



Research article

Natural insecticides for the control of urticating ant, *Tetramorium aculeatum* Mayr (Hymenoptera: Formicidae) in a coffee plantation of Southwestern Ethiopia



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ABSTRACT

Coffee, *Coffea arabica* L. is a vital crop in Ethiopia's social, cultural, and national economy. Despite its dominant role in the country, the crop is challenged by various insect pests, which reduce coffee yield and quality. The urticating ant or biting ant, *Tetramorium aculeatum* Mayr (Hymenoptera: Formicidae), is an important pest in coffee that affects farming activities in Southwestern Ethiopia. The objective of this study was to evaluate the efficacy of some natural insecticides for the control of biting ants. Nine insecticide treatments were evaluated both under ex-situ and in-situ conditions in CRD and RCBD designs, respectively. Under the ex-situ evaluation, all the insecticide treatments caused significantly higher mortality of biting ants and caused complete mortality within 24 h. Under field conditions, the insecticides showed significant variations in the percent of evacuated nests, and the number of newly constructed nests. In both efficacy tests of ex-situ and in-situ experiments, the mortality of biting ants and percent of evacuated nests in the coffee trees treated with oxymatrine and nimbicidine were found to be comparable and effective as the deltamethrin 2.5% EC. All the insecticide treatments significantly reduced the number of newly constructed nests compared to the control plot. On the other hand, deltamethrin 2.5% EC significantly decreased newly constructed nests after 30 days of application compared to the other insecticide treatments. Generally, the natural insecticides effectively minimized the biting ant infestation, but further studies are essential on the frequency of spray to use them for sustainable management approaches of biting ant in the coffee plantation.

1. Introduction

Coffee (*Coffea* L., Rubiaceae) is mostly grown in the tropical and subtropical regions in 80 countries on over 10 million hectares of land (Waller et al., 2007; FAO, 2013), and it is an economically important crop grown over 50 developing countries, for which it is the primary source of foreign currency (Waller et al., 2007). Ethiopia is the center of origin and the largest producer of *Coffea arabica* L. in Africa, and the fifth globally, next to Brazil, Vietnam, Colombia, and Indonesia (ICO, 2017). Coffee farming in Ethiopia takes place over a vast area under different production systems and cultivation practices (ECFF, 2017). Coffee is an important commodity in many African countries, both in export earnings and generating income for smallholder farmers (ICC, 2015). In Ethiopia,

coffee farming provides livelihoods for around 15 million farmers and generates a quarter of its export earnings (Moat et al., 2017).

Even though *C. arabica* is an important cash crop in Ethiopia, and numerous production constraints have been affecting the production and productivity of coffee. Both abiotic and biotic factors are significant constraints of coffee production in the country among which insect pests such as biting ant, *Tetramorium aculeatum* Mayr (Hymenoptera: Formicidae) is the one that affects coffee production (Damte and Minase, 2010; Teshome et al., 2018; Mendesil, 2019). Biting ant does not cause direct damage to the coffee plant but indirectly affects farm activities by biting workers during coffee berry picking and pruning in ant-infested farms (McNutt, 1975; Hill, 2008). The biting ant, also known as the urticating ant, is an African ant feared by plantation laborers and that causes yield loss (McNutt, 1975; Cammaerts et al., 1994; Hill, 2008).

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According to Van Emden (2013), the biting ant on the coffee tree builds delicate nests (each containing a few hundred small brown ants) in the bushes. The ant does not favor honeydew-producing insects (not saccharophilic). However, its bite causes such severe pain to workers that they may abandon the task they were performing (Crowe and Tadesse, 1984; Hill, 2008; UCDA, 1996; Damte and Minase, 2010; Van Emden, 2013; Teshome et al., 2018). The indirect losses attributable to the biting ant alone reach 15–30% in Bebeka Coffee Estate Share Company coffee plantation (BCESC), in Ethiopia (Damte and Minase, 2010; Wakjira et al., 2015; Teshome et al., 2018).

