



**ECOLOGY AND MANAGEMENT OF BITING ANT, *Tetramorium
aculeatum* MAYR (HYMENOPTERA: FORMICIDAE) IN COFFEE,
Coffea arabica L. IN SOUTHWESTERN ETHIOPIA**

MSc THESIS

SISAY KIDANU DEMMIREW

**HAWASSA UNIVERSITY
COLLEGE OF AGRICULTURE**

HAWASSA, ETHIOPIA

MARCH, 2019

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SISAY KIDANU DEMMIREW

MAJOR ADVISOR: ESAYAS MENDESIL (PhD)

CO-ADVISOR: FERDU AZEREFEGNE (PhD)

**A THESIS SUBMITTED TO THE SCHOOL OF PLANT AND
HORTICULTURAL SCIENCES**

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**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF MASTER OF SCIENCE IN PLANT SCIENCE
(SPECIALIZATION: CROP PROTECTION)**

HAWASSA, ETHIOPIA

MARCH, 2019

**COLLEGE OF AGRICULTURE
SCHOOL OF PLANT AND HORTICULTURAL SCIENCES**

ADVISORS APPROVAL SHEET -I

This is to certify that the thesis entitled “**Ecology and Management of Biting ant, *Tetramorium aculeatum* Mayr (Hymenoptera: Formicidae) in Coffee, *Coffea arabica* L. in South western Ethiopia**” submitted in partial fulfillment of the requirements for the degree of Master of Science with specialization in **Crop Protection**, to graduate program of the School of Plant and Horticultural sciences, College of Agriculture, and is a record of original research carried out by **Sisay Kidanu Demmirew** under our supervision and no part of the thesis has been submitted for any other degree or diploma. Therefore, we recommend that the student has fulfilled the requirements and hence hereby can submit the thesis to the department.

Esayas Mendesil (PhD)

Name of major advisor

Signature

Date

OR

Ferdu Azerefegne (PhD)

Name of co-advisor

Signature

Date

APPROVAL SHEET-II
Department of Graduate Committee
HAWASSA UNIVERSITY

We, the undersigned, members of the Board of Examiners of the final open defense by **Sisay Kidanu Demmirew** have read and evaluated his thesis entitled “**Ecology and Management of Biting ant, *Tetramorium aculeatum* Mayr (Hymenoptera: Formicidae) in Coffee, *Coffea arabica* L. in Southwestern Ethiopia**” and examined the candidate. This is therefore, to certify that the thesis has fulfillment of the requirements for the degree of Master of Science in Plant Science (Crop Protection).

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DEDICATION

I dedicate this thesis to my family, Kidanu Demmere and Genet Tadesse for their sacrifice in my endeavors and success.

STATEMENT OF THE AUTHOR

First, I declare that this thesis is a result of hard work output and endeavors as well all source materials used for this thesis have been acknowledged. This thesis has been submitted in partial fulfillment of the requirements for the advanced M.Sc. degree at Hawassa University and is deposited at the University Library to be used by other researchers and students as per the rule of Library. I confidentially declare that this thesis is not submitted to any other institution whenever for the reward of any academic, degree or certificate. I have followed all ethical and technical principles of scholarships in the preparation, data collection, data analysis and compilation of this thesis. Any scholarly material that is included in this thesis has been given recognition through citation.

Name: Sisay Kidanu

Signature:

Place: College of Agriculture, Hawassa University, Hawassa, Ethiopia.

Date of submission:

ABBREVIATIONS AND ACRONYMS

BCESC	Bebeka Coffee Estate Share Company
CPDE	Coffee Plantation Development Enterprise
CSA	Central Statistics Agency
CBD	Coffee Berry Disease
DZARC	Debrezeit Agricultural Research Center
ECFF	Environment and Coffee Forest Forum
EIAR	Ethiopian Institute of Agricultural Research
FAO	Food and Agricultural Organization
ICC	International Coffee Council
ICO	International Coffee Organization
JARC	Jimma Agricultural Research Center
RCBD	Randomized Complete Block Design
PLC	Private Limited Company
UCDA	Uganda Coffee Development Authority

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Ecology and Management of Biting ant, *Tetramorium aculeatum* Mayr (Hymenoptera: Formicidae) in Coffee, *Coffea arabica* L. in Southwestern Ethiopia

Sisay Kidanu (B.Sc in Plant Science)
Major advisor: Esayas Mendesil (PhD)
Co-advisor: Ferdu Azerefegne (PhD)

ABSTRACT

Biting ant, Tetramorium aculeatum Mayr (Hymenoptera: Formicidae) is an important pest in coffee that affects farming activities in Southwestern Ethiopia. The objectives of this study were to assess farmers' knowledge and pest management practices of biting ant understand the ecology of biting ant in Southwestern Ethiopia, and evaluate the efficacy and potential of some insecticides. Survey was conducted on 180 coffee farmers in 12 districts. Spatial distribution of biting ant was studied in eight coffee farms and analyzed by semivariance using geostatistics (GS⁺10). Nine insecticides treatments were evaluated both under ex-situ and in-situ conditions in CRD and RCBD designs, analyzed by analysis of variance. Most of the farmers interviewed (58.9%) recognize the problems of biting ant in coffee and some (23.3%) of them are knew this ant for about ten years. Significant variations were observed in the levels of infestation and abundance of the biting ant in different ecology of coffee producing districts and production systems. Lower altitudes and private coffee plantations had higher number of nests. The biting ants within coffee plant are aggregate on the tree. The semivariogram of the biting ant characterized with localized discontinuity and long range correlation in some coffee farms and vice versa in the others indicating spatial dependence of biting ant nests between samples pairs. The spatial distribution trends of the nest in the field showed aggregated along the center and in some farms near corners and declined from there across to the inner field. Under the ex-situ evaluation, all the insecticide treatments caused significantly ($p \leq 0.01$) higher mortality (100%) of biting ants and caused complete mortality within 24 hours. Under field condition the insecticides showed significant variations on percent of evacuated nests. However, the two botanical insecticides, oxymatrine and nimbidine, performed significantly ($p \leq 0.01$) as par with the other tested insecticides up to 18th day after application. On the other hand, deltamethrin 2.5% at the rate of 0.75ml/l of water caused significantly highest percent of evacuated nests and less number of newly constructed nests of biting ant (0.2) after thirty days of application. Generally deltametrin 2.5% at 0.75ml/l can be used for heavily infested farms where pruning and manually destructing the nest difficult and the future it is important to conduct insecticide evaluation with additional botanical based insecticides, and also undertake residual effect analysis of deltamethrin insecticide in coffee environment.

Key words: *Abundance, Aggregate, Agro-ecology, Insecticides, Spatial distribution*

1. INTRODUCTION

1.1. Background and Justification

Coffee (*Coffea arabica* L.) is an important commodity for a number of tropical lower and middle income countries and a relevant source of export revenues and tax collection (ICC, 2018). It is produced by about 25 million farmers worldwide; the vast majority being smallholder producers (Da Silveira *et al.* 2017) and coffee production is fundamental for over 50 developing countries, for which it is the main foreign currency earner (Gichimu and Omondi, 2010). In general, developing countries produce coffee beans, and developed countries import and consume them (Kazuki, 2015). Ethiopia is the largest producer of coffee in Africa and the fifth largest coffee producer in the world next to Brazil, Vietnam, Colombia and Indonesia (ICO, 2017).

Coffee farming in Ethiopia takes place over a vast area, under a wide variety of production systems and with many different cultivation practices. The main coffee growing areas are found within Oromia Region and Southern Nations Nationalities and Peoples' Region (SNNPR) and the majority of coffee production is from main coffee zone of the South west, followed by the Sidama and Yirgacheffe coffee area in the Southeast coffee zone of the country (ECFF, 2017).

In Ethiopia, there are four main coffee production systems: forest, semi-forest, garden and plantation, which account for 8-10, 30-35, 50-55 and 5-8% of coffee production, respectively (Taye, 2013). Forest coffee is a wild coffee grown under the shade of natural forest trees, where as semi-forest coffee farming is a system where farmers thin and select forest trees to let sufficient sunlight to the coffee trees and provide adequate shade. A farmer who prunes and weeds the forest area once a year claims to be the owner of the

semi-forest coffee. Garden coffee is found near a farmer's residence. It is normally fertilized with organic material and usually inter-cropped with other crops. Plantation coffee is planted by the government or private investors for export purposes. Fertilizers and herbicides are usually used in the coffee plantation farming system (Arega, 2006).

In agricultural systems, particularly that of coffee, herbivorous insects has significant impacts on plant productivity and become a constant problem for farmers (Vega *et al.*, 2015). The major insect pests like coffee leaf miner, antestia bug, coffee berry borer and ant (which affect the yield of coffee indirectly) are the one which needs priorities/great attention for management (Million and Bayisa, 1985; Esayas *et al.*, 2008; Wakjira *et al.*, 2015).

Although Million and Bayisa (1985) did not specify the species, they indicated the existence of nuisance ants in Mizan Teferi area, Southwestern Ethiopia. Tebkew *et al.* (2011) reported two arboreal ant species viz. the biting ant (*Tetramorium aculeatum*) and the weaver ant (*Oecophylla longinoda*) at Bebeke and Tepi coffee plantations in Southwestern Ethiopia. These ants were also recorded on coffee and other crops in some parts of Africa particularly in Congo, Tanzania and Uganda (De pury, 1968; Hill, 1975). Both species do not cause direct damage to the coffee plant but indirectly affecting farm activities by biting workers during coffee berry picking and pruning in ant infested farms (Tebkew and Belay 2010). The ant, *T. aculeatum*, also known as *Macromischoides aculeatus*, is an African ant feared by plantation laborers and cause yield loss (Cammaerts and Dejean, 1993). According to Van Emden (2013), the biting ant on coffee builds delicate nests (each containing a few hundred small brown ants) in the bushes. This does not cause direct damage to the crop; neither does the ant promote honey dew 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, 1987; UCDA, 1996; Tebkew *et al.*, 2011; VanEmden, 2013; Fisseha, 2014). The indirect losses attributable to the biting ant alone reach 30%, 29% and exceed 15% in Bebek coffee estate share company plantation (Tebkew *et al.*, 2011; Fisseha, 2014; Wakjira *et al.*, 2015) respectively.

In a recent survey work Wakjira *et al.* (2015) reported that farmers around Tepi area were well familiar with the biting ant and considered the biting ant as a pest because it prevents them from coffee berry picking and cause irritation, swelling and itching lasting for several hours to days. As reported by these authors, the biting ants exist throughout the year in coffee plantations. Moreover, the authors indicated that pruning and smoking management methods were used by farmers to control biting ants in the coffee plantations.

One of the major constraints upon establishing effective pest management approaches for smallholder farmers is the lack of adequate information about farmers' knowledge, perceptions and practices in pest management (Morse and Buhler, 1997). In addition knowledge about spatial distribution of insect pest is important to develop pest management programs (Naeimamini *et al.*, 2014). Furthermore, information on the spatial structure of populations may help in developing rational chemical control and release strategies for natural enemies (Fauvergue and Hopper, 1994).

In order to develop safe and effective control methods against the biting ant, and to minimize effect inflicted by this pest in coffee production system, it is imperative to assess farmers' knowledge and pest management practices. Furthermore, it is very important to study the ecology of biting ant for development of pest management for biting ant. The farmers' knowledge, perceptions, control practices and spatial distribution trend of this ant

pest was not well known before and thus study on the ecology and develop management measures to enhance coffee production and marketing in the country is relevant.

1.2. Research Objectives

1.2.1. General objective

- To study the ecology of biting ant in coffee and potential/efficacy of botanical based insecticides in controlling biting ant in of Southwestern Ethiopia

1.2.2. Specific objectives

- To assess farmers' knowledge and perceptions about biting ant, their damage and management practices.
- To assess the agro-ecological, spatial and within plant distributions of biting ant in Southwestern of Ethiopia.
- To evaluate some insecticides for management of biting ant under ex-situ and in-situ condition

2. LITERATURE REVIEW

2.1. Coffee and Its Economic Importance

Coffee is mostly grown in the tropical and subtropical regions and is grown in 80 countries in over 10 million hectares of land (FAO, 2013) and it is economically important crop grown over 50 developing countries, for which it is the main source of foreign currency (Gichimu and Omondi, 2010). *Coffea arabica* constitute 70% of the coffee that is traded globally and it is considered to be of higher quality and fetches a higher market value than *C. canephora* due to a better aroma and less caffeine levels (Medina *et al.*, 2006). Coffee is an important commodity in many African countries both in terms of export earnings and generating income for smallholder farmers (ICC, 2015).

Ethiopia is one of the main coffee producer as well as consumer in the world, the country produced 420 million kilograms of coffee beans and consumed up to about 220 million kilograms (ICO, 2016), that is, more than half of its total production. In Ethiopia coffee farming provides livelihoods for around 15 million farmers and generates a quarter of the country's export earnings (Moat *et al.*, 2017).

2.2. Formicidae

The Formicidae are ants, social insects which can be easily recognized with narrow waists, bulbous abdomens, and elbowed antennae. Ants live underground, in dead wood, or sometimes in plant cavities. Most ants are black, brown or red (Debbie, 2016). They live in colonies that range in size from few individuals to millions occupying vast space of land. They construct their nests in soils, rotten logs, under bark and on tree trunks (Shattuck, 1999). With few exceptions, ant colonies divide labor between sterile workers, queens, and male reproductive, called alates. Winged queens and males fly in swarms to mate. Once

mated, queens lose their wings and establish a new nest site; males die. Workers tend to the colony's offspring, even rescuing the pupae should the nest be disturbed. The all-female workforce also gathers food, constructs the nest, and keeps the colony clean. Ants perform important tasks in the ecosystems where they live. Some ants defend their plant partners from attacks by herbivores. Feeding habits vary in the ant family. Most ants prey on small insects or scavenge bits of dead organisms. Many also feed on nectar or honeydew, the sweet substance left behind by aphids (Debbie, 2017).

One of the outstanding survival characteristics of the ant is its ability to adapt its self to a varying environment. Another important factors in the survival of ants is their divisions of labors, further division of labor among the workers increases the efficiency of task performance by allowing parallel task processing and specialization on particular tasks such as foraging, defense or broods care (Anonyms, 2017). Furthermore, in the ants, this division of labor is more efficient because functional differences between queens and workers are amplified by morphological differences. Queens (usually winged) start new colonies and produce offspring, while workers (never winged) raise the brood, build the nest, and forage for food (Peeters and Molet, 2010).

