiana*) and various medicinal plants are considered promising models to control insect pests and several studies have emphasized their evaluation, limited studies have been carried out against *B. brassicae* using entomopathogenic fungi, *Azadirachta indica*, and *Justicia schimperiana* alone and in combination. The aim of this study was, therefore, to determine the efficacy of *A. indica* and *J. schimperiana*, and *B. bassiana* (entomopathogenic fungi) against *B. brassicae* (aphid) attacking Ethiopian rape (*Brassica carinata* A. Braun).

## 2. Material and Methods

**2.1. Cultivation of Fungal Pathogen.** Cultures of entomopathogenic fungi (*B. bassiana*) were obtained from the Ethiopian Biodiversity Institute. The process of cultivation of fungal pathogen was carried out at the microbiology laboratory, School of Biological Science and Biotechnology, Haramaya University. Pathogenic fungi were taken from the mother slant and inoculated on potato dextrose agar (PDA) in test tubes under aseptic conditions in a laminar airflow chamber [20]. The test tubes containing PDA medium were autoclaved at 121°C for 15–20 minutes and incubated at 25 ± 2°C and 60 ± 10% relative humidity after inoculation. After adequate growth, it was subcultured in the newly prepared medium in 250 mL conical flasks for mass multiplication of the fungal pathogen and subsequently used for evaluation.

**2.2. Preparation of Fungal Spore Suspension.** Fungal spore suspension was prepared in accordance with the methods described by [21] (pp.1–7) with minor modifications. The entomopathogenic fungi (*B. bassiana*) were grown on PDA

for 3 and 4 weeks. The conidia were harvested by scraping the surface of 3 and 4 weeks old culture gently with an inoculation needle. The conidia were suspended in distilled water containing 0.1% TritonX-100 (added as surfactant). The mixture was stirred with a magnetic shaker for 10 minutes. The hyphal debris was removed by filtering the mixture through a fine-mesh sieve. The conidial concentrations of final suspensions were determined by direct count using haemocytometer. Three droplets of a diluted suspension containing conidia at the rate of  $1 \times 10^8$ ,  $1 \times 10^7$ , and  $1 \times 10^6$  conidia/mL were placed on PDA and incubated at 25 ± 2°C and 65 ± 10% relative humidity in the dark for 24 hrs. After staining with lactophenol cotton blue, germination was checked under a microscope, and the spores were used for the bioassay.

**2.3. Bioassay of Fungal Pathogen on Cabbage Aphids at Laboratory.** Bioassays in the laboratory were prepared in accordance with the methods described by [8] (pp.175–180). Fungal isolates with concentrations of  $1 \times 10^8$ ,  $1 \times 10^7$ , and  $1 \times 10^6$  conidia/mL were used for bioassays. Ethiopian rape (*B. carinata* A. Braun) was surface sterilized with 0.5% v/v sodium hypochlorite (NaOCl) for 30 seconds, rinsed with sterilized distilled water, and air-dried. The detached leaf method was used for the treatment of aphids. The leaves were placed on agar in 90 mm Petri dishes. The agar was nonnutritive and just supplied water to the leaves to maintain relative humidity during the test. The efficacies of entomopathogenic fungi (*B. bassiana*) were tested for nymph and adult aphids on fresh leaves. Nymph and adult aphids were brought to the laboratory with their host plant leaves. The oldest leaves were replaced with fresh leaves every 3 days using the same procedure.

After treating each leaf by spraying it with 10 mL of conidial concentrations ( $1 \times 10^8$ ,  $1 \times 10^7$ , and  $1 \times 10^6$  conidia/mL), the leaves were placed on PDA in Petri dishes. The fungal pathogens were sprayed gently and slowly using the hand sprayer to avoid falling of the insect pests from the plant and also for proper contact of the spray fluid on the insect pests [20]. The aphids of the control were treated with 0.1% Triton X-100 solution and pure distilled water. The experiment was conducted under CRD (completely randomized design) with 3 replications of each treatment. The efficacy was observed after 1 day, 2 days, 3 days, 4 days, 5 days, and 6 days of spray, and control treatment was compared with each other. Any dead aphids were removed and incubated on damp filter paper within sealed Petri dishes in an incubator at 25 ± 2°C and 65 ± 10% relative humidity for 6 days and inspected for the presence of mycelium on the cadavers.