In Ethiopia, coffee pest management relies on cultural practices. In addition, natural enemies play a vital role in suppressing insect pests of coffee, and thus insecticides spray is not common (Mendesil, 2019). Furthermore, there are no registered insecticides for the control of *T. aculeatum* in Ethiopia except applications allowed thoroughly as an emergency label, suggesting an urgent need for insecticides screening. Natural insecticides are eco-friendly, economical, usually target specific, biodegradable, and can be an alternative for the control of biting ant (Ujváry, 2010). These botanical derived insecticides are cheap and affordable, which are important qualities of pest control products for smallholder farmers in Africa (Stevenson et al., 2017). To develop effective and easily adopted methods against the biting ant in the coffee production system, it is essential to evaluate different natural insecticides to manage this ant. Therefore, this study aimed to test the efficacy and evaluate the performance of natural insecticides consisting of nine natural and pyrethroid insecticide treatments for the control of *T. aculeatum* both under ex-situ and in-situ conditions.

2. Materials and methods

2.1. Description of the study area

The ex-situ and in-situ experiments were conducted at Bebeka Coffee Estate Share Company coffee plantation (BCESC), located in Southern Nations, Nationalities, and People's Region (SNPPR), in Bench Maji zone, 595 km to the Southwest of the capital Addis Ababa. Out of the total area of 10,030 ha owned by the company, over 5,252 ha are covered with modern coffee plantation. The plantation is located at 6°51'30"-7°11'00"N and 35°15'53"-35°15'66"E at altitudes ranging from 900 – 1380 m.a.s.l. The mean annual rainfall is 1800 mm and the mean maximum and minimum temperature are 27.5 °C and 16.43 °C, respectively (BCESC, 2018).

2.2. The ex-situ evaluation of insecticides against biting ant

The tested insecticides, oxymatrine and nimbidine were obtained from Agrisher trading plc (Addis Ababa, Ethiopia), Deltamethrin from Bayer Crop Science Ethiopia PLC (Addis Ababa, Ethiopia) and Tracer was obtained from Dow Agro science Company (Addis Ababa, Ethiopia). The biting ants were collected with their nests from unsprayed coffee farm

variety Geisha planted 2 m × 2 m spacing with all recommended agro-nomic practices. The ant colonies were maintained in the plastic bucket (cage) in the laboratory for one day before the tests. The experiment was conducted in the laboratory at ambient temperature. Thirty ants were released into the enclosure. In total, 1,350 ants (30 ants * 9 treatments * 5 replications) were used in this experiment. The collected ants were placed inside the plastic bucket of 20 cm × 10 cm × 10 cm, and each treatment was applied after the insecticides rates were calculated and mixed with water following the listed amounts (Table 1). The cages were laid on a 1.5 m × 1.5 m (2.25 m²) area flat table at 0.75 m above the ground. The experiment was arranged in a completely randomized design (CRD) with five replications, and it was repeated after three weeks. The ants were observed at a two-hour interval to record the mortality (Wakjira et al., 2015). The number of dead ants and live ants were counted in the treated and untreated cages. An ant was considered dead if it could not right itself after being placed on its dorsal surface and not moving.

2.3. The in-situ study

The insecticide evaluation experiment was conducted from February – April 2018 on a farm highly infested by *T. aculeatum*. It was carried out on the Geisha coffee variety on which the highest ant infestation was observed. A total of nine treatments, together with unsprayed control (water) tested in the in-situ experiments (Table 1). The in-situ study was conducted at two different coffee farms (South Bench and Guraferda). The coffee variety was Geisha planted in 2 m × 2 m spacing using randomized complete block design (RCBD) in three replications. The spacing between plots and blocks were 4 and 6 m, respectively. Before treatment application, ant nests were checked, and trees with colony nests were marked. Data on the number of evacuated ant nests and the number of newly constructed ant nests were recorded. A 100 m² area was used for each treatment application. Unsprayed plots (trees with active nests) were also included as a control. The amount of insecticides required to spray 100 m² was calculated, and all sprayings were made using a manually operated knapsack sprayer of 15 L capacity (CHINAGROS, International Company, China) for each treatment. The conventional volume of spray in coffee 400 L ha⁻¹ of water (Junior et al., 2012) was used for spraying.

2.4. Data collected

2.4.1. Ex-situ experiment

In the ex-situ experiments, the number of dead ants and live ants were counted in the treated and untreated cages with an interval of two hours after each spray. In this experiment, the number of dead ants in each replicate was converted into proportions of the total number of introduced ants in each cage (30 worker ants) and expressed as a percentage. The data was compiled, and percent mortality was calculated using Eq. (1) as described by Karar et al. (2010):

Table 1. Lists of insecticides treatments evaluated both at ex-situ and in-situ conditions.