Some species are valued in their role as biological pest control agents. Their ability to exploits resources may bring ants in to conflict with humans; however, as they can damage crops and buildings. Like all insects, they have six legs, each leg has three joints and the legs are very strong so they can run very quickly. Ants use their antenna not for smell only, but also for their sense of smell. The head of the ant has a pair of large strong jaws. The jaws open and shut sidewise like a pair of scissors. Adult ants cannot chew and swallow solid foods. Instead they swallow the juice which they squeeze from pieces of food and throw away the dry part that is left over (Anonyms, 2017).

2.2.1. General biology of ants

Ants undergo complete metamorphosis with four stages; egg larva, pupa and adult. The immature forms (eggs, larvae, and pupae) of all sizes and stages of development of most social Hymenoptera are immobile, and completely dependent on adult workers that provide for their needs. However, unlike wasps and social bees, the offspring of ants are kept in a collective nest rather than in individual cells, which prevents them from being properly cared for by workers. Besides the feeding, displacement, and cleaning of the offspring carried out by workers, larvae and pupae also depend on the help of workers in order to hatch from their eggs and emerge from the ecdyses during the moulting process (Lopes *et al.*, 2005).

The wingless worker ants are the most common adults seen. However, there are three types of adults; queens, males and workers. After the mating flight, males die, and inseminated queens disperse in search of potential nest sites such as a dead tree or stump. Here, the newly mated queen excavates a chamber, seals herself in, and begin laying eggs. Colony growth is slow at first and only after several years does the colony reach maturity and begin producing a new generation of winged ants to begin the cycle again (Klotz *et al.*, 2009).

Worker ants, which are sterile females, are rarely winged and often are extremely variable in size and appearance within a given species, polymorphic (many forms or sizes), monomorphic (one form) and dimorphic (two forms). The functions of the worker are to construct, repair and defend the nest and feed the immature and adult ants of the colony, including the queen. In some species is queen is replaced by a daughter queen. Depending on the species, ants can have one or more queens. The sole function of the male is to mate with an unfertilized female reproductive. Males are produced in old or very large colonies

where there is an abundance of food. After reaching maturity, the male usually does not remain in the colony very long (Koehler *et al.*, 2013).

2.2.2. Foraging behavior of ants

The foraging ecology of ants is the result of the collective behavior that leads the colony to find and exploit new food sources. Arboreal ants collect mostly plant-derived food, including nectar and fluids collected from the edges of wounds on leaves, as well as caterpillar frass and lichen. The ants often cluster at the edges of herbivory wounds on leaves, drinking leaf fluids and forage in trees, and rarely descend to the ground. When moving along a trail, between food sources and nests, the ants follow the trails exactly and do not deviate. When exploring, apparently searching for new food sources, ants go up and down every possible stem and branch in the vegetation, and travel all over leaves. Foraging trails move along a convoluted path of vines, twigs, and branches (Hill, 1987).

Most ants eat a wide variety of foods, although some have specialized tastes. Often ants' preference for certain foods will vary throughout the year depending on how much brood is being produced in the nest. Ants use scouts to locate food. When a scouting ant finds promising food, she carries it or a piece of it back to the nest. Some ants leave scent trails composed of various chemical compounds known as pheromones that others can follow to the food source (Koehler *et al.*, 2013). Arboreal and terrestrial ants differed markedly in their preferences for protein or carbohydrate based baits and that arboreal ants preferred protein based baits and terrestrial ants preferred carbohydrate based baits (Daniel and Diana, 2002).

2.2.3. Beneficial effects of ants

Ants play an important role in ecosystem (Folgarait, 1998). Ant biodiversity is high in many coffee plantations and ants attack and prey on many coffee pests, including the

coffee berry borer *Hypothenemus hampei* (Ferrari) (Philpott and Armbrrecht, 2006). Small ants may enter the berry through the beetle entrance hole and predate the larvae and adults inside. Not harvested old berries, infested with the borer may fall to the ground and may serve as reservoir of borer populations, and ant predation at this stage could be very important for limiting the borer in the next season (Pardee and Philpott 2011). Predatory ant can be utilized to control arthropods crop pests especially those found in or on the ground, for example soil pupating diptera and Lepidoptera. Ants were the first insects to be used as biological control agents and are still in use today and they feed on other pests for examples fleas, caterpillars, termites, dead insects and decomposing tissues from dead animals (University of California, 2007). Mansfield *et al.* (2003) observed the use of ants as egg predators of the cotton bollworm *Helicoverpa armigera* Hubner in Australia cotton crops and stated also that fifty species of predacious ants were utilized by farmers for the control of insect nests and include *Oecophylla*, *Dolichoderus*, *Anopolepis*, *Wasmania* and *Azteca* spp.

Some ants are potentially beneficial protecting coffee from pest outbreaks. Individual ant species thus act as important predators in coffee and cacao agro ecosystems, but few studies summarize how the loss of ant diversity with intensification of these multi strata systems may affect their role as predators (Stacy and Inge, 2006). The weaver ant *Oecophylla longinoda* (Latreille) in Africa and *Oecophylla smaragdina* in Asia and Tropical parts of Australia, are tropical arboreal nesting ants that are effective biological control agents due to their highly aggressive and terrestrial behavior. They have been successfully deployed to manage insect pests in a broad range of tropical perennial tree crop agro ecosystems including citrus, cocoa, coconut and mango (Van Mele, 2008).

2.2.4. Harmful effects of ants

Harmful effects of ants to the environmental and humans includes invading and causing damage to buildings destroying crops and stinging humans or in some cases even kill an animal (Anonyms, 2012). Ants interfere with biological control efforts promoting hemipteran populations that cause crop loss (Way, 1963). Many ants chase away pollinators (Altshuler, 1999). Invasive species, those species that demonstrate ecological, environmental or economic impacts (Colautti and Richardson 2009) are now widely recognized as one of the primary threats to biodiversity and the functioning of ecosystems. Invasive ants currently comprise <1% of all known ant species (Lach *et al.*, 2010). Leaf cutting ants considered one of the most destructive herbivores in neo-tropical and sub-tropical forests (Hanson, 1995).

2.3. Coffee Ants

Ants are present in almost all coffee orchards, all the time. Since they feed on the honeydew produced by green scales, ants protect this food generating source against green scale's natural enemies, they do the same for mealy-bugs (Mike *et al.*, 2010). The author also stated that ants do damage to irrigation drip emitters and tubing, to tree bark and perhaps to the root system of coffee trees. Ants are normally not direct pests of coffee, but because of their habit of biting and stinging they can hinder harvesting, pruning and cultivation within the crop (Uganda Coffee Development Agent, 1996; Hill, 1987). Abundance and diversity of predatory arthropods, such as ants, may also be important in controlling populations of coffee pests. Ants are extremely important biological control agents in a number of different agricultural systems including in coffee agro-forests (Philpott and Armbrrecht 2006) and brief descriptions for ants associated with coffee crop are given below.

2.3.1. Leaf-cutting ants (*Atta cephalotes* L.)

Leaf-cutting ants can cause damage on many agricultural crops, notably citrus (*Citrus* spp.), Cacao (*Theobroma cacao*), Cassava (*Manihot esculenta*), Coffee (*C. arabica*), Maize (*Zea mays*) and Cotton (*Gossypium hirsutum*) and forest plantations (Cherrett 1986; Holldobler and Wilson, 1990). Moreover, in addition to direct crop damage, their impacts on agriculture include loss of land surface and destruction of farm roads because of their large colonies, leading to accidents involving machinery and livestock. Total losses caused by leaf-cutting ants are probably in the billions of dollars (Holldobler and Wilson, 1990). Occurrence of species and their population densities, as well as population dynamics, trophic interactions and community structure within a habitat may depend on processes at larger spatial scales than included within individual farms (Kareiva and Wennergren, 1995). In the case of leaf-cutting ants as agricultural pests, the specifics and intensity of these landscape level effects, if known, could contribute to develop ecologically-based management approaches to deal with them. The density, distribution and colony size of *Atta* spp. and *Acromyrmex* spp. are of importance in relation to their potential impact on the ecosystem.

2.3.2. Little fire ant (*Wasmannia auropunctata*)(Roger)

Little fire ants interfere with production in agricultural system such as coffee and citrus where they promote scale insects and interfere with biological control organisms (Fasi, 2009). Workers have refused to harvest or tend orchards infested with this species in Hawaii. Another serious impacts of little fire ant is their affects on subsistence gardens often a mixture of tree (coconut, banana, mango and ground crops (maize, taro, yam etc.). The spread of little fire ants in to coffee plantations will be a huge blow to an industry

already struggling to address the coffee borer beetle, another invasive pest that threatens the coffee industry and reduces its yields (Strohecker, 2012).

2.3.3. African weaver ant (*Oecophylla longinoda*) (Latreille)

Weaver (Tailor) ant is less common than the biting ant but more troublesome in some robusta coffee areas than the biting ant. They also nest on the other trees such as citrus and mango. Adult worker is yellowish red in color about 10mm long. The insects fasten leaves together to certain their nests. Leaves are hold together by fine web of white silk produced only by the larvae. They are the most aggressive and ferocious insects which attack workers in the fields (UCDA, 1996). This ant poses great challenge to harvesting of the pods as well as damage to leaves of kolanut trees. It was reported by Asogwa *et al.* (2011) that Taylor ants (Hymenoptera: Formicidae) weave kolanut leaves to build nest and scale insect are sometimes harbored in these nests for protection against natural enemies. These ants are both agents of dissemination and sources of inoculums for some pathogens (Olunloyo, 1979). High ant incidence in most cases constitutes great nuisance as they bite the farmers and thus disturb normal cultural work on the farm.

2.3.4. Biting ant (*Tetramorium aculeatum* (Mayr.))

In the Afrotropical and Malagasy regions, *Tetramorium* is hyperdiverse by the definitions of Wilson (2003). *T. aculeatum* is small ant, which is frequently, found nesting in coffee trees. The adults are characterized by having two sharp, posterior pointing spines at the back of the thorax. The immature stages are confined solely to the nest and are of no direct importance as pests. When nests are disturbed by movement of the leaves the ants rush forth with great activity (McNutt, 1975). In Uganda these insects are particularly common on Robusta coffee but apparently do not damage the coffee, nor do they seem to encourage infestations of scale insects or mealy-bugs. However, at picking and pruning, laborers can

be so severely bitten by ants that they often refuse to work. Thus actual bite appears to be a combination of both biting and stinging and causes severe skin irritation. The wound is a compound one consisting of a bite by the mandibles followed by formic acid injection in to it, making it most irritating. This ant is not saccharophilic and does not encourage infestation of scales and mealy bugs (Hill, 1987; Tebkew *et al.*, 2009).

According to Tebikew *et al.* (2009) biting ant undergoes complete metamorphosis. The egg is white in color and circular in shape with estimated size of approximately one fourth of the size of teff seed. It is accumulated in one direction inside the nest and not common to see eggs in the nest when it is compared to larva and pupae. Larvae are maggot like with sharp head and wide at the back. The mature larva reaching the pupal stage has easily observable yellow head. The larvae vary in size some are big and the others are small. Bigger sized larvae are the future king and queens while smaller sized larvae are the future workers. Larvae at different larval instars were observed in a single nest. The different larval instars can be identified based on their length, thickness and color. The smallest instar larvae have light reddish and the largest instar has light blackish colored line at the back of larvae. Larvae pass through 5-6 instars. The exarate pupa has yellow head and white body. Mature pupa is reddish in color. Adult: The adult reaches 3-4mm of size and has brown abdomen and black body. The presence of a similar species with bigger size up to 5mm) and two spines like structure on the upper part of the thorax has been reported (Hill, 1987; Tebkew *et al.*, 2009). The queen of biting ant category is bigger and has wing (Tebikew *et al.*, 2009).

2.4. Management Methods of Ants

2.4.1. Cultural

Increasing the diversity of plants favors the establishment of beneficial fauna, especially birds, contributing to the natural suppression of ants (University of California, 2013). Weed control, clearing of any dried and fallen trees, cutting coffee branches touched the ground, coffee tree pruning and coffee shade regulation or reduction are some of the agronomic practices helps in reduction of ant nests (Tebkew *et al.*, 2009).

2.4.2. Physical and Mechanical

Disturbance of nests or berries that preclude the access of foragers to nutrient resources represents an easy and inexpensive way to protect plants grown on a small scale and in urban gardens. The use of wool, sticky paste and grease for bearings temporarily prevents ant foraging on plants (Ballari and Farji, 2006).

2.4.3. Ant poisoned baits

Baits are effective because they take advantage of the ants' natural foraging and feeding behavior. Because ants orally transfer food between colony members, the active ingredient contained in bait, is naturally spread throughout the colony (Williams *et al.* 2001), but moist conditions and rainfall can decrease the efficacy of baits (Kafle *et al.* 2009). Baits containing integrated growth regulators (fenoxycarb and methoprene) generally take 3-5 months to suppress populations (Wakjira *et al.*, 2015). The probable reason why ants refrain themselves from entering the poisoned baits is that victimized individuals might have released certain chemical/s that warns their live con-specifics. Mutton poisoned with carbaryl 185wp killed significantly more number of biting ants (30ants/tree) than the other

insecticides. However, the ants do not carry food and die before returning to their nests (Tebkew and Belay, 2010).

2.4.4. Chemical methods

A spray insecticide gives the satisfaction of seeing dead ants and insecticides are applied by most farmers in different formulations: powders, granular and liquid baits (Combrony, 1992). The author also reported that dusting or spraying the branches with malathion (0.6 – 1%) or with 0.1% diazinon or with parathion powder at 1% spray at 0.5% were found effective for the control of *O. longinoda*, *T. aculeatum* and ant genus *Atrpomyrmex* (Carpenter ants) that directly harmful to the bush due to the excavated galleries and other harmful Brazilian species cut young shoots and young leaves (Combrony, 1992). Comparison of three insecticides Ethiolathion (malathion) 50% EC, Ethiozinon/Diazinon/ 605EC and Fenitrethion/ Ethiotrethion/ 50EC direct spraying to control biting ant at Bebek coffee plantation in southwestern Ethiopia showed that Ethiozinon / Diazinon 60% EC at a rate of 1.6 litre per hectare gave a better control 92% (Teferi *et al.*, 2004).