**2.4. Preparation of Extracts.** Neem (*A. indica*) and Hochst. Ex Nees (*J. schimperiana*) were identified and authenticated properly at the Herbarium of Haramaya University. The plant leaves were separated, washed, and cleaned thoroughly with tap water and then with distilled water and air-dried in shade at the botanical laboratory for several weeks. Plant extracts were prepared in accordance with the methods

described in [22] (p. 26), [23] (pp. 2261–2268). About 40 g of the powder was separately soaked in 200 mL of ethanol (70%), in a 400 mL stoppered reagent bottle, and the mixtures were shaken for 72 hrs using an electrical shaker. The resulting mixture was first filtered with cheesecloth, then with Whatman No. 1 filter paper. The filtrates were then separately concentrated in a vacuum using a rotary evaporator at 30–40°C. The extracts were then transferred carefully to labeled vials and allowed to permit evaporation of residual solvents at room temperature for 3 and 4 days. Solutions of 200 mg/mL were prepared by reconstituting 1 g of each of the dried crude powder in 5 mL of an aqueous solution. From this stock solution, 150 mg/mL working solutions were prepared. The solution thus prepared was stored at 4°C for pesticidal testing [24].

**2.5. Bioassay of Plant Extracts on Cabbage Aphids under Greenhouse Condition.** The experiment was conducted in the month of October and December in the field. During these months, average relative humidity was  $65 \pm 10\%$ . The experimental block was divided into 21 plots each  $6 \text{ m}^2$  in size to accommodate six treatments and controls, which were all replicated 3 times. Treatments were arranged in a randomized complete block design [25]. Seeds of Ethiopian rape (*B. carinata*) were collected from local farmers of Haramaya district. The seeds were grown on plastic pots by providing all the standard nutrients and cultural practices for 30 days. The water was supplied to the plants 3 times per day. Each 30-day-old plant was infested with 15 adult and nymph aphids. After 3 days of infestation, 150 mg/mL of concentration were sprayed on each plant. Spraying was carried out with a fine hand sprayer machine every day for the whole experimental period [26]. The efficacy of concentration was then observed after 1 day, 2 days, 3 days, 4 days, 5 days, and 6 days of spraying and compared with the control treatment. A recommended pesticide for cabbage (Karaate) was served as a positive control, and distilled water was used as a negative control.

**2.6. Coapplication of Entomopathogenic Fungal Isolates with Plant Extracts.** Bioassays for compatibility of entomopathogenic fungal isolates with plant extracts were prepared in accordance with the methods described by [27] (pp. 1–6). A fungal isolate with the highest concentration ( $1 \times 10^8$  conidia/mL) and plant extracts with a concentration of 150 mg/mL were used for the test. The treatments were set as follows: *A. indica* + BEI1, *A. indica* + BEI1, *J. schimperiana* + BEI1, and *J. schimperiana* + BEI2. The bioassays were performed under the laboratory conditions (at  $25 \pm 2^\circ\text{C}$  and  $65 \pm 10\%$  relative humidity). Plant extracts and entomopathogenic fungi were applied with the abovementioned concentrations, using a fine hand sprayer machine.

**2.7. Evaluating the Percentage Dead of Insect Pests.** To estimate the percentage of dead insect pests, it is useful to know if an insect dead is due to mycosis and plant extracts. The proportion of insects (adult and nymph aphids) killed by the

entomopathogenic fungi and plant extracts alone as well as a combination of both is measured using the following formula [28–30]:

$$P = \left[ \frac{(C - T)}{C} \right] \times 100. \quad (1)$$

where  $PP$  = the estimated percentage of insects killed by the entomopathogen fungi and/or plant extracts,  $C$  = the percentage of the control insects that are living, and  $T$  = the percentage of treated insects that are living after the experimental period.