Treatment codes	Trade name	Active ingredient/Common name/	Manufacturer	Rates used mL/L of water
T1	Prosuleroxymatrin 2.4%	oxymatrine	Sineria China Ltd.	20.0
T2	Prosuleroxymatrin 2.4%	oxymatrine	Sineria China Ltd.	25.0
T3	Azadirachtin 0.03%	nimbidine	M/S. T.Stanes and Company Ltd.	4.00
T4	Azadirachtin 0.03%	nimbidine	M/S. T.Stanes and Company Ltd.	5.00
T5	Tracer 480SC	spinosad	Dow Agro Sciences	1.00
T6	Tracer 480SC	spinosad	Dow Agro Sciences	1.50
T7	Decis 2.5% EC	deltamethrin	Bayer Company	1.00
T8	Decis 2.5% EC	deltamethrin	Bayer Company	0.75
T9	Control	unsprayed	-	—

$$\% \text{ Mortality} = \frac{(\text{Population recorded before spray} - \text{Population recorded after spray}) * 100}{\text{population recorded before spray}} \tag{1}$$

Mortality data of the ex-situ experiment were corrected for control mortality as indicated in Eq. (2) (Abbot, 1925).

$$\% \text{ Corrected mortality} = \frac{(\%T - \%C)}{100 - \%C} * 100 \tag{2}$$

Where: T is mortality in treated cages and C mortality in untreated cages.

2.4.2. In-situ experiment

In the in-situ experiments, data on the number of pre-treatment nests with active ant colonies were recorded, and empty nests were removed. After treatment application, at three days interval, the number of evacuated nests was recorded for three consecutive weeks. Also, the numbers of newly constructed nests were recorded after thirty days of treatment application. Data on the number of evacuated nests in the in-situ experiment were converted to proportions of evacuated nests treated to