3. MATERIALS AND METHODS

3.1. Description of the Study Areas

A survey of biting ant was conducted in the major coffee producing areas of Southwestern Ethiopia from September to October of 2017. The majority of coffee production is from the largely forested main coffee zone of the Southwest of the country (ECFF, 2017). Jimma Zone is one of the four zones known for its high coffee production and lies between latitudes 7°15' N and 8°45' S and longitudes 36° 00' E and 37°40' E. The zone generally lies in altitude ranges between 900 and 3334 m above sea level. As a result, Jimma zone enjoys several types of agro-ecological zones from moist to dry arid zones (Addisu *et al.*, 2016). The Sheka zone has three agro-ecologies (lowland, midland and highland) between 7° 24' to 7° 52'N and 35°13' to 35°35'E at an altitude ranging from 900 to 2800 masl (Belachew *et al.*, 2016).

Iluababora zone is found in Oromia regional state, out of its land, 10% is highland, 67% is midland and 23% is lowland and its altitudes ranges from 500 to 2575 masl (Teshager *et al.*, 2013). The ex-situ and in-situ experiment were conducted at BCESC plantation, is located in SNNPR region in Benchmaji zone, 595km to southwest of Addis Ababa. Out of the total area 10,030 hectares owned by the company, over 5,252 hectares is 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 masl, mean annual rainfall 1800 mm and the mean maximum and minimum temperature are 27.5°C and 16.43°C (BCESC, Undated).

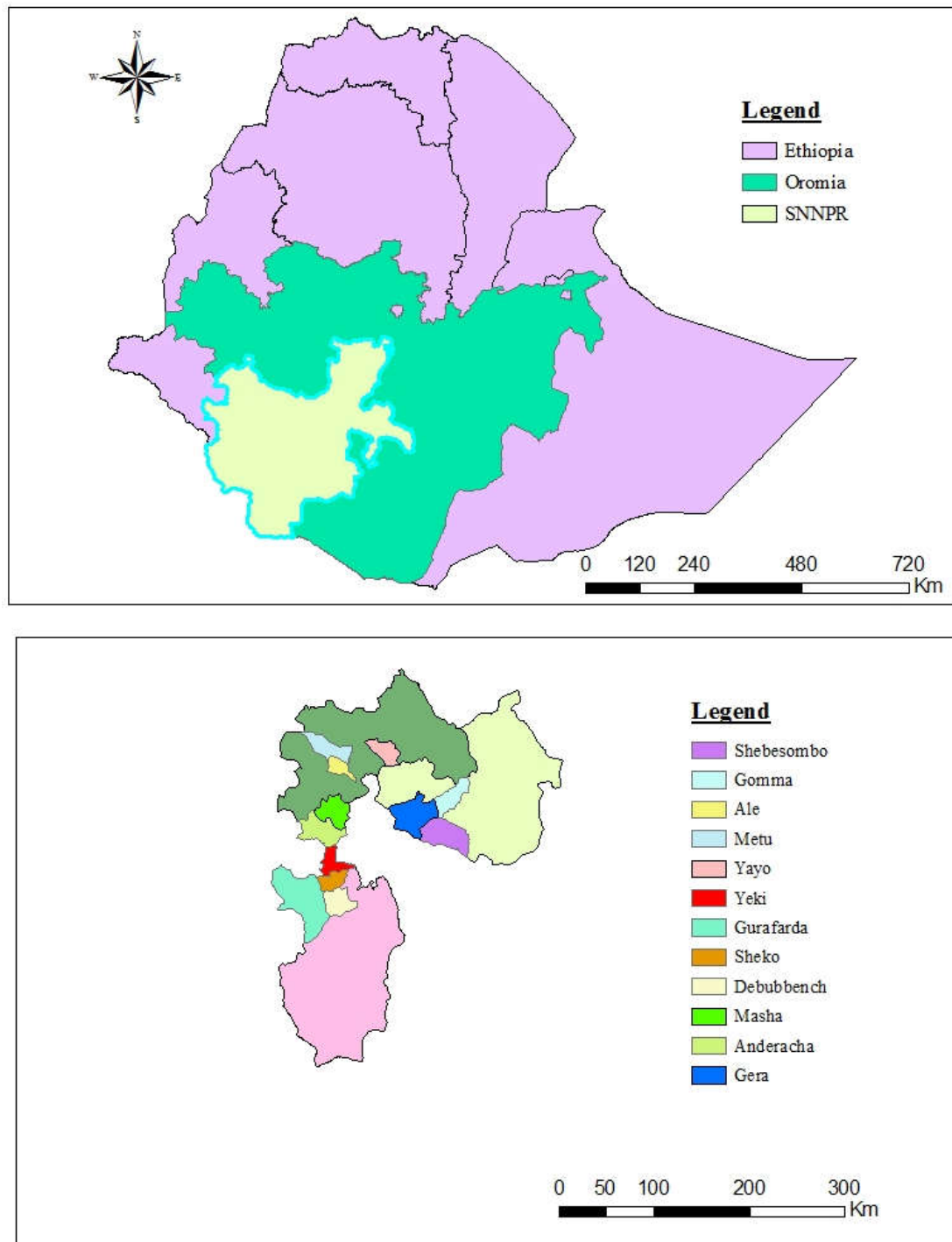


Figure 1. Map of the study areas in southwest of Ethiopia

3.2. Farmers' Knowledge of Biting ant and Management Practices

Three stages sampling were used to select respondents for the interview. In the first stage, four major coffee producing zones were selected purposively based on coffee production potentials (Jimma, Iluababora, Benchmaji and Sheka). In the second stage, sample districts were selected purposively based on coffee production potentials. From Jimma zone (Gomma, Gera and Shebe districts), from Iluababora zones (Metu, Yayo and Gore districts) from Sheka zone (Yeki, Masha and Gecha) and From Benchmaji (Debub bench, Sheko and Guraferda districts) were selected(Fig.1). In the third stage, from each districts three kebeles were selected and five households were selected from each kebeles randomly and a total of 180 respondent farms were monitored and interviewed about biting ant. Semi-structured questionnaire was used during the survey to gather information about demographic characteristics of the respondents, agronomic practices, farmers' knowledge and management practices of biting ant.

3.3. Agro-ecological Based Distribution of Biting ant

Systematic random sampling procedures were used in the farm described above in section 3.2, and GPS data were also recorded (Table1). The survey areas were categorized in to three altitudes groups, Low 1000-1500; Midland 1500-1750; High above 1750 masl (Tadesse, 2017) and also in to three shade levels category (High > 50 trees per acre, Moderate 21-50 trees per acre and low 0-21 trees per acre (Jonsson *et al.*, 2015). Abundance and percent of infestation of the ant for each group were calculated by averaging the records from fields within different groups. The same procedure was followed to determine Abundance and percent of infestation for different coffee production systems.

Table 1. List of surveyed regions, zones, districts, their coordinates and altitudes 2017

Region	Zones	Districts	No. of Farms	Latitude (N)	Longitudes(E)	Altitudes (m)
SNNPR	Benchmaj	South bench	15	6°52'48"-6°57'20"	35°21'39"-35°28'25"	991-1293
		Guraferda	15	6°46'04"-7°05'176"	35°11'42"-35°19'45"	1083-1410
		Sheko	15	7°02'02"-7°07'18"	35°22'55"-35°26'10"	1060-1750
	Sheka	Yeki	15	7°10'55"-7°11'17"	35°25'09"-35°25'17"	1189-1209
		Anderacha	15	7°28'31"-7°37'02"	35°18'25"-35°24'26"	1218-1784
		Masha	15	7°29'25"-7°46'17"	35°22'25"-35°33'40"	1730-2020
Oromia	Ilubabor	Metu	15	7°52'51"-8°19'23"	35°29'04"-35°35'25"	1558-1780
		Alle	15	8°05'31"-8°10'33"	35°30'24"-35°32'53"	1769-1959
		Yayo	15	8°21'34"-8°23'37"	35°47'42"-35°52'41"	1350-1488
	Jimma	Gera	15	7°46'57"-7°47'14"	35°36'25"-36°36'26"	1910-1969
		Gomma	15	7°49'37"-7°50'50"	36°35'37"-36°35'52"	1596-1695
		Shebsombo	15	7°29'25"-7°31'83"	36°23'98"-36°29'09"	1461-1790

In each farm, 20–40 coffee trees were selected randomly from different sampled sites which were spaced at least 10 to 20m apart. Canopy of each of tree was divided in to three approximately equal sections and labeled as upper, middle and lower. The farms were situated within 5 to 10 km of each other depending on coffee farm availability. The total number of ant nests, nests with colony and nests without colony in each canopy section were counted and recorded. Finally, one nest with colony per plant (on three plants) was randomly selected to determine colony composition (the number of eggs, larvae, pupae, adult and reproductive forms).

3.3.1. Correlation and Regression

A simple correlation analysis was done to assess the association of various geo-climatic factors with biting ant percentage infestation and abundance. Complete ten years rainfall, maximum temperature and minimum temperature data were available for eight of twelve surveyed districts and hence only these areas geo-climatic factors were included in the correlation. Geo-climatic data were obtained from the National Meteorology Agency,

2017. Multiple regression analysis was conducted to determine the effects of rainfall, maximum and minimum temperature, altitudes and shade level on biting ant infestation.

3.4. Spatial Distribution in the Field

The study was conducted at Bebek coffee estate share company plantation, where high number of *T. aculeatum* nests was recorded during the survey. The experimental site remained free from pesticides treatments during the study period, but all other recommended agricultural practices were performed. Eight coffee farms were systematically selected from the coffee plantation and assessment and recordings were done from four different coffee varieties. Coffee trees in these fields were planted 2x2m spacing, their ages ranged from 9 -12 years coffee plants during the study. In each field, using Garmin 60 GPS for coordinate's data, biting ant nests were counted in rectangular quadrates (6 x 6 m) at 4m interval in a 60 x 60 m grid for a total of 36 samples per field.

3.5. Spatial Distribution within Tree

Thirty coffee trees of similar phenology and field management were systematically selected as sample trees. Geisha coffee variety was used for distribution study, since this variety harbored high number of *T. aculeatum* nests (Personal observation). The canopy of each tree were partitioned in to two strata of upper and lower halves, with each stratum split in to four directions, namely North, West, South and East. Hence, there were a total of eight sections in one tree for observations. From each section, ten secondary branches were randomly chosen, making a total of 80 branches per tree for estimation of the *T. aculeatum* nest density. Counting and recording for the presence of nests was conducted in situ as the number of nests for each tree. Since the sample data consisted of the number of nests per branches and the total number of branches were 80 per tree.

3.5.1. Distribution Pattern

Variance – to – mean ratio (s^2/x), which is the simplest index of dispersion and the most fundamental, indicates a random dispersion when it is equal to 1.0; a uniform dispersion when it's less than 1.0 and an aggregated when it is more than 1.0 (Myers, 1978).

3.6. Evaluation of Insecticides against Biting Ant

3.6.1. Treatments and Experimental Design for Insecticides Evaluation

The insecticides evaluation experiment was conducted in highly *T. aculeatum* infested farm identified during the distribution survey, at BCESC coffee plantation. It was carried out on coffee variety that highest ant infestations observed. Four insecticides each at two rates were evaluated and totally nine treatments, together with unsprayed control were used in the experiments (Table 2). Under ex-situ condition the biting ants were collected with their nests and placed inside the plastic bucket of 20cm x 10cm x 10 cm and each chemical concentration were applied and the cages were laid on 1.50m x 1.5m x 0.75 m table. The experiment was arranged in completely randomized design with five replications and it was repeated after three weeks. The ants were observed at two hour interval to record the mortality (Wakjira *et al.*, 2015).

The in-situ study was conducted at two different locations of the BCESC coffee plantation using randomized complete block design (RCBD) with three replications. The space between plots and blocks were 4 and 6 m, respectively. All sprayings were made using manually operated knapsack sprayer of 15 liters capacity for each treatment. Prior to treatment application, ant nests were checked and trees with colony nests marked. A 10 mx10 m area was used for each treatments application under field condition. Unsprayed plots (trees with active nests) were also included as a control. Data on number of pre treatment nests with active ant colony nests were recorded and empty nests cleared. After

application, numbers of evacuated nests were recorded for three consecutive weeks and the number of newly constructed nests also recorded after thirty days of application.

Table 2. Lists of insecticides treatments evaluated both at ex-situ and in-situ conditions

Treatment codes	Trade name	Common name	Rates used ml/lt of water
T1	Prosuleroxymatrin 2.4%	oxymatrine	20.0
T2	Prosuleroxymatrin 2.4%	oxymatrine	25.0
T3	Azadirachtin 0.03%	nimbidine	4.00
T4	Azadirachtin 0.03%	nimbidine	5.00
T5	Tracer 480SC	spinosad	1.00
T6	Tracer 480SC	spinosad	1.50
T7	Decis 2.5% EC	deltamethrine	1.00
T8	Decis 2.5% EC	deltamethrine	0.75
T9	Control	Un sprayed	---

The conventional volume in coffee 400 L. ha⁻¹ of water (Junior *et al.*, 2012) was used for spraying.

Under insecticides efficacy evaluation under ex-situ experiment, the number of dead ants in each replicate was converted in to proportions of the total number of introduced treated ants and expressed as percentage. The data was compiled and percent mortality was calculated through formula (Karar *et al.*, 2010):

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

Mortality data of ex-situ experiment were corrected for control mortality using Abbott's correction formula (Abbott, 1925).

$$\% \text{ Mortality} = \frac{(\%T - \%C)}{100 - \%C} * 100$$

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

For ex-situ experiment, cumulative (mean of 1st and 2nd round experiment) data was used for analysis. Data from in-situ experiment was converted to proportions of evacuated nests of the treated total number of active nests (nests with ant colony) and expressed as percentage. Before analysis, data were checked for the normality and all the data met the assumption except, number of newly constructed nests after spray under in-situ experiment. Data of newly constructed nests were square root transformed prior to statistical analysis, in order to stabilize the variances.

3.7. Data Analysis

Descriptive statistics and comparative statistical tools including chi square were used to compare the different socio-demographic and farm characteristics, knowledge on biting ant and pest management practices of farmers across the surveyed districts. The survey data was analyzed using Statistical Package for Social Science (SPSS) version 20.0 software where as correlation and regression between geo-climatic factors, altitude, shade level and *T. aculeatum* nest infestation percent were analyzed using the SAS Statistical Analysis Software (version 9.0) and Minitab 17 software respectively. Stepwise regression analysis was performed to select the best explanatory variables.