**2.8. Data Analysis.** The values were then analyzed using Statistical Package for Social Sciences (SPSS) version 20 software. One-way analysis of variance (ANOVA) was used on the mortality data to test the level of significance (efficacies against these insect pests), and the comparison of means test was used. The significant differences were marked by different alphabets.

### 3. Results and Discussions

The effects of different treatments were examined and observed for the efficacy of plant extracts and entomopathogenic fungi on Ethiopian cabbage (*B. carinata* A. Braun). This cabbage was infested by adult and nymph stages of insect pest (*B. brassicae*). The data was then observed on these stages of insect pests after treatments, and also insect count was recorded. Significant differences were observed in the mortality of aphid insect pests recorded at different intervals of days.

**3.1. Efficacy of Plant Extracts against Pest Aphids.** The present experiment has demonstrated the possibility of using extracts from medicinal plants to use as pesticidal constituents in the management of *B. brassicae*. Related works have previously stated the pesticidal activity of various medicinal plants and their compounds against different groups of risky insects [31]. Our results revealed that a given concentration of extracts displayed considerable effectiveness to reduce *B. brassicae* infestation. *A. indica* extract was powerful enough to reduce the number of aphid levels after treatment. Owing to the fact that their secondary metabolite and mode of action, *A. indica* could be very suitable for killing aphid pests by feeding poisons to nymphs and adult aphids, and therefore, they show a considerable selectivity toward nymphs and adults aphid pests. Reference [31] stated that *A. indica* compounds present various effects ranging from repellency to toxicity against a wide spectrum of insect pests. High mortality routinely showed potential pesticidal activity of plant extracts [32]. Combined with an alternative technique such as biological control [33], the pest load could be brought down to economic threshold levels using plant extracts [25]. This study brought about promising results using plant crude extracts against aphid pests. Aphids were the most susceptible insect with mortality observed after 24 h for all the plant extracts tested [34]. This study also supports

TABLE 1: Efficacy of plant extracts against aphid pest.

Extracts	Stages of aphid pest	Treatments observation (at conc. 150 mg/mL)						P (estimated percentage)
		1 day	2 days	3 days	4 days	5 days	6 days	
<i>A. indica</i>	Adult	8.65 <sup>Aa</sup>	7.23 <sup>Ab</sup>	7.57 <sup>Ab</sup>	6.33 <sup>Ac</sup>	3.05 <sup>Ad</sup>	2.77 <sup>Ad</sup>	81.53
	Nymph	10.62 <sup>Ba</sup>	9.66 <sup>Ba</sup>	8.02 <sup>Ab</sup>	7.87 <sup>Bb</sup>	3.66 <sup>Ac</sup>	3.11 <sup>Ac</sup>	79.27
<i>J. schimperiana</i>	Adult	9.11 <sup>Aa</sup>	8.77 <sup>Aa</sup>	7.74 <sup>Ab</sup>	7.17 <sup>Ab</sup>	4.33 <sup>Ac</sup>	3.41 <sup>Ad</sup>	77.3
	Nymph	11.01 <sup>B</sup>	10.71 <sup>B</sup>	8.97 <sup>B</sup>	8.01 <sup>Ac</sup>	4.86 <sup>Ad</sup>	4.00 <sup>Ad</sup>	73.33
Karate (P.c.)	Adult	2.3 <sup>Aa</sup>	1.6 <sup>Aa</sup>	1 <sup>Ac</sup>	0	0	0	93.33
	Nymph	2.9 <sup>Aa</sup>	2.1 <sup>Bb</sup>	1.4 <sup>Bc</sup>	0	0	0	90.67
Distilled water (N.c.)	Both	NK	NK	NK	NK	NK	NK	NK