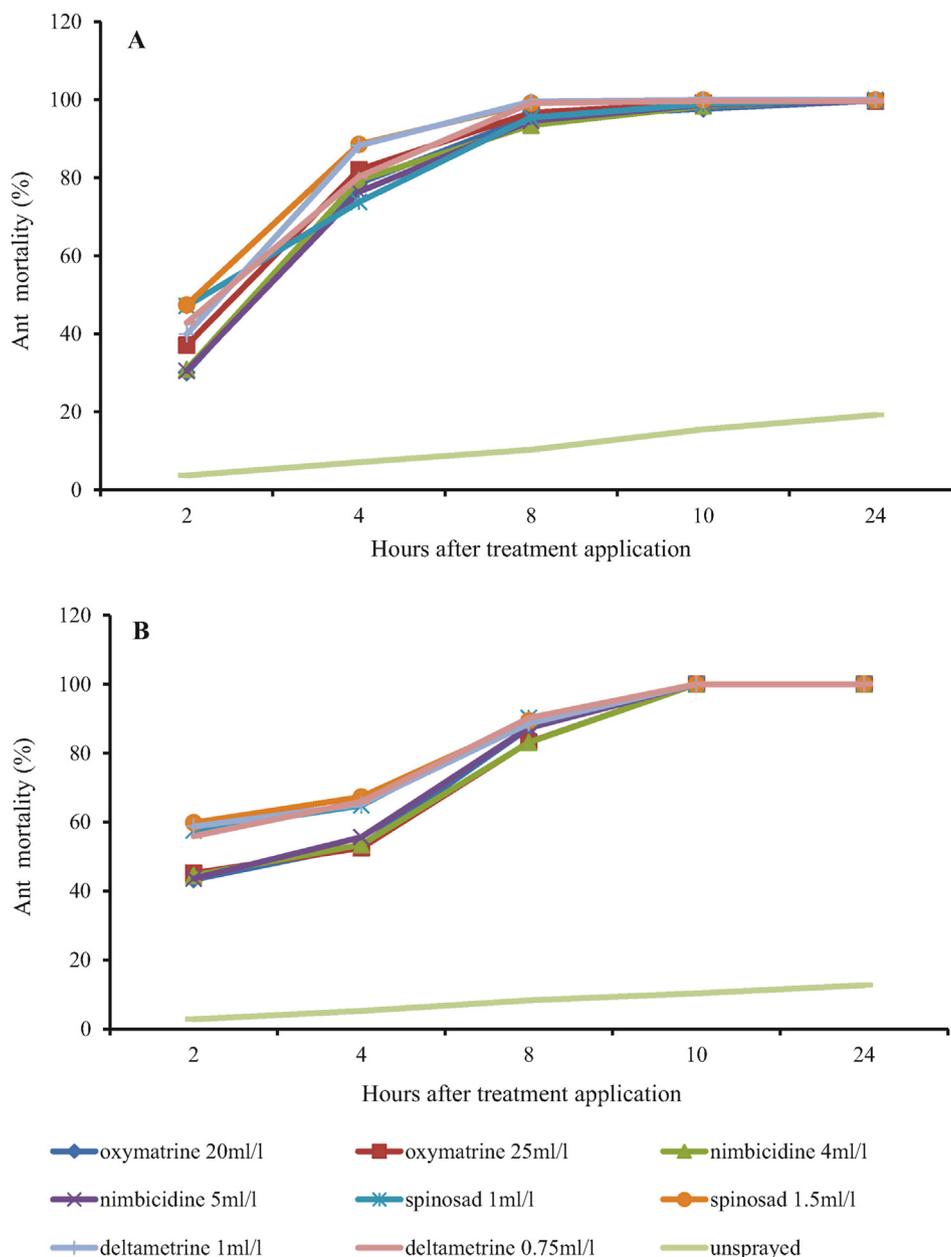


Figure 1. Effects of different insecticides on mortality of biting ant under ex-situ at different hours after application in Experiment 1 (A) and Experiment 2 (B), 2018.

$$\% \text{ of evacuated nests} = \frac{(\text{NoAN recorded before spray} - \text{NoAN recorded after spray}) * 100}{\text{NoAN recorded before spray}} \quad (3)$$

the total number of active nests (nests with ant colony) and expressed as percentages using Eq. (3).

NoAN = The number of active nests of biting ant (nests with biting ant colony)

2.5. Data analysis

For the ex-situ experiment, the cumulative mortality data were used for analysis after being corrected by Abbot's formula for experiment 1 and 2. Before analysis, data were checked for normality (Shapiro Wilk normality test), and all the data met the assumptions. Percent of biting ant mortality in the ex-situ and percent of evacuated and newly constructed nests after treatment in the in-situ experiments were analyzed using the SAS Statistical Analysis Software (version 9.0). Significant differences between the means were determined using Tukey's Studentized Range (HSD) Test.

3. Results and discussion

3.1. Ex-situ insecticides efficacy test

In experiment 1, all the insecticide treatments caused mortality of ants compared to control (Figure 1A) ($p < 0.001$). The percentage mortality of ants in the insecticide-treated cages ranged from 30.1 to 100% (Figure 1A). At 2 h after treatment application, all the insecticides caused an ant mortality of below 50%. However, all the insecticides resulted in a higher percentage of ant mortality (>90%) 8 h after treatment application. Spinosad at 1.5 mL/L and deltamethrin at 1 and 0.75 mL/L caused 100% ant mortality 10 h after treatment application. All the insecticides resulted in over 99% ant mortality 24 h after treatment application (Figure 1A).

In experiment 2, spinosad and deltamethrin at both rates caused significantly ($p < 0.001$) higher percentage mortality of ants ranged 55.9–59.8% compared to oxymatrine and nimbidine (43.3–45.2%) at 2

h after treatment application (Figure 1B). On the other hand, all the insecticides caused 100% ant mortality 10 and 24 h after treatment application (Figure 1B). However, colonies in the untreated cages were alive and only 2.8–12.7% (cumulative values) of the population died within 24 h of application.

Various studies demonstrated the efficacy of insecticides against ants. For example, variation in the susceptibility of ants to fipronil insecticide was reported by Hannum and Miller (2008). They tested the insecticide against the black carpenter ant, *Camponotus pennsylvanicus*, and found 100% mortality colonies of ants 40 h after treatment application. In another study, Nondillo et al. (2014) reported the efficacy of Hydrathylnon (toxic bait) and thiamethoxam (chemical barrier) against *Linepithema micans* (Forel) (Hymenoptera: Formicidae), which is the predominant ant species in vineyards in southern Brazil. The authors also noted that the effectiveness of the treatment depends on the active ingredient used and observed a reduction in the population of ants in the treatment with thiamethoxam one week after application compared with the control treatment.