Spatial distribution of insects in the fields, abundances (number of nests per tree) were plotted at each x-y coordinate using GS⁺10 software and geostatistics, a method to analyze the degree of spatial dependence among samples (Liebhold *et al.*, 1993). Spatial patterns of abundance were evaluated by means of the semivariance $\gamma(h)$, calculated for each specific distance h :

$$\gamma(h) = \frac{1}{2N(h)} \sum [z(x_i) - z(x_{i+h})]^2$$

Where $z(x_i)$ is the abundance at point x_i , $z(x_{i+h})$ is the abundance at point x_{i+h} , and $N(h)$ is the total number of sample pairs that are separated by a distance h . The resulting plot of $\gamma(h)$ versus all possible distances between sample pairs is called the semivariogram and describes the spatial structure of the population sampled. The semi-variograms (computed from *T. aculeatum* nests counts) were produced using GS⁺¹⁰ version software (Gamma Delta Software 2004). Isotropic semivariograms were calculated for all data sets. Semivariograms were computed using active lag distance (the range over which autocorrelation calculated). The fits of spherical, exponential, linear and Gaussian model identified. Then the three model parameters, the nugget variance (the y intercept of the model), the Sill (the model asymptote), the range (the separation distance over which spatial dependence is apparent; m) for the model best fitting my data displayed.

For the analysis of spatial distribution of nests within trees, initially, the data was analyzed to determine the trends of population distribution based on the variance to mean ratio. To determine the distribution of nests in relation to height (strata) and orientations (directions), the data (mean number of nests per branch in each section) were subjected to analysis of variance. The experimental design was split-plots, with a stratum as the main plot (n=2), orientation as the sub-plot (n=4) and trees as blocks (n=30).

Data from ex-situ and in-situ experiments were analyzed using the SAS Statistical Analysis Software (version 9.0). For in-situ combined analysis of variance over locations was done to see the insecticides x location interaction effects, the homogeneity of error variance was tested using Bartlett test and met the assumption of ANOVA. Significant differences between the means were determined using Tukey Studentized Range (HSD) Test.

4. RESULTS AND DISCUSSION

4.1. Farmers' Knowledge of Biting ant and Management Practices in Southwest Ethiopia

4.1.1. Demographic and Socio-economic characteristics of the farmers

Among the surveyed farmers, the majority were male (76.7%), this was also the case in all the surveyed districts except for Guraferda where about 53% of the respondents were female households (Table 3). This indicates that men dominated coffee farm activities in southwestern Ethiopia. Similarly, Schepers (2014) in his/her survey in Northwest of Cameroon reported that male dominated in coffee farming households and stated that this unequal representation could be explained due to the general view coffee is a 'Mans' crop and women are mainly engaged in food crops such as beans and maize. Women run coffee farming inherited, when their husband deceased as the author reported. There was highly significant difference in age of the farmers across surveyed districts. The majority of the interviewed farmers were between 20 and 50 years of age (93.9%). In the south western of the country farmers practice coffee productions for different purposes. Most (70%) of the farmers grow coffee both for market and home consumption, whereas about 28.9% of the farmers produced coffee for market only, and these are mostly coffee plantation owners and 1.1% of the farmers for consumption only (Table 3).

Table 3. Demographic and Socio-economic characteristics of the respondents during the survey (n=180)

Variables	Districts													χ^2
	South bench	Gura ferda	Sheko	Yeki	Ande- racha	Masha	Metu	Alle	Yayo	Gera	Gomma	Shebe sombo	Overall mean	
Gender(%)														
Male	93.3	46.7	93.3	93.3	80.0	80.0	80.0	73.3	80.0	80.0	53.3	66.7	76.7	20.49*
Female	6.7	53.3	6.70	6.70	20.0	20.0	20.0	26.7	20.0	20.0	46.7	33.3	23.3	
Age (%)														
<20years	0.0	6.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	67.01***
b/n20-50	60.0	66.7	100	100	100	100	100	100	100	100	100	100	93.9	
>50years	40.0	26.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.6	
Purpose of production														
Market	100	0.00	0.0	66.7	93.3	73.3	0.0	0.0	0.0	13.3	0.0	0.0	28.9	1.76***
Consumption	0.0	0.0	0.0	0.0	0.0	0.0	6.7	0.0	0.0	6.7	0.0	0.0	1.1	
For both	0.0	100	100	33.3	6.7	26.7	93.3	100	100	80	100	100	70	
Farming experience														
<10years	13.3	46.7	46.7	20.0	20.0	33.3	40.0	40.0	20.0	20.0	73.3	13.3	32.2	24.32*
>10 years	86.7	53.3	53.3	80.0	80.0	66.7	60.0	60.0	80.0	80.0	26.7	86.7	67.8	

Statistically significant * at $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

4.1.2. Farm characteristics and Agronomic practices of the Farmers

Highly significant variations exist in coffee cropping systems across the study districts (Table 4). Majority of the farmers planted coffee as a sole crop (78.45%) and about 1.7 and 2.2% of them grew coffee as an intercropping and mixed pattern, respectively (Table 4). From the surveyed fields, most of the coffee trees were five to ten years old (55.6%) whereas 3.3, 28.3 and 12.8% of the coffee plants were less than five years, between 10-20 and greater than 20 years, respectively.

Coffee farmers planted different varieties in the study districts. There was highly significant variation across districts in the use of coffee varieties (Table 4). About 46.1% of the farmers used local coffee type, whereas 53.9% of them used improved varieties. Majority of the farmers in Guraferda, Sheko, Alle, Yayo and Shebe sombo districts used local varieties, whereas farmers in South bench, Yeki, Anderacha, Metu, Gera and Gomma most farmers used improved varieties. The sources of local varieties were from local market and improved varieties were usually obtained from government organization such as research centers, agricultural offices and farmer' unions.

The majority (84.4%) of the farmers used permanent shade trees in their coffee farms, whilst 15.6% of the farmers used temporary shade type. In about 56.7% of the assessed farms, coffee were grown under medium shade level, whereas 19.4 and 23.9% of the farms were shaded with low and high shade level, respectively (Table 4). Philpott (2005) reported that reducing or eliminating shade cover in Coffee agro-forestry systems affects pest outbreaks. Thus shade tree pruning largely affect certain aspects of arboreal ant communities in coffee agro-forestry system, with important implications for biological control. The author also stated that shade tree pruning also caused a dramatic change in the abundance of the most common ants such as *Crematogaster spp.*

Table 4. Farm characteristics and agronomic practices of respondents during the survey 2017

Variables	Districts													χ^2
	South bench	Gura ferda	Shek o	Yeki	Ande- racha	Masha	Metu	Alle	Yayo	Gera	Goma	Shebe sombo	District mean	
Cropping System (%)														
Coffee as sole	100	73.3	100	80.0	100	100	100	100	100	100	100	100	96.1	78.45**
Intercropping	0.0	0.0	0.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7	
Mixed pattern	0.0	26.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.2	
Coffee Plant age (%)														
<5 years	0.0	0.0	0.0	0.0	0.0	13.3	6.7	13.3	6.7	0.0	0.0	0.0	3.3	67.02**
5-10 years	46.7	53.3	33.3	73.3	86.7	60.0	80.0	53.3	26.7	60.0	66.7	26.7	55.6	
10-20 years	46.7	40.0	26.7	13.3	13.3	26.7	13.3	33.3	26.7	13.3	26.7	60.0	28.3	
>20 years	6.7	6.7	40.0	13.3	0.0	0.0	0.0	0.0	40.0	26.7	6.7	13.3	12.8	
Variety used %														
Local variety	0.0	93.3	100	20.0	0.0	26.7	13.3	93.3	93.3	6.7	20.0	86.7	46.1	119.90***
Improved var.	100	6.7	0.0	80.0	100	73.3	86.7	6.7	6.7	93.3	80.0	13.3	53.9	

Table continued....

Variables	Districts													χ^2
	South bench	Gura ferda	Shek o	Yeki	Ande- racha	Masha	Metu	Alle	Yayo	Gera	Goma	Shebe sombo	Distric t mean	
Variety source %														
Local	0.0	86.7	100	13.3	0.0	26.7	13.3	93.3	93.3	6.7	20.0	86.7	45.0	119.13 ***
Organizations	100	13.3	0.0	86.7	100	73.3	86.7	6.7	6.7	93.3	80.0	13.3	55.0	
Shade type %														
Permanent	100	86.7	100	53.3	53.3	86.7	80.0	100	93.3	66.7	93.3	100	84.4	38.91* **
Temporary	0.0	13.3	0.0	46.7	46.7	13.3	20.0	0.0	6.7	33.3	6.7	0.0	15.6	
Shade level %														
Low	0.0	0.0	20.0	0.0	20.0	20.0	33.3	20.0	40.0	20.0	26.7	33.3	19.4	39.14*
Medium	53.3	80.0	46.7	73.3	73.3	60.0	66.7	60.0	26.7	60.0	60.0	20.0	56.7	
High	46.7	20.0	33.3	26.7	6.7	20.0	0.0	20.0	33.3	20.0	13.3	46.7	23.9	
Statistically significant * at p<0.05, **p<0.01, ***p<0.001														

4.1.3. Farmers knowledge and perceptions about biting ant

More than half of the respondents (58.9%) were aware of about biting ant, but there were significant differences between the districts. All interviewed farmers in Southbanch, Guraferda, Sheko and Yeki districts had knowledge about biting ant and 93.3% of the farmers in Anderacha districts were aware of this ant. These farmers were able to identify biting ant based on their common behavior that they bind two or more coffee leaves to construct their nest and during disturbance rush out to bite. About 23% of the farmers knew this ant in their farms for about 10 years, about 21% of the farmers for five years, and 14.4 % of them for over 20 years in their coffee farms. According to Wakjira *et al.* (2015) all the interviewed farmers in the surveyed areas of southwestern Ethiopia reported that they knew the biting ant since their childhood and all these farmers considered this ant as a pest because it prevents them from collecting coffee due to its bite that cause burning, swelling and itching.

Most (47.8%) farmers reported that the biting ant occurred in all coffee phenology throughout the year (Table 5). This result corroborates the findings of Wakjira *et al.* (2015) who reported the occurrence of ants throughout the year in the coffee plantation according to the interviewed farmers. Half of the farmers (50.6%) considered biting ant as a pest causing damage indirectly through interfering different agronomic and management practices via biting the workers embarked which leads to loss coffee yield and the binding of two to three leaves together for nesting may lead to leaf damage and drop (Table 5).

Table 5. Farmers' knowledge and perceptions about biting ant in Southwestern Ethiopia

Variables	Districts													χ^2
	South bench	Gura ferda	Sheko	Yeki	Ande-racha	Mash a	Metu	Alle	Yayo	Gera	Goma	Shebe sombo	District mean	
Know this ant %														
yes	100	100	100	100	93.3	40.0	66.7	20.0	0.0	0.0	6.7	80.0	58.9	123.82
No	0.0	0.0	0.0	0.0	6.7	60.0	33.3	80.0	100	100	93.3	20.0	41.1	***
Since when ant know (%)														
Before 5 yrs	0.0	26.7	46.7	13.3	33.3	33.3	53.3	20.0	0.0	0.0	6.7	20.0	21.1	1.76**
Before 10 yrs	60.0	26.7	33.3	60.0	26.7	6.7	13.3	0.0	0.0	0.0	0.0	53.3	23.3	
Before 20 yrs	40.0	46.7	20.0	26.7	33.3	0.0	0.0	0.0	0.0	0.0	0.0	6.7	14.4	
Don't know	0.0	0.0	0.0	0.0	6.7	60.0	33.3	80.0	100	100	93.3	20.0	41.1	
Plant stage this ant occur %														
Flowering	0.0	0.0	0.0	0.0	0.0	6.7	0.0	0.0	0.0	0.0	0.0	0.0	0.6	2.36**
Maturity	0.0	0.0	0.0	0.0	0.0	0.0	20.0	0.0	0.0	0.0	0.0	0.0	1.7	*
Harvesting	0.0	20.0	0.0	0.0	13.3	6.7	0.0	6.7	0.0	0.0	0.0	60.	8.9	
In all stage	100	80.0	100	100	80.0	26.7	46.7	6.7	0.0	0.0	6.7	26.7	47.8	
Don't know	0.0	0.0	0.0	0.0	6.7	60.0	33.3	86.7	100	100	93.3	13.3	41.1	
Damage cause %														
Interfere works	0.0	6.7	0.0	0.0	0.0	6.7	0.0	0.0	0.0	0.0	6.7	66.7	7.2	2.34**
Intact leaves	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.7	0.0	0.0	0.0	0.0	0.6	
All listed above	100	93.3	100	100	93.3	33.3	66.7	6.7	0.0	0.0	0.0	13.3	50.6	
Don't know	0.0	0.0	0.0	0.0	6.7	60.0	33.3	86.7	100	100	93.3	20.0	41.7	

Statistically significant * at $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

4.1.4. Management methods of biting ant

Farmers were using different methods to manage biting ant in their coffee farms. Farmers are cognizant that farm management experiences have influence on the ant and the experience could be used to build farmers understanding to manage the biting ant. In this study, about 27.4% of the farmers practice pruning to reduce the ant infestation in their fields and the rest destroy nests manually using wooden sticks (9.4%), smoking (6.6%) and about 19.8% of them used both pruning and nest destruction by stick.

On the other hand, 35.8% of the interviewed farmers did not use any management practices (Table 6). Wakjira *et al.* (2015) reported pruning as the management methods used by 50% of the respondents where as 3% of them used smoking and about 47% did not practice any control measure against biting ant.