Capital letter superscripts compare means in the column, while small letter superscripts compare means in the row. Mean values with different letters show significant differences at  $P < 0.05$ . NK = not killed, A = *Azadirachta*, J = *Justicia*, P.c. = positive control, and N.c. = negative control.

that both plant extracts revealed promising efficacy against aphids (nymph and adult stages). According to [35] (pp. 16077–16082), the mixture of *A. indica* and wild garlic was more effective in reducing population densities of aphids than either plant extract applied alone. In this study, however, *A. indica* alone shows promising results against aphids (nymph and adult stages).

The results reveal that *A. indica* and *J. schimperiana* were effective in killing cabbage aphid (*B. brassicae*) with varying inhibitory effects as depicted in Table 1. The adult aphids were reduced gradually from 8.65/plant to 2.77/plant after six days of spraying indicating the highest efficacy with 81.53% by *A. indica*. The treatment of nymph aphids was decreased from 10.62/plant to 3.11/plant after six days of spraying with *A. indica* showing efficacy with 79.27% ranked the second, followed by *J. schimperiana* against adult aphids that reduced from 9.11/plant to 3.41/plant ranked the third efficacy with 77.3%.

### 3.2. Efficacy of Entomopathogenic Fungi against Adult Aphids.

The effectiveness of entomopathogenic fungi (*B. bassiana*) on aphid pests differs depending upon the strains, site of isolation, incubation time and dose, and the target pest stages [36, 37]. According to this study, experiments were deliberated as a measure of mortality of aphids (nymph and adult stages) at various concentrations of entomopathogenic fungi isolates. Thus, it is time- and dose-dependent mortality testing. The effectiveness of fungal isolates was based on the duration of exposure to kill aphids with the same concentrations of inoculums recorded [38–40]. In the laboratory bioassay against aphids, none of the entomopathogenic fungi products caused aphid mortality by day 3, but mortality had occurred by day 6 depending on the time of exposure [41]. According to this study, however, the entomopathogenic fungi products initiated aphid mortality starting from day 1 and gradually increased up to day 6. As stated in [42] (pp.138–142), entomopathogenic fungi isolates revealed promising efficacy against mortality rates of adult aphids. Based on this, the information stated in the present study would be helpful to plan for use of entomopathogenic fungi for aphid pest control practices. Nearly 75% of nymph aphids were not killed by entomopathogenic fungi (BE1 and BE2) at all concentrations. This is due to the morphological

structures of the nymphal stages of the insect pest [43] (pp.25–30). Reference [44] (pp.1200–1207) said that the presence of waxy protections resists penetration of chemicals contact with nymphal stages of aphids. The results showed that mortality caused by the concentration of  $1 \times 10^8$  conidia/mL of fungi isolates was significantly higher than those of  $1 \times 10^6$  and  $1 \times 10^7$  conidia/mL. Therefore, a concentration of  $1 \times 10^8$  conidia/mL is the recommended concentration to control aphids. The concentration that reveals promising results (mortality) is the optional concentration to control aphids [45].

The data in Table 2 showed that the adult aphids after spraying of entomopathogenic fungi (BE1) reduced from 11.2/plant to 6.5/plant after six days of spray at  $1 \times 10^8$  conidia/mL showing the highest efficacy of 56.67%. The adult aphids sprayed with entomopathogenic fungi (BE2) extract ranked second, where the adult aphids decreased from 10.89/plant to 7.08/plant after six days of spraying, showing an efficacy of 52.8% at the same concentration. The entomopathogenic fungi (BE1) ranked third, where the adult aphids decreased from 13.66/plant to 7.03/plant after six days of spraying, showing an efficacy of 53.13%. More than half of the adult aphids were killed up to  $1 \times 10^7$  conidia/mL except BE2 that killed less than half (39.67%) of adult aphids at  $1 \times 10^7$  conidia/mL. Relatively, about 75% of the adult aphids were not killed by entomopathogenic fungi (BE1 and BE2) at  $1 \times 10^6$  conidia/mL, and there was no significant difference ( $P < 0.05$ ) among the insecticidal activities.