3.2. In-situ insecticides evaluation

3.2.1. Percent of evacuated nests

The insecticide treatments significantly reduced nest populations of the biting ant under field conditions. The treatments at different levels significantly ($p < 0.01$) reduced the active nests for all the assessed dates (Tables 2 and 3). The percentages of evacuated nests in all the sprayed plots were significantly higher than the control. The percentage of evacuated nests increased along the days after treatment application (Tables 2 and 3). In Farm-1 (South Bench district), the percentage of evacuated nests at 21 days ranged from 52.8% in spinosad 1 mL/L treated plots to 89.9% in deltamethrin 1 mL/L treated plots. However, except spinosad 1 mL/L, all the treatments showed similar performance in the percentage of evacuated nests of biting ants. On the 21st date after treatment, oxymatrine and nimbidine at both rates showed similar efficacy with spinosad and deltamethrin in terms of the percent of evacuated nests of ants (Table 2).

Table 2. Effects of insecticides on nests of biting ant at the in-situ condition in Farm-1 (South Bench district), 2018.

Treatments	Percent of evacuated nests						
	3DAA	6DAA	9DAA	12DAA	15DAA	18DAA	21DAA
oxymatrine 20 mL/L	16.7a	32.4a	44.5a	51.4a	56.8a	62.0a	64.4ab
oxymatrine 25 mL/L	16.9a	36.9a	51.7a	59.5a	66.2a	72.1a	74.8ab
nimbidine 4 mL/L	13.5ab	29.5a	35.5a	42.5a	49.2a	55.8a	58.0ab
nimbidine 5 mL/L	12.0ab	28.5a	38.5a	43.7a	48.4a	55.9a	59.9ab
spinosad 1 mL/L	10.5ab	19.8ab	30.9ab	35.8ab	41.7ab	48.0ab	52.8bc
spinosad 1.5 mL/L	11.7ab	28.8a	42.0a	58.6a	52.3a	57.6a	67.1ab
deltamethrin 1 mL/L	18.7a	38.3a	52.4a	58.6a	69.5a	80.4a	89.9a
deltamethrin 0.75 mL/L	14.8a	28.7a	42.9a	52.2a	61.9a	73.5a	84.6ab
unsprayed control	3.2b	5.7b	8.2b	10.7b	13.9b	16.5b	18.9c
ANOVA							
F value	4.84	6.01	7.63	8.37	8.02	8.28	9.13
DF	8	8	8	8	8	8	8
P =	0.0036	0.0012	0.0003	0.0002	0.0002	0.0002	0.0001

CV = Coefficient of Variation; DAA = days after application; Means with the same letter(s) within a column are not significantly different at 5% level of probability (Tukey HSD). ** = highly significant ($p < 0.01$); * = Significant ($P < 0.05$); ns = non significant.

Table 3. Effects of insecticides on nests of biting ant at the in-situ condition in Farm-2 (Guraferda district), 2018.

Treatments	Percent of evacuated nests						
	3DAA	6DAA	9DAA	12DAA	15DAA	18DAA	21DAA
oxymatrine 20 mL/L	7.5	31.7a	41.6a	58.2a	65.5a-c	69.8ab	73.3bc
oxymatrine 25 mL/L	6.7	26.3a	34.2a	48.2a	56.1bc	61.2b	64.3c
nimbicidine 4 mL/L	6.1	24.8a	33.1a	45.3a	51.3c	55.3b	58.2c
nimbicidine 5 mL/L	9.4	26.9a	34.9a	45.3a	52.6c	57.9b	61.8c
spinosad 1 mL/L	8.2	25.1a	37.9a	53.8a	65.3a-c	70.4ab	75.9a-c
spinosad 1.5 mL/L	5.8	28.8a	35.5a	51.3a	61.6a-c	69.7ab	73.9bc
deltamethrin 1 mL/L	7.0	31.2a	40.7a	57.8a	71.4a	81.7a	91.4ab
deltamethrin 0.75 mL/L	7.5	29.1a	42.5a	55.2a	68.4ab	82.4a	94.9a
unsprayed	3.8	6.8b	9.7b	13.8b	16.7d	19.6c	22.6d
ANOVA							
F value	2.38	7.52	11.89	14.34	29.56	33.47	27.77
DF	8	8	8	8	8	8	8
P<	0.0666	0.0003	0.0001	0.0001	0.0001	0.0001	0.0001

CV = Coefficient of Variation; DAA = days after application; Means with the same letter(s) within a column are not significantly different at 5% level of probability (Tukey HSD). ** = highly significant ($p < 0.01$); * = Significant ($P < 0.05$); ns = non significant.