Table 6. Management methods farmers practiced to biting ant in the surveyed districts

Variables	Districts													χ^2
	South bench	Gura ferda	Shek o	Yeki	Ande- racha	Mash a	Met u	Alle	Yay o	Gera	Gom a	Shebe sombo	District mean	
Control practices (%)														
Smoking	0.0	6.7	6.7	0.0	0.0	16.7	0.0	100	0.0	0.0	100	15.4	6.6	2.34 ***
Manual stick	0.0	0.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	53.8	9.4	
Pruning	0.0	6.7	0.0	93.3	57.1	83.3	9.1	0.0	0.0	0.0	0.0	0.0	27.4	
All above	0.0	0.0	6.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	
All from 2-3	100	0.0	0.0	0.0	42.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	19.8	
No control	0.0	86.7	66.7	6.7	0.0	0.0	90.9	0.0	0.0	0.0	0.0	30.8	35.8	
Statistically significant * at p<0.05, **p<0.01, ***p<0.001														

4.2. Agro Ecological Based Distribution of Biting ant

The common signs for presence of biting ant are the nests of ants constructed by binding a single, two or more coffee live or dead leaves together (Fig. 2). During the current survey biting ant occurred in almost all surveyed coffee fields of Southwest Ethiopia with considerable variations in their distribution in the surveyed districts (Table 7). The proportion of infested farms ranged from 6.7 (Masha district) to 100% (Southbench) in SNNPR and 0 (Gera) to 60% (Metu) in Oromiya region. There was also a variation in percentage of biting ant nest infestation in surveyed farms. In SNNPR the highest biting ant nest infestation was recorded in Benchmaji ($69.8 \pm 6.9\%$), whereas the lowest infestation was recorded in Masha ($1.3 \pm 1.0\%$). In Oromia region, the highest infestation was observed in Metu ($13.3 \pm 3.7\%$), whilst the lowest in Goma ($2.3 \pm 1.1\%$). On the other hand, no ant nest infestation was observed in Gera (Table 7). The average number of biting ant nests per coffee tree ranged from 0.03 in Masha to 11.5 in South bench in SNNPR, whereas in Oromia region it ranged from 0.01 in Gomma to 0.45 biting ant nests per coffee tree in Metu (Table 7).

The highest number of ant nests was recorded in Benchmaji zones in SNNPR, especially Southbench district. This district is characterized by modern coffee plantations. In modern plantation, the use of chemical inputs and mono-cropping of coffee, may affect natural enemies that might contribute for suppression of ant population. Over all, biting ant was high at lower altitude of SNNPR districts. About 91.67% of the surveyed districts had varied levels of nest incidences which indicate that the ant has the potential to hamper productivity of coffee.

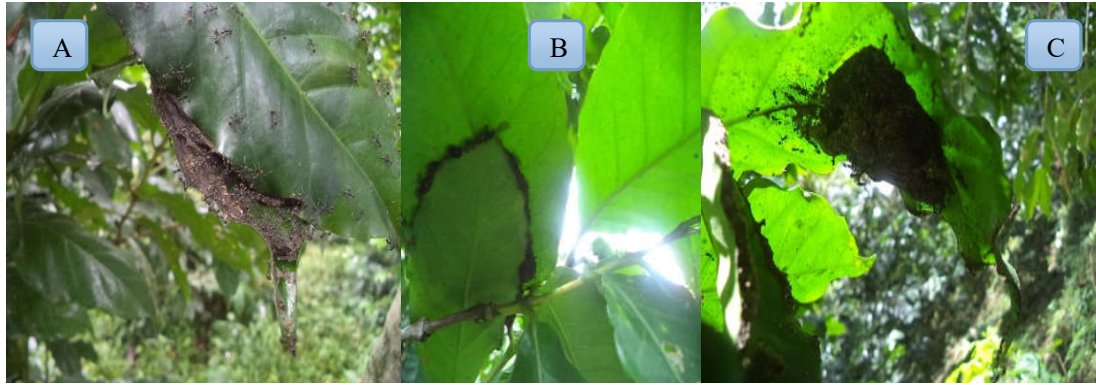


Figure 2. Biting ant nests binding three coffee leaves (A), binding two leaves (B) & single leaf(C)

Among the coffee fields surveyed, the lowest proportion of infested fields, biting ant infested coffee plants and abundance was recorded in Gera (null) followed by Gomma and Masha districts (Table 7). Generally, out of the twelve surveyed coffee growing districts, eleven were infested by biting ant, with the highest proportion of infested fields and biting ant infestation in South bench district, and the lowest in Masha and Gomma districts. It was also observed that unlike other surveyed districts, in Gomma biting ant nests were built on shade trees rather on coffee trees. High infestation of biting ants in those surveyed areas might be due to coffee management practices, and due to different species of coffee shade trees, which might harbor biting ants in the farms. Earlier studies have indicated that the biting ant was most common and abundant in southwestern of Ethiopia (Million and Bayisa, 1985; Wakjira *et al.*, 2015).

In addition, in study conducted in Bebek coffee estate share company (Southbench district), Tebkew *et al.* (2009) reported mean number of biting ant per tree ranged from 0.35-3.65, 0.80 – 7.65 and 1.15 - 9.90 in lower, middle and upper canopy, respectively.

Table 7. Distribution of biting ant in different surveyed districts of Southwest Ethiopia

Regions	Zones	Districts	No. of surveyed fields	% of infested farms	% of biting ant nest infestation	Abundance of biting ant nest
SNNPR	Benchmaj	South bench	15	100	69.8 ± 6.9	11.5
		Guraferda	15	73.3	46.7±8.3	3.74
		Sheko	15	80.0	41.0±7.9	2.10
	Sheka	Yeki	15	73.3	22.0±6.6	1.20
		Anderacha	15	53.3	11.7±4.1	0.43
		Masha	15	6.7	1.3±1.0	0.03
Oromia	Ilubabor	Metu	15	60.0	13.3±3.7	0.45
		Alle	15	33.3	2.7±1.6	0.14
		Yayo	15	33.3	3.7±1.6	0.08
	Jimma	Gera	15	0.0	0.0±0.0	0.00
		Gomma	15	13.3	2.3±1.1	0.01
		Shebsombo	15	26.8	7.3±4.4	0.22
Mean				46.11	18.48	1.66

Note: % of infested field = (Number of infested fields/number of total surveyed fields)*100

% of biting ant infestation = (Number of infested tree/total number assessed tree)*100

Abundance = Mean number of biting ant nests per coffee tree

Different levels of biting ant infestations were observed among the four coffee production systems. In coffee plantation system high percent of infested fields (53.3%) was recorded followed by garden (38%), and semi forest production systems (36.8). On the other hand, the lowest infestation was observed in forest production system (23.5%) (Fig.3). Low level biting ant infestations in the forest production system might be attributed to minimum human interference such as low/no pesticide use that might enhance the effect of natural enemies. In Mexico, Philpott (2006) observed ant patchiness in coffee agro ecology varied based on management intensity where coffee intensification affects the occurrence of

dominant ants species in coffee plants. Million (1987) also reported that insect pests in intensive coffee plantation is a challenge and this is mainly due to changes in cultural practices associated with new varieties planted in the plantation.

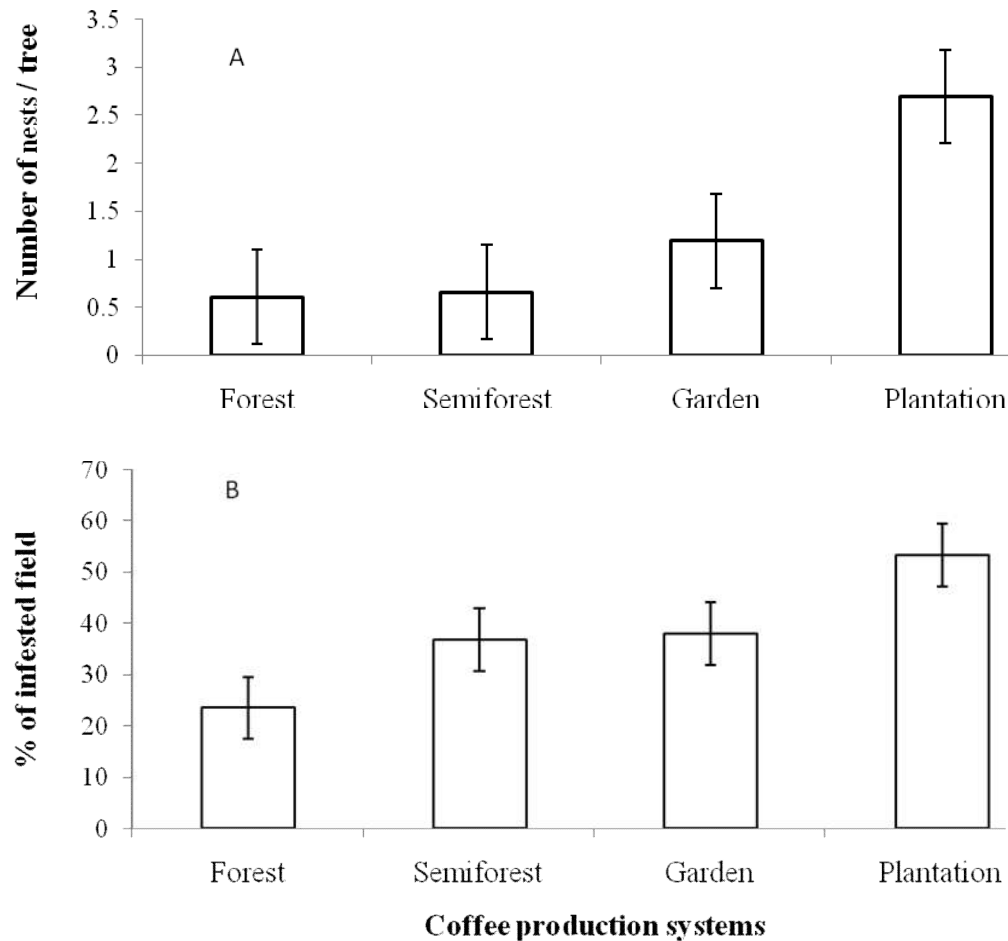


Figure 3. Nest abundance ($\pm$ SE) (A) and Percent of infested field ($\pm$ SE) (B) of *T. aculeatum* in the four coffee production systems in Southwestern Ethiopia 2017

At BCESC plantation different species of shade trees were planted including Wanza (*Cordia africana*), Silky Oak (*Grevillea robusta*) birbira (*Milletia feruginea*), Sesa (*Albizia gummifera*), bisana (*Croton macrostachus*). It was noted that the biting ant dwells more on heavily shaded coffee trees as its nests are less disturbed (destroyed) by rainstorms.

Tebkew and Belay (2010) observed that *M. ferruginea*, *G. robusta* and *Ficus* sp harbor biting ant, however coffee trees under heavy shade had greater number of nests than the under light shade which indicate that the intensity of shade is more important in determining coffee infestation. In this study it was also observed that the biting ant also dwells on black pepper (*Pepper nigrum*), rubber tree (*Hevea brasiliensis*), *Citrus* spp., and guava (*Psidium guajava*).

A colony in a particular nest had a number of worker population and may or may not have reproductive forms (queen/king). Mean number of colony composition in a nest in South bench district were 14.7 workers , 1.2 eggs, 2.5 larvae, 0.9 pupae and 0.04 reproductive forms (Fig. 4 and 5). Except in few instances, most colonies or broods have no reproductive forms. According to Mueller *et al.* (2001) a single colony of biting ant can colonize up to 12 adjacent trees.

Ant colonies with broods in the middle canopy had more reproductive forms. Reproductive forms might not be always found with broods; suggesting that the reproductive forms may be either sexually immature or the time was not reproduction season (Fig. 5). Males of biting ant swarm at the beginning of a wet sunny season, which is a conducive time for the solitary queen to establish her first colony (Leston 1979).

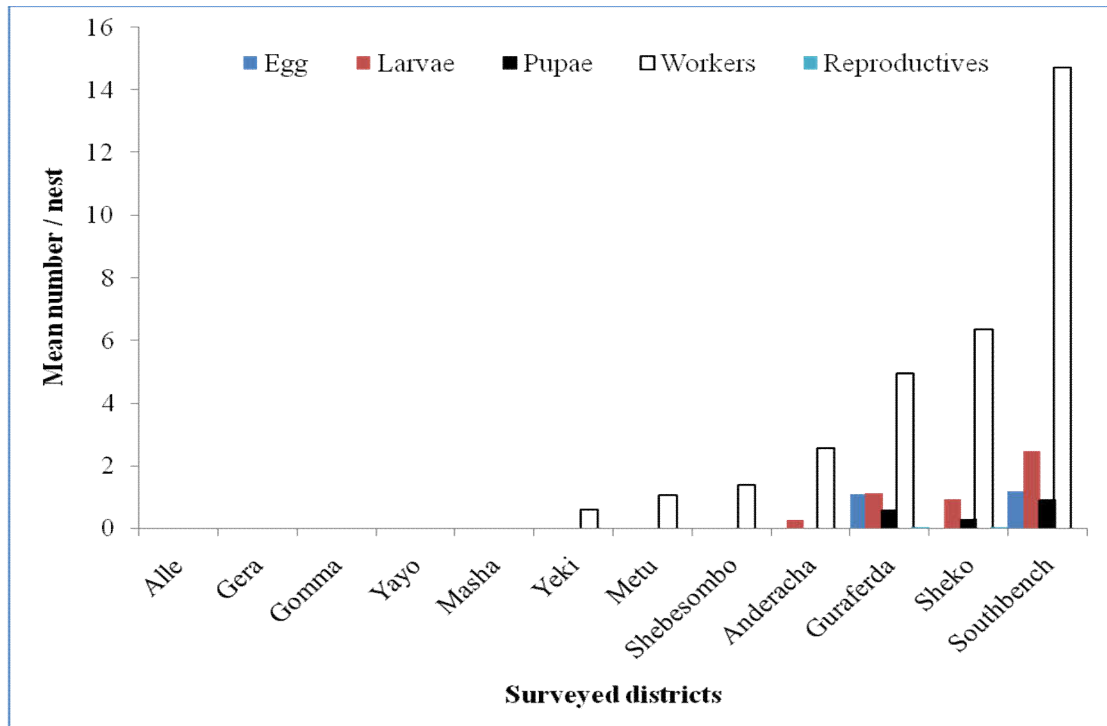


Figure 4. Mean colony composition of biting ant per nest in Southwestern Ethiopia in 2017

4.2.1. Correlation analysis

Minimum temperature and shade level showed significant and positive correlation to percentage infestation of biting ant (Table 8). Beer *et al.* (1998) indicated that shade trees create hostile conditions for pests and harbor a variety of predatory insects. There were significant and negative correlations between biting ant abundance and altitude. In addition, percent infestation was positively correlated with biting ant abundance (Table 8). This result was in agreement with Wakjira *et al.*, (2016) who observed the distribution of biting ant was higher at lower elevation (Bebeka 1100 m.a.s.l) and lower at higher elevation (Teppi 1200 m.a.s.l). Furthermore, Fisseha (2014) found higher number of biting ant active nests under shaded coffee farms compared to the un-shaded. The author also reported that some shade tree species such as *Gravilia robusta* and *Ficus* spp. attract the biting ant and the shade trees may also be infested by this ant.