The nymph aphids after spraying of entomopathogenic fungi (BE1) reduced from 13.67/plant to 11.55/plant after six days of spray at  $1 \times 10^8$  conidia/mL showing the second most efficacy of 23%; and the nymph aphids sprayed with entomopathogenic fungi (BE2) ranked highest, where the nymph aphids decreased from 13.13/plant to 11.01/plant after six days of spraying, showing an efficacy of 26.6% at  $1 \times 10^8$  conidia/mL. The entomopathogenic fungi (BE2) ranked third, where the nymph aphids decreased from 13.87/plant to 11.81/plant after six days of spray, showing the efficacy of 21.2%.

### 3.3. Coapplication of Entomopathogenic Fungal Isolates with Plant Extracts.

In this study, the higher inhibition values



TABLE 2: Efficacy of various concentrations of entomopathogenic fungi against aphids pest.

Pest stages	<i>B. bassiana</i>	Conidia/mL	Treatments observation						<i>P</i> (estimated percentage)
			1 day	2 days	3 days	4 days	5 days	6 days	
Adult	BEI1	108	11.2 <sup>Aa</sup>	10.4 <sup>Ab</sup>	10.1 <sup>Ab</sup>	8.33 <sup>Ac</sup>	7.76 <sup>Ac</sup>	6.5 <sup>Ad</sup>	56.67
		107	13.66 <sup>Ba</sup>	12.78 <sup>Ba</sup>	11.02 <sup>Bb</sup>	10.87 <sup>Bb</sup>	8.19 <sup>Bc</sup>	7.03 <sup>Ad</sup>	53.13
		106	13.87 <sup>Ba</sup>	13.22 <sup>Ba</sup>	12.98 <sup>Cb</sup>	12.43 <sup>Cb</sup>	11.78 <sup>Cc</sup>	11.03 <sup>Bc</sup>	26.47
	BEI2	108	10.89 <sup>Aa</sup>	10.42 <sup>Aa</sup>	10.22 <sup>Aa</sup>	9.22 <sup>Ab</sup>	8.11 <sup>Ac</sup>	7.08 <sup>Ad</sup>	52.8
		107	11.77 <sup>Ba</sup>	11.19 <sup>Ba</sup>	10.78 <sup>Aa</sup>	10.63 <sup>Ba</sup>	10.12 <sup>Bb</sup>	9.05 <sup>Bb</sup>	39.67
		106	13.98 <sup>Ca</sup>	13.77 <sup>Ca</sup>	13.02 <sup>Bb</sup>	12.99 <sup>Cb</sup>	12.15 <sup>Cc</sup>	11.08 <sup>Cc</sup>	26.13
Nymph	BEI1	108	13.67 <sup>Aa</sup>	13.11 <sup>Ab</sup>	13.01 <sup>Ab</sup>	12.77 <sup>Ac</sup>	12.16 <sup>Ad</sup>	11.55 <sup>Ad</sup>	23
		107	14.11 <sup>Ba</sup>	13.77 <sup>Aa</sup>	13.67 <sup>Aa</sup>	13.01 <sup>Ab</sup>	12.77 <sup>Ab</sup>	12.03 <sup>Ac</sup>	19.8
		106	14.77 <sup>Ba</sup>	14.73 <sup>Ba</sup>	14.66 <sup>Ba</sup>	14.23 <sup>Bb</sup>	14.12 <sup>Bb</sup>	14.01 <sup>Bb</sup>	6.6
	BEI2	108	13.13 <sup>Aa</sup>	13.00 <sup>Ab</sup>	12.91 <sup>Ab</sup>	12.61 <sup>Ac</sup>	12.23 <sup>Ac</sup>	11.01 <sup>Ad</sup>	26.6
		107	13.87 <sup>Ba</sup>	13.51 <sup>Ba</sup>	13.03 <sup>Ab</sup>	12.79 <sup>Bc</sup>	12.22 <sup>Ac</sup>	11.81 <sup>Bd</sup>	21.27
		106	14.13 <sup>Ba</sup>	13.75 <sup>Ba</sup>	13.16 <sup>Ab</sup>	12.84 <sup>Bc</sup>	12.47 <sup>Bd</sup>	12.11 <sup>Cd</sup>	19.27
Triton X-100		NK	NK	NK	NK	NK	NK	NK	