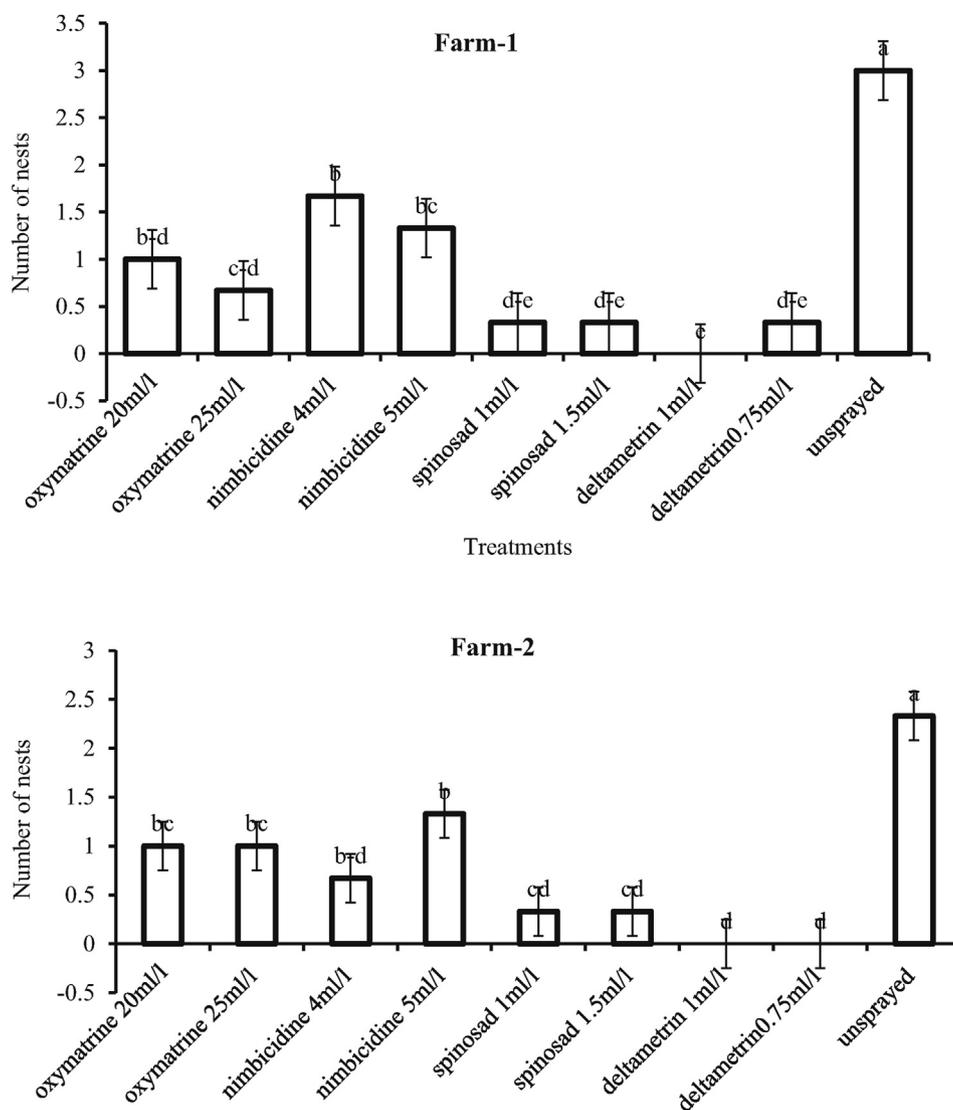


Figure 2. Mean number of newly constructed ant nests per 100 m² plot after thirty days of treatment application at the in-situ condition in the two farms: Farm-1 (South Bench districts) and Farm-2 (Guraferda district), 2018. Values with different letter(s) are significantly different at $p < 0.05$ (LSD test).