Table 8. Pearson Correlation Coefficients among biting ant distributions with rainfall, temperature, different altitudes and shade levels in southwest of Ethiopia

	RF	MAXT	MINT	ALT	SHL	INF	ABU
RF	1.00	-0.35 ^{ns}	-0.17 ^{ns}	0.39 ^{ns}	0.01 ^{ns}	-0.01 ^{ns}	0.10 ^{ns}
MAXT		1.00	0.42 ^{ns}	-0.93*	0.17 ^{ns}	0.44 ^{ns}	0.39 ^{ns}
MINT			1.00	-0.52 ^{ns}	0.34 ^{ns}	0.61*	0.41 ^{ns}
ALT				1.00	-0.27 ^{ns}	-0.49 ^{ns}	-0.57*
SHL					1.00	0.56*	0.47 ^{ns}
INF						1.00	0.66*
ABU							1.00

* =Significant at P<0.05; **= significant at P<0.01; ns=non significant; RF= Rainfall; MAXT=Maximum temperature; MINT= Minimum temperature; ALT = Altitude; SHL = Shade level; INF=Percent of infestation; ABU= Abundance of nests

Source of meteorological data = National Meteorological Agency, 2017.

4.2.2. Regression analysis

The results revealed that, 38.80% of the variability of infestation of biting ant was explained by one of the best explanatory variables. In the regression analysis all the explanatory variables did not show any significant differences except minimum temperature (Table 9). The adjusted R^2 for the analysis was relatively low (34.88%), indicating the weakness of the model in explaining the total variation in the infestation percent. Multicollinearity causes a failure in the proper specification and in the effective estimation of the structural association in the dependent and independent variables that can be explained by the regression analysis. In such cases, one or two best explanatory variables could carry adequate information that appropriately explains the dependent

variable, while the rest of the explanatory variables carry redundant or unnecessary information. Hence such variables need to be dropped by selecting the best explanatory variables (Esayas and Abush, 2009). The best explanatory variable was selected using step wise regression and the result showed that only minimum temperature was important in explaining the total variation in biting ant infestation percent (Table 9 and 10). Highly significant regression coefficient (11.97%) was obtained for the minimum temperature when only minimum temperature was fitted in the model. Therefore, the result showed that minimum temperature has the most significant influence on the infestation rate of biting ant in coffee. Weather may directly influence the physiology or behavior of an insect or indirectly affect the insect population through its effect upon host plant and natural enemies (Martinat, 1987).

Table 9. Analysis of variance based on stepwise regression analysis

Source	DF	Sum square	Mean square	F-value	P-value
Regression	1	5677	5677.1	8.24	0.013
Min T	1	5677	5677.1	8.24	0.013
Error	13	8955	688.8		
Total	14	14632			

Final model: Constant + Minimum Temperature

$R^2 = 38.80\%$ Adjusted $R^2 = 34.09\%$

Table 10. Estimates of the regression coefficient and t- probability for the explanatory variable selected by stepwise regression analysis

Parameter	Estimate	Standard error	T-statistics	T-probability
Constant	-122.3	56.6	-2.16	0.050
MinT	11.97	4.17	2.87	0.013

The fitted regression model was: Y (% of infestation) = $-122.3 + 11.97(\text{minimum temperature})$

4.3. Spatial Distribution of Biting ant in the Fields and Within Coffee Tree

4.3.1. Spatial distribution of biting ant nests in the field

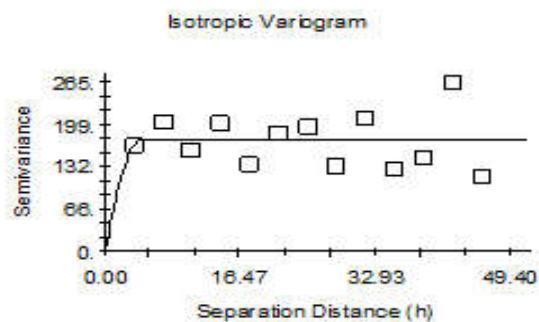
4.3.1.1. Autocorrelation of biting ant nests (Semivariogram)

Omni directional semivariograms (isotropic) spatial dependence among samples for each measurement of the ant nest abundance and the spatial dependence proved with semivariograms in a different range of ant nest in this study.

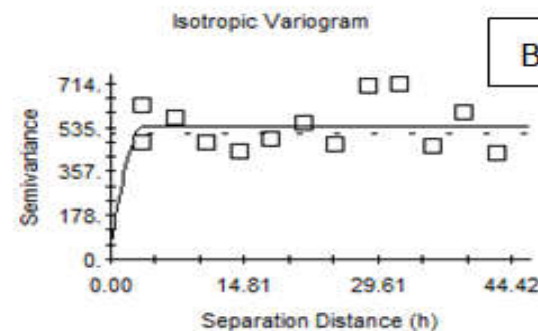
In this study biting ant spatial distribution analysis showed a low nugget value and long range of correlation in some farms, whereas high nugget effect and short range correlation in the other (Table 11) indicating high variations in the number of biting ant nests at the intercept points in some farms and increased with distances between sample pairs (Fig. 5). Those phenomena are characterized as well behaved or spatially continuous. Srivastava (1996) stated that variograms enable us to judge the independence of infestations in neighboring plants, and the author also stated that an increasing variogram is the sign of correlation between neighboring plants. Under these assessments, the range of spatial dependence (distance between data pairs at which spatial independence is reached) varied greatly among the data sets from 3.52 to 53.03 m (Table 11) suggesting that there were interdependence of samples showing the existence of spatial dependence between sample pairs in number of biting ant nests within the ranges. Out of all parameters, the range is the most important to pest management mapping because it defines the extreme outermost distance within which samples must be placed from each others.

In spatial distribution study of coffee variety Geisha in MF-4 farm, isotropic semivariogram with a range of 4.23m and Spherical model fitted (Fig. 5abc). Similarly, in farms MF-4 and 1, Geisha variety semivariogram showed a range of 3.5 and 3.52m both

spherical models fitted respectively (Fig. 9 and 10) suggesting that most of the spatial dependence of biting ant nests between samples occurred under 4.23m on Geisha coffee varieties in a different farms. After 4.23 meters no more spatial dependence exists. Localized discontinuity spatial structure was observed on Geisha variety confirming that most of the spatial dependence occurred at short distances (Fig. 5abc). Fisseha (2014) stated that biting ant prefer Geisha coffee variety, which could be due to thick foliage serve as habitat conditions that favorable sites for nesting and seasonal migration. On the other hand, on farm planted with Catimor variety biting ant nest spatial distributions in farm MF-1 showed a range of 47.65 and 6.61m and exponential model and spherical models fitted (Fig. 5d and 6e). Such a spatial structure could be caused by localized high densities. In farm MF-3 and 2 of coffee variety 7440, isotropic semivariogram with the range of 13.36 and 43.36m and Gaussian models fitted (Fig. 6fg), suggesting moderate spatial dependence where as variety F-59 of farm MF- 2, isotropic semivariogram with the range of 53.07m and Linear model fitted (Fig. 6h), showed the semivariogram was with high localized discontinuity (nugget value). Lasmar *et al.*, (2012) reported that leaf cutting ant nests distribution semivariograms analysis showed moderate spatial dependence on eucalyptus, which was due to the high nugget effect value. Random distribution occurs when environmental conditions are similar at any point in space and the presence of one organism does not affect one other (Begon *et al.*, 1996).



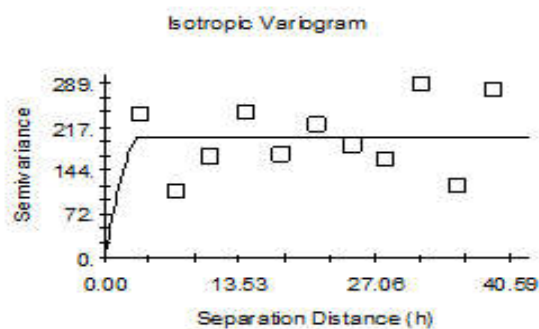
A



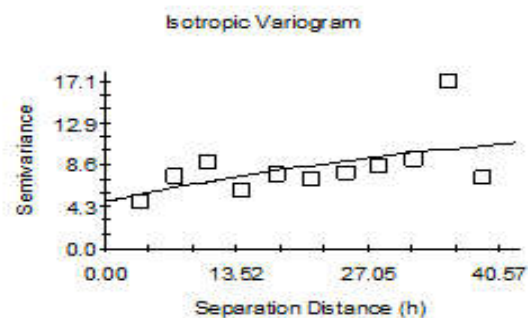
B

Spherical model ($C_0 = 0.10000$; $C_0 + C = 172.90000$; $A_0 = 4.23$; $r^2 = 0.002$; $RSS = 20827$.)

Spherical model ($C_0 = 60.00000$; $C_0 + C = 540.90000$; $A_0 = 3.54$; $r^2 = 0.000$; $RSS = 114066$.)



C



D

Spherical model ($C_0 = 2.10000$; $C_0 + C = 199.20000$; $A_0 = 3.52$; $r^2 = 0.000$; $RSS = 36199$.)

Exponential model ($C_0 = 4.83000$; $C_0 + C = 15.05000$; $A_0 = 47.65$; $r^2 = 0.284$; $RSS = 71.8$.)

Figure 5. The autocorrelation and model fit biting ant nests for Geisha (A, B & C) and Catimor (D) varieties of different farms in BCESC

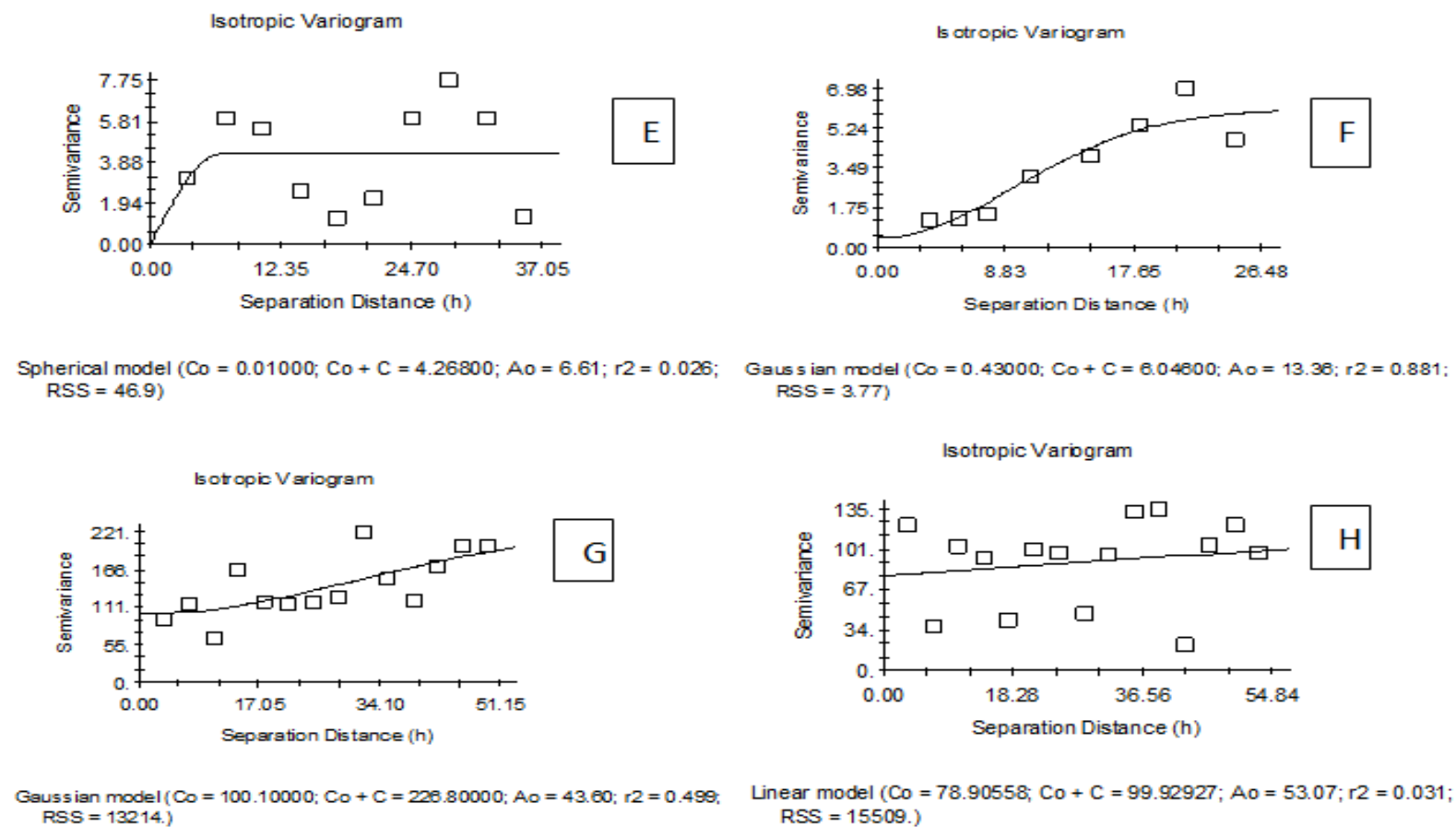


Figure 6. The autocorrelation and model fitted for biting ant nests for Catimor (E), 7440 (F&G, and F-59(H) varieties of different farms in BCESC

4.3.1.2. Interpolation of biting ant nests

The spatial distribution pattern of biting ant nest population trends showed varies spatial structures in different farms. In some farms high localized discontinuity spatial structure were observed at the corners and declined from there across the field's (Fig. 8ad and 9gh) where as in some farms continuous similar nest densities observed along the edge (Fig.8e,f,g and h). This is probably because of the ability of biting ant developing nests on several plants adjacent to the coffee farms. The presences of different species of coffee shade trees in the farms may help the ant (queens) to locate sites on the tree which dwells on it. In addition to shade trees, black pepper was planted mostly at the border of coffee farms and the ants were abundant on this crop binding its leaves as nests (Fig. 7) and such abundance around the center and edges of farms might be migration and different environment factors effects.