Capital letter superscripts compare means in the column, while small letter superscripts compare means in the row. Mean values with different letters show significant differences at  $P < 0.05$ . NK = not killed and BEI = *Beauveria bassiana*.

TABLE 3: Coapplication of entomopathogenic fungal isolates with plant extracts.

Extracts	Stages of aphid pest	Treatments observation						<i>P</i> (estimated percentage)
		1 day	2 days	3 days	4 days	5 days	6 days	
AB <sub>1</sub>	Adult	7.01 <sup>Aa</sup>	6.67 <sup>Ab</sup>	5.12 <sup>Ac</sup>	3.42 <sup>Ad</sup>	2.66 <sup>Ae</sup>	1.74 <sup>Af</sup>	88.4
	Nymph	8.13 <sup>Ba</sup>	6.81 <sup>Ab</sup>	5.22 <sup>Ac</sup>	4.55 <sup>Bd</sup>	3.27 <sup>Be</sup>	3.01 <sup>Bf</sup>	79.93
AB <sub>2</sub>	Adult	7.50 <sup>Aa</sup>	7.07 <sup>Aa</sup>	6.10 <sup>Ab</sup>	5.81 <sup>Ab</sup>	4.09 <sup>Ac</sup>	2.41 <sup>Ad</sup>	83.9
	Nymph	7.91 <sup>Aa</sup>	7.63 <sup>Ba</sup>	7.05 <sup>Bb</sup>	6.71 <sup>Bb</sup>	5.86 <sup>Bc</sup>	4.02 <sup>Bd</sup>	73.2
JB <sub>1</sub>	Adult	8.15 <sup>Aa</sup>	7.43 <sup>Ab</sup>	7.02 <sup>Ab</sup>	6.43 <sup>Ac</sup>	4.07 <sup>Ad</sup>	3.66 <sup>Ae</sup>	75.6
	Nymph	9.22 <sup>Ba</sup>	8.73 <sup>Bb</sup>	8.07 <sup>Bc</sup>	7.77 <sup>Bd</sup>	5.26 <sup>Be</sup>	4.16 <sup>Bf</sup>	72.3
JB <sub>2</sub>	Adult	8.27 <sup>Aa</sup>	7.67 <sup>Ab</sup>	7.04 <sup>Ac</sup>	6.17 <sup>Ad</sup>	4.53 <sup>Ae</sup>	3.33 <sup>Af</sup>	77.8
	Nymph	9.21 <sup>Ba</sup>	8.61 <sup>Bb</sup>	8.07 <sup>Bc</sup>	7.46 <sup>Bd</sup>	6.11 <sup>Be</sup>	5.24 <sup>Bf</sup>	65.1

Capital letter superscripts compare means in the column, while small letter superscripts compare means in the row. Mean values with different letters show significant differences at  $P < 0.05$ . AB<sub>1</sub> = *A. indica* + BEI1, AB<sub>2</sub> = *A. indica* + BEI2, JB<sub>1</sub> = *J. schimperiana* + BEI1, and JB<sub>2</sub> = *J. schimperiana* + BEI2.