In the early days of treatment applications, oxymatrine at 20 and 25 mL/L and nimbecidine at 4 and 5 mL/L significantly increased the percentage of evacuated nests, which were comparable to deltamethrin and spinosad. Unsprayed plots consistently had a low percentage of evacuated nests throughout the assessment periods (Table 2&3). Wakjira et al. (2015) reported that integration of cultural coffee management practice (pruning) with a spray of botanical insecticide (oxymatrine 2.4SL) during the dry season could minimize the biting ant population in the coffee plantation at Tepi.

In Farm-2 (Guraferda district), the percentage of evacuated nests increased along the days after application, with significant differences among treatments (Table 3). Deltamethrin 2.5% EC applied at 0.75 mL/L and 1 mL/L concentration caused the highest nest evacuation from the 6–21 days after treatment application. Teshome et al. (2018) suggested spraying coffee with deltamethrin 2.5% at 1 mL/L combined with handpicking of nests and dipping in detergent as a management option for biting ant in coffee plantations.

3.2.2. Newly constructed nests of biting ant after treatments

After thirty days of treatment application, significantly fewer newly constructed nests were recorded in both farms than unsprayed plots (Figure 2). In Farm-1 (South Bench district), all the insecticide treatments significantly ($p < 0.05$) reduced the number of newly constructed nests compared to the control plot (3 mean number of nests) (Figure 2). Deltamethrin and spinosad at both concentrations showed superior results in reducing the number of newly constructed nests compared to the other treatments.

In Farm-2 (Guraferda district), there was no newly constructed ant nest in coffee trees treated with deltamethrin at both concentrations, while less than 1.5 mean number of nests were recorded in the other treatments per 100 m² plot. On the other hand, 2.3 mean number of nests were recorded in unsprayed coffee trees (Figure 2).

This study demonstrated that all the insecticides reduced newly constructed ant nests in coffee trees after thirty days of treatment application. This might be attributed to the residual toxicity of the insecticides that contributed to the reduced number of newly constructed ant nests. In an earlier study, Kenne et al. (2003) found that spraying insecticides in citrus, guava and mango tree plantations eliminated the dominant arboreal ant. Miguelena and Baker (2014) stated that it is vital to consider the residual activity of insecticides observed in the indoor experiment, which may not be the same if applied outdoors. Most likely, the insecticides lose their efficacy faster. According to Miguelena and Baker (2014), ant mortality using insecticides treatment is important. It opens the possibility for the transfer of the insecticide from exposed to unexposed ants via contacts through social interactions. In Ethiopia, given the traditional coffee production systems coupled with the diverse species of natural enemies played a crucial role in suppressing coffee pests, chemical insecticides are not encouraged for coffee pest control. Therefore, oxymatrine, nimbecidine and spinosad, which are a natural insecticide with reduced risk, contribute to the sustainable management of the biting ants in coffee plantations.

4. Conclusion

The biting ant problem is more severe in the Southwest part of Ethiopia, and it may continue to be a problem unless practical and easily applicable control methods are developed. The present study demonstrated that, in the ex-situ experiment, all the insecticides caused significantly higher mortality of biting ants compared to the untreated control. After 8 h of treatment, over 80% of mortalities were recorded, while total mortality was observed after 24 h of the insecticide treatments. In the in-situ experiment, the percentages of evacuated nests in all the sprayed plots were significantly higher than the untreated control. The percentage of evacuated nests increased along the days after treatment application. At Farm-1 (South Bench district), the percentage of evacuated nests at 21 days of treatment application ranged from 52.8% to

89.9%, while in Farm-2 (Guraferda district) percentage of evacuated nests at 21 days of treatment application ranged from 58.2% to 94.9%. Furthermore, all the insecticides significantly decreased newly constructed nests after 30 days of application in both farms than unsprayed plots. Generally, the evaluated natural insecticides (oxymatrine, nimbecidine and spinosad) were found to have the potential to be used to manage biting ant in the coffee plantation, but further studies on the frequency of spray to use them for sustainable management approaches is required.

Declarations

Author contribution statement

Sisay Kidanu: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ferdu Azerefeagne; Esayas Mendesil: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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