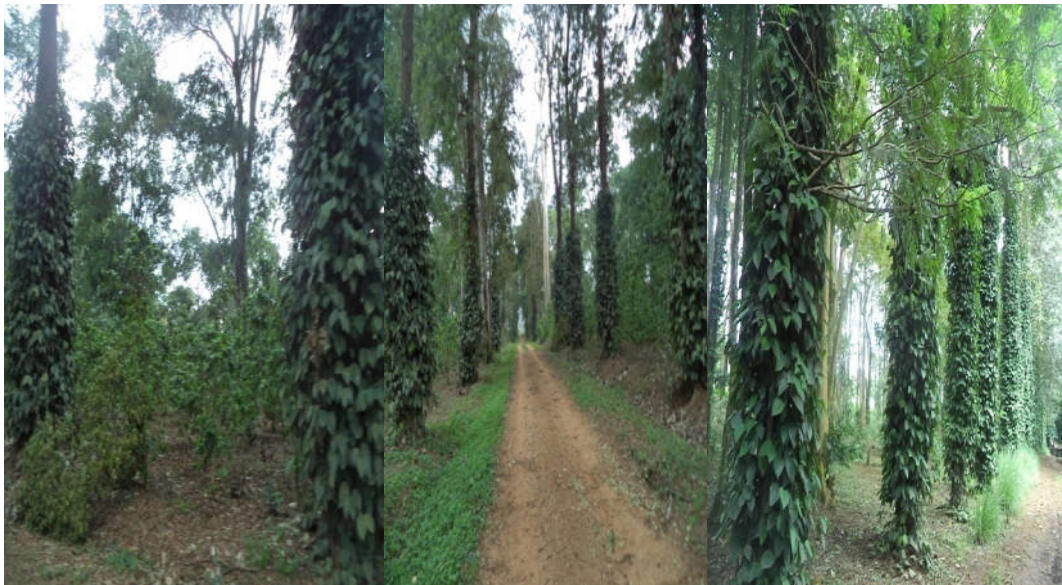


Figure 7. Coffee farms border planted with black pepper spice crop (Source photo: Sisay, 2018)

The interpolation result also showed localized high density of biting ant nests at the farm centers surrounded by low continuous densities in the half of the farms (Fig.8a and 9e), suggesting that lack of proper agronomic practices such as imbalance of shade level could contribute to aggregate to the center of the farm. *M. ferruginea*, *G. robusta* and *Ficus* sp, *C. macrostachus* and *C. africana* harbor biting ant, however coffee trees under heavy shade had greater number of nests than under light shade which indicate that the intensity of shade is more important (Tebkew and Belay, 2010; Fisseha, 2014). In some farms aggregated high localized density of nests observed both at the corner and center of the fields and varies densities in the other part of the fields (Fig.8 a, b and d).

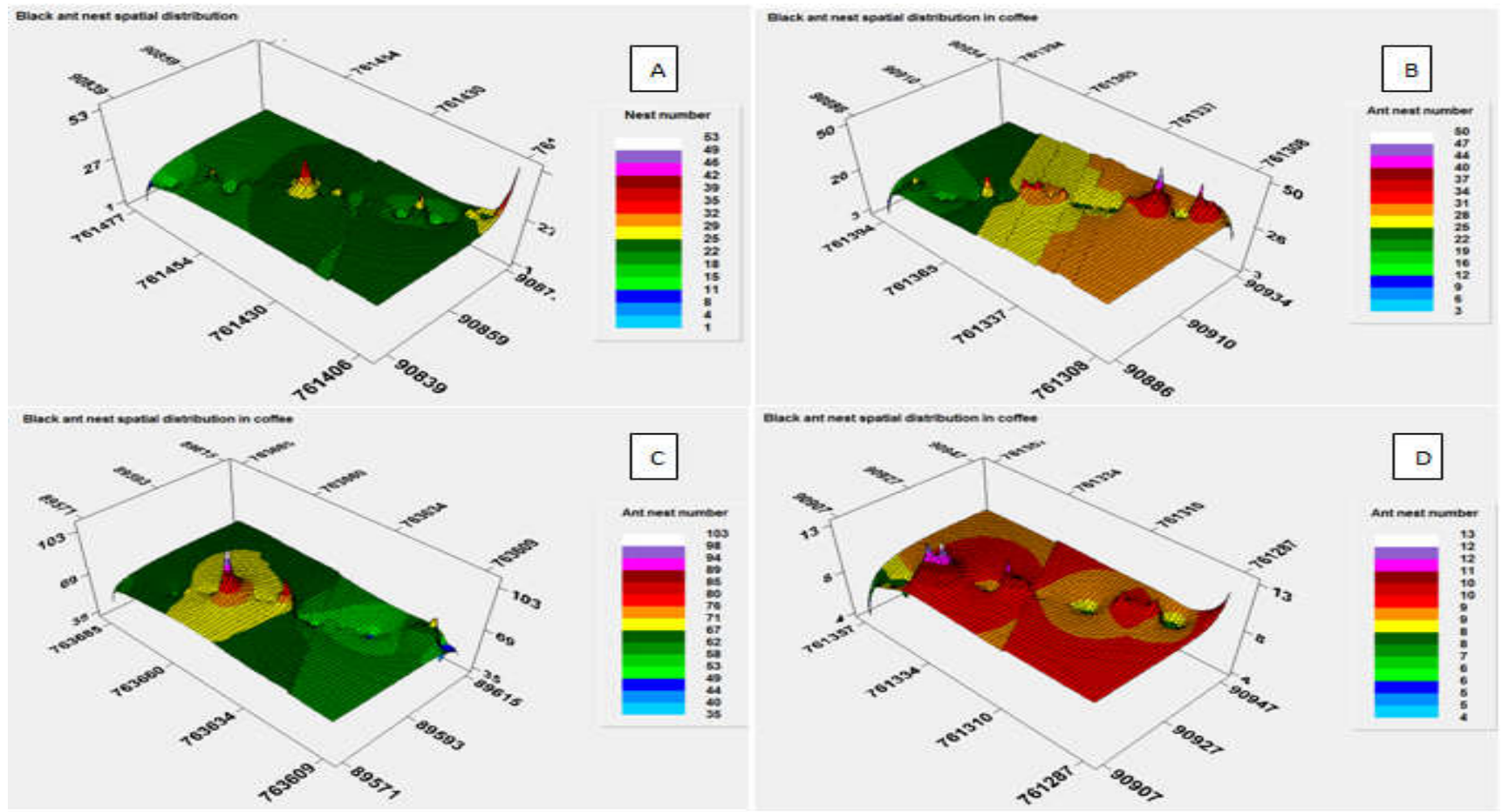


Figure 8. The interpolation of biting ant nest spatial distribution for Geisha(A,B,C) and Catimor (D) coffee varieties in different farms in Bebeka coffee plantation

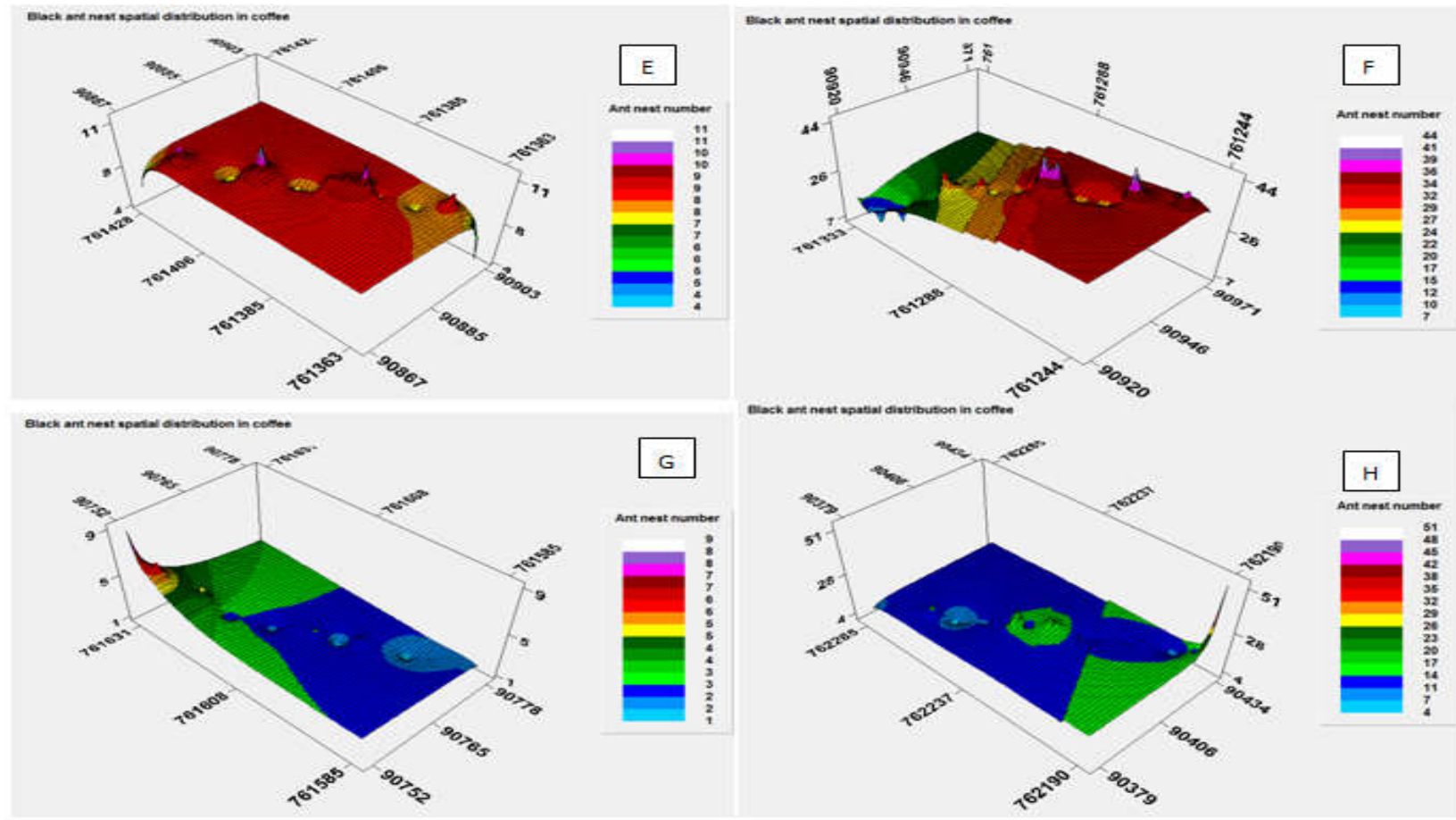


Figure 9. The interpolation of biting ant nest spatial distribution for Catimor(E), 7440 (F,G) and F-59 (H) coffee varieties in different farms in Bebek coffee plantation

The ratio (nugget/sill) value of the most farms (< 0.25), indicate high correlation where as the biting ant nest distribution in 7440 and F-59 varieties (MF-2 farm) showed moderate correlation (ratio 0.25-0.75) (Table 11). According to Cambardella *et al.* (1994) stated that the ratio nugget/sill is an indicator of the variable autocorrelation and the ratio value indicate low correlation if the value is close to one (>0.75) and high correlation for value close to zero (0.25). Residual sums squares and R^2 provides a measure of how well the model fits the variogram data. When geostatistics autofit the model, it uses residual sums square to choose parameters for each of the variogram models and residual sums squares that is robust and judge the effect of changes in model parameters (Robertson, 2008).

Table 11. Geostatistical description of *T. aculeatum* nests in different coffee varieties in BCESC during 2018

Coffee varieties	Farms code	Model	Nugget	Sill	Ratio	Range	R^2	RSS
7440	MF-2	Gaussian	100.1	226.80	0.306	43.60	0.499	13214
	MF-3	Gaussian	0.43	6.046	0.066	13.36	0.881	3.770
Catimor	MF-1	Exponential	4.83	15.05	0.243	47.65	0.284	71.80
	MF-1	Spherical	0.01	4.268	0.002	6.61	0.026	46.90
Geisha	MF-4	Spherical	60.00	540.90	0.100	3.54	0.000	114066
	MF-1	Spherical	2.10	199.20	0.010	3.52	0.000	36199
	MF-4	Spherical	0.10	172.90	0.001	4.23	0.002	20627
F-59	MF-2	Linear	78.91	99.93	0.441	53.07	0.031	17318

RSS = Residual sum square

Ratio = Nugget/Sill

4.3.2. Within plant distribution of biting ant

4.3.2.1. Distribution pattern

The spatial distribution pattern of the biting ant nests on coffee plant was determined in accordance with variance to mean ratio. The variance to mean ratio (s^2/x), which is the simplest index of dispersion and the most fundamental (Taylor, 1984), indicates a random dispersion when it is equaled 1.0; a uniform dispersion when it is less than 1.0; and an aggregated dispersion when it is more than 1.0 (Myers, 1978). In this study, the variance to mean ratio of the biting ant nest greater than one (16.61) on coffee plant indicating the distribution pattern of biting ant is aggregated on trees (Appendix Table 2). This is in agreement with Tebkew *et al.* (2011) who found aggregated type of distribution of biting ant on coffee tree.

4.3.2.2. Within plant distribution

The strata, upper and lower half of the coffee tree canopy, were significantly different in number of the ant nests, where the upper canopy harbored significantly more than the lower half (Table 12). The distribution of biting ant on the coffee trees was not affected by the directions (north, west, south and east), and interactions of directions (quadrants) and stratum (coffee plant height) (Table 13).

Table 12. ANOVA for coffee tree height (strata) and directions (quadrants) effects on biting ant nest on coffee tree distributions

No	Tree strata	Mean number of ant nests
1	Upper	6.43 (2.58)a
2	Lower	5.00 (2.36)b
	Mean	5.71
Direction (Quadrants)		
1	East	6.23 (2.58)
2	West	5.18 (2.34)
3	North	5.47 (2.46)
4	South	5.96 (2.49)
	Mean	5.91
	Cv (%)	18.97
	Lsd 5%	1.23 ^{ns}

Means followed by the same letter(s) within a column are not significantly different from each other at 5% level of probability Tukey Studentized Range (HSD). Figures in parenthesis are square root transformed value ($\sqrt{x+0.5}$)

Table 13. ANOVA for coffee tree height (strata) and directions (quadrants) interactions effects on biting ant nest on coffee tree distributions

No	Tree strata	Direction (Orientation)	Mean number of ant nests
1	Upper	East	6.58 (2.63)
		West	5.96 (2.45)
		North	5.93 (2.53)
		South	7.17 (2.68)
2	Lower	East	5.83 (2.51)
		West	4.24 (2.20)
		North	5.13 (2.40)
		South	4.65 (2.28)
	Mean		5.69
	Cv (%)		18.81

Means followed by the same letter(s) within a column are not significantly different from each other at 5% level of probability Tukey Studentized Range (HSD). Figures in parenthesis are square root transformed value ($\sqrt{x+0.5}$)

The causes of aggregation of biting ants might be due to their inherent active aggregation behavioral response such as in a situation where the presence of one individual attracts the others, perhaps for the purpose of feeding or reproduction and also due to some heterogeneity of the environment like microclimate and preferred part of the plants (Southwood, 1978). The difference in mean count of biting ant nests between upper and lower halves of the plant canopies seemed to be most influenced by physical factors such as exposure to the sun (Soemargono *et al.*, 2008).