were obtained from plant extracts, and entomopathogenic fungi (*B. bassiana*) indicate results that are simultaneously intended for the control of insect pests. We observed that the combination of plant extracts with entomopathogenic fungi significantly increased the mortality rates. Similarly, previous studies showed that the coapplication of plant extracts and entomopathogenic fungi significantly increased the mortality of several insect pests compared to individual application of plant extracts or entomopathogenic fungi [24, 46–48]. As explained by [49], *A. indica* products can be mixed with other bioproducts or with synergists to increase their efficacy due to the unique mode of action derivatives interfering with the hormonal system of insects. From the result, the combination of entomopathogenic fungi and *A. indica* extract showed promising results against nymphs and adult aphids. Similarly, the coapplication of entomopathogenic fungi and *A. indica* extract showed effective pesticidal activities and may become an effective component of insect pest management programs against various insect pests [48]. In this study, about 75% of nymph and adult aphids were killed by coapplication of the products. The higher efficacy provided by the coapplication is due to the

additive and synergistic interactions between plant extracts and entomopathogenic fungi [48, 50].

*A. indica* leaf extract was significantly effective when applied singly or in combination with entomopathogenic fungi isolates (Table 3). *A. indica* caused higher mortality rates among the treated aphid pests due to potent secondary metabolites secreted by the plant. Based on the present results, although all treatments caused significant mortality of aphid pests, we claim that the coapplication of plant extracts and entomopathogenic fungi yield higher mortality of the insect pests at selected concentration. The result showed that the adult aphids sprayed with *A. indica* + BEI1 reduced from 7.01/plant to 1.74/plant after six days of spraying with the highest efficacy of 88.4%. The adult aphids sprayed with *A. indica* + BEI2 extract ranked second, where it decreased from 7.50/plant to 2.41/plant after six days of spray, showing an efficacy of 83.9%. The coapplication of *A. indica* + BEI1 ranked third, where the nymph aphids decreased from 8.13/plant to 3.01/plant after six days of spraying, showing an efficacy of 79.93%.

#### 4. Conclusion

The results of this study revealed that *A. indica* and *J. schimperiana* have promising pesticidal activities by showing high efficacy compared to entomopathogenic fungi. Among plant extracts, the maximum percent of mortality was recorded for *A. indica* against adult aphids. Similarly, conidial suspension of entomopathogenic fungi (BEI1) at  $10^8$  conidia/mL was found to have higher activities for adult aphids as compared to entomopathogenic fungi (BEI2) at the same concentration. This study also put forward the coapplications of entomopathogenic fungi along with the plant extracts. These coapplications have revealed encouraging results compared to the application of entomopathogenic fungi and plant extracts alone. Among coapplication, *A. indica* + BEI1 was found to be the most effective product with high mortality rates, especially in its later days of application. Adult aphid was the most sensitive toward all treatments. The sensitivities of the tested aphids were based on the duration of exposure and the dose of products used during treatments. Therefore, based on the present result, entomopathogenic fungi and plant extracts should be used for further tests against other pests alone and in combination to fully realize their possible pesticidal activities and attain quality products.

#### Data Availability

No data were used in this study.

#### Conflicts of Interest

The authors declare that they have no conflicts of interest.

#### Authors' Contributions

G. Gebreyohans and J. M. Sasikumar contributed to study conception and design; G. Gebreyohans contributed to data collection; J. M. Sasikumar and N. I. Batu contributed to methodology; G. Gebreyohans, J. M. Sasikumar, and N. I. Batu contributed to analysis and interpretation of results; and G. Gebreyohans and N. I. Batu contributed to drafting the manuscript. All authors reviewed the results and approved the final version of the manuscript. Moreover, all authors have read and agreed to the published version of the manuscript.

#### Acknowledgments

The authors would like to thank the Ethiopian Biodiversity Institute, the Ethiopian Ministry of Education and the Haramaya University for providing them with financial and institutional support. Special thanks also go to the authors' dear families for their constant appreciation and inspiration. This research received no external funding.

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