4.3.3. Evaluation of Insecticides against Biting ant

4.3.4. Ex-situ insecticides efficacy test

The ex-situ experiment was conducted in to two rounds and the cumulative data of first and second round experiments are presented. All the insecticides caused mortality of biting ant and were significantly higher than that of the untreated check at all time intervals after treatment applications (Table 14). Although some of the insecticides acted fast and killed higher proportion of the insect, all had similar efficacies after 4hours of treatment. After 8 hours of treatment mortalities were around to 90% and complete mortality was recorded after 24 hours of the insecticide treatments. Mortality of biting ants in the untreated control is negligible. Miguelena and Baker (2014) indicated that liquid insecticides had differences in their capacity to kill off workers ants and potential efficacy against dark rover ants.

Variation in the susceptibility of ants to fibronil insecticide was reported by Hannum and Miller (2008) who tested the insecticide against the black carpenter ant (*Camponotus pennsylvanicus*). They also noted that all the colonies of ants were dead (100% mortality) 40 hour after application.

Table 14. Effects of different insecticides on mortality of biting ant under ex-situ at different hours after application cumulative (Mean of 1st and 2nd Round Experiment), 2018

Treatments	Mean percent mortality of biting ant				
	2 hr	4 hr	8 hr	10 hr	24 hr
oxymatrine 20ml/l	36.7d	65.9a	91.5a	98.8a	99.9a
oxymatrine 25ml/l	41.2bcd	67.3a	90.1a	99.6a	99.8a
nimbicidine 4ml/l	37.7cd	66.4a	88.3a	99.2a	100.0a
nimbicidine 5ml/l	37.1d	66.0a	91.0a	99.3a	99.8a
spinosad 1ml/l	52.3a	69.0a	92.8a	99.5a	100.0a
spinosad 1.5ml/l	53.6a	77.9a	94.3a	100.0a	100.0a
deltametrine 1ml/l	49.3abc	76.8a	94.1a	100.0a	100.0a
deltametrine 0.75ml/l	49.4ab	73.1a	94.7a	99.9a	99.8a
unsprayed	3.3e	2.9b	3.2b	3.6b	3.1b
Mean	40.1	62.8	82.2	88.9	89.2
MST	1179.7**	2634.9**	4411.8**	5112.8**	5212.9**
MSE	32.9	67.9	56.7	1.6	0.1
Cv (%)	14.3	13.1	9.2	1.4	0.3
Lsd(0.05)	11.9	17.2	15.7	2.6	0.6

CV = Coefficient of Variation; hr = hours after application; Means with the same letter(s) within a column are not significantly different at 5% level of probability (Tukey Studentized Range (HSD) test). MST= mean square of treatment, MSE= mean square of error. ** = highly significant ($p < 0.001$); * = Significant ($P < 0.05$)

4.3.5. In-situ insecticides evaluation

The insecticide treatments significantly reduced nest populations of the biting ant under field condition (Table 15). The treatments were significantly ($p < 0.01$) different for all assessed dates of applications (Table 15). The combined analysis of variance also revealed highly significant insecticides treatment and location interaction for date of treatment application of evacuated nests. Percent of evacuated nests in all the sprayed plots were

statistically different from the control. The percent of biting ant evacuated nests reduction was observed starting from 3 to 21 days after treatment application (Table 15).

In the early days of application, oxymatrine at 20 and 25ml/l as well as nimbidine at 4 and 5ml/l of water significantly increased the percent of evacuated nests compared to the other insecticides. Unsprayed plots consistently had low percent of evacuated nests throughout the assessment periods. Wakjira *et al.* (2015) reported that integration of cultural coffee management practice (pruning) with spray of botanical insecticide (oxymatrine 2.4SL) during dry season can minimize biting ant population in coffee plantation at Tepi. In the combined analysis of the two locations the insecticide treatment deltamethrin 2.5% applied at 1 and 0.75ml/l concentration had significantly higher percent of evacuated nests, at 15th, 18th and 21st days after application, as compared to other insecticide treatments. Fisseha (2014) who conducted his experiment under field condition suggested the use of hand picking plus dipping nests in detergent and also spraying with deltamethrin 2.5% at 1ml/l for heavily biting ant infested coffee farm.

Table 15. Effect of insecticides evaluated to control biting ant at farm 1 (Southbench and Guraferda districts combined analysis) 2018

Treatments	Percent of evacuated nests							Newly constructed nests/100m ²
	3 da	6da	9da	12da	15da	18da	21da	30da
oxymatrine 20ml/l	13.4a	32.6ab	42.7ab	54.9ab	61.6ab	66.6ab	69.9bc	1.0(1.2)b-d
oxymatrine 25ml/l	13.2a	32.5ab	43.5ab	54.9ab	62.5ab	68.2ab	71.4bc	0.8(1.1)b-d
nimbicidine 4ml/l	11.2a	28.7ab	35.4ab	45.9ab	53.1b	58.8b	62.1c	1.2(1.3)bc
nimbicidine 5ml/l	12.1a	27.5ab	37.7ab	46.6ab	53.4b	59.4b	64.3c	1.3(1.3)b
spinosad 1ml/l	10.8a	23.2b	33.4b	44.6b	53.7b	59.6b	64.7c	0.3(0.8)c-d
spinosad 1.5ml/l	10.2a	30.3ab	40.2ab	51.6ab	59.6ab	66.3ab	71.3bc	0.3(0.8)c-e
deltametrin 1ml/l	14.2a	35.3a	46.1a	58.0a	69.7a	79.1a	87.2a	0.0(0.7)e
deltametrin 0.75ml/l	12.5a	29.6ab	40.7ab	52.2ab	63.4ab	74.7a	84.7a	0.1(0.8)e
unsprayed	3.5b	4.3c	7.5c	10.7c	13.9c	16.6c	19.3d	2.6(1.7)a
Mean	11.2	27.1	36.4	46.6	54.56	61.1	66.1	0.9
MSE	2.7	5.4	6.3	6.6	6.7	7.2	7.8	0.5
Cv (%)	24.1	20.1	17.2	14.3	12.3	11.8	11.7	19.8
Lsd(5%)	5.2	10.4	11.9	12.7	12.8	13.8	11.9	1.0

CV = Coefficient of Variation; da = days after application; Means with the same letter(s) within a column are not significantly different at 5% level of probability Tukey Studentized Range (HSD) test. MSE= mean square of error. ** = highly significant (p<0.001); * = Significant (P<0.05)

The study revealed that different types of insecticides were effective against the biting ant under in-situ condition. Kenne *et al.* (2003) found that spraying insecticides in citrus, guava and mango tree plantations eliminated the dominant arboreal ant. The study also showed that all the tested insecticides caused almost total mortality in an ex-situ (indoor) experiment within 24 hours and under field condition (in-situ) the rate of mortality (evacuated nests) reduced as the days after application increased. Miguelena and Baker

(2014) stated that it is important to consider that the residual activity observed in indoors experiment but the same products being applied outdoors are likely to lose their efficacy considerably faster. According to Miguelena and Baker (2014) suggested that ant mortality by insecticides treatments is important because it opens the possibility for transfer of the insecticide from exposed to unexposed ants via contacts through social interactions.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusion

As coffee is increasingly economically important crop in Ethiopia, problems associated with insect pests need to be addressed properly. Biting ant problem is more serious in southwest of the country than in the past and it will continue to be a problem unless effective, economical and easily applicable control methods are developed. The survey results revealed that there were variations among farmers' perceptions and knowledge about the biting ant across the study districts in the Southwestern part of Ethiopia. It was also noted that coffee farmers in the surveyed districts use different cultural methods and agronomic practices to reduce the infestation of the biting ant in their farms. The infestation levels varied in different ecology of coffee producing districts (12 surveyed districts) and production systems. Lowland districts and large coffee plantation production systems showed relatively higher percentage infestation of biting ant (especially, South bench and Yeki districts).

There was spatial dependence among samples of each measurement of the biting ant nests abundance in a wide range of pest densities. Biting ant spatial distribution analysis showed a localized discontinuity spatial structure and long range of correlations in some farms and vice versa in other farms at BCESC coffee plantation. The spatial distribution trends in the farm showed highest along the farms center and in some farms near farm corners and declined from there across to the inner field. The abundance around the farms center and along the corners is probably because of the building of nests on several potential wild or cultivated host plants commonly found in and adjacent to the coffee farms. In addition to shade trees, black pepper planted mostly at the borders of coffee farms and ant nests were abundant on black pepper. The distribution pattern of nests within the coffee on tree was

aggregated. The four directions of the plant with plant strata showed non significant difference, in abundance of ant nests.

The insecticides evaluation experiments revealed important differences in the performance of the different products tested. The treatments showed significant difference in ant mortality and reduction of active nests in both ex-situ and in-situ experiments. Under ex-situ study all insecticide treatments caused complete mortality within 24 hours. The insecticides efficacy was lower in the in-situ (field condition). During early stage of treatments the botanical insecticides oxymatrine and nimbidine had similar performance showing in reduction of active nests compared to the other evaluated insecticides.

5.2. Recommendations

1. If proper agronomic practices are implemented, they can contribute to reduce biting ant but it is very important to provide training for coffee farmers.
2. Conducting regular surveys of biting ant in coffee producing areas across wide agro-ecologies is important.
3. Carry out agro-ecological and spatial distribution of this ant in un-addressed major coffee producing areas.
4. Deltamethrin 2.5% at 0.75ml/l can be used for heavily infested farms where pruning and manually destructing the nest difficult.
5. Further studies on the impacts of weather variables on biting ant biology.
6. This study result calls further investigation to evaluate rates and frequency of different botanical derived insecticides because oxymatrine and nimbecidine showed potential performance.
7. Investigating the residual effects of deltamethrin 2.5% on coffee environment before widely application is important.
8. Future research should be focused studies on temporal distribution, foraging behavior, bio-control and integrated pest management approach.

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7. APPENDIXES

7.1. Survey questionnaire for biting ant assessment in coffee farms of south western of Ethiopia

1. General information

1.1. Date -----recorded by-----region-----
zone-----district-----Aver. Daily temperature-----
-----RH-----Name of respondent (farmer) ----- sex-----
age-----

2. Field information and assessment

- 2.1. Field no----- field estimated area (ha) ----- crop growth stage-----
- 2.2. Cropping system ----- a) sole b) intercropping c) mixed cropping
- 2.3. Major crops grown-----
- 2.4. For how long you have been growing coffee a) <3 years b) 3-6years c) 6-10years
d) >10 years
- 2.5. Aim of coffee production a) for market only b) for home consumption only c) for both
- 2.6. Coffee variety famers used a) improved varieties b) local varieties
- 2.7. Coffee variety sources a) govt. organization eg. Research center, Agri. Offices) b) local market
- 2.8. Coffee production system a) Forest b) Semi forest c) Garden d) Plantation
- Shade trees used a) Temporary shade tree b) Permanent shade tree
- Shade level in the farm a) low b) medium c) High
- 2.9. Agronomic practices done mention-----
- 2.10. Did black biting ant occur in your coffee farm a) yes b) no if yes since when (year) -----
- 2.11. At what coffee stage the ants occur? A) Seedling b) vegetative c) flowering d) maturity e) throughout the year
- 2.12. What damage do the ant cause on coffee tree and on the workers-----
-----what management method used to control this ant (mention them) -----

Appendix Table 1. Distribution pattern of *T. aculeatum* nests on Geisha coffee variety at BCESC plantation, 2018

<u>Sample-tree</u> <u>no</u>	<u>No.nests</u> <u>/tree(x)</u>	<u>Mean</u> <u>(m)</u>	<u>x-m</u>	<u>(x-m)²</u>
1	28	45.70	-17.70	313.29
2	101	45.70	55.30	3058.09
3	89	45.70	43.30	1874.89
4	108	45.70	62.30	3881.29
5	95	45.70	49.30	2430.49
6	43	45.70	-2.70	7.29
7	81	45.70	35.30	1246.09
8	87	45.70	41.30	1705.69
9	51	45.70	5.30	28.09
10	68	45.70	22.30	497.29
11	43	45.70	-2.70	7.29
12	21	45.70	-24.70	610.09
13	42	45.70	-3.70	13.69
14	28	45.70	-17.70	313.29
15	52	45.70	6.30	39.69
16	23	45.70	-22.70	515.29
17	21	45.70	-24.70	610.09
18	42	45.70	-3.70	13.69
19	19	45.70	-26.70	712.89
20	15	45.70	-30.70	942.49
21	22	45.70	-23.70	561.69
22	28	45.70	-17.70	313.29
23	30	45.70	-15.70	246.49
24	32	45.70	-13.70	187.69
25	67	45.70	21.30	453.69
26	33	45.70	-12.70	161.29
27	30	45.70	-15.70	246.49
28	25	45.70	-20.70	428.49
29	23	45.70	-22.70	515.29
30	24	45.70	-21.70	470.89
Mean(m)				45.70
Variace(s ²)				772.63
Ratio(s ² /m)				16.61



Appendix figure 1. Status of Insecticides efficacy evaluations in ex-situ and in-situ at BCESC plantation

Appendix Table 2. ANOVA table showing mean square values of spatial distribution within coffee trees (strata and directions)

Source	Mean Square	
	DF	Number of nests
MP	1	121.837**
SP	3	13.5263 ^{ns}
MP*SP	3	9.015278 ^{ns}
Error	174	6.74497

DF= degree freedom; MP = main plot; SP subplot; ** = highly significant ($p < 0.001$); ns = non significant

Appendix Table 3. ANOVA table showing mean square values of insecticides efficacy test against biting ant at ex-situ condition

Source	Mean Squares					
	DF	2ha	4ha	8ha	10ha	24ha
Treatments	8	1179.76**	2634.98**	4411.80**	5112.86**	5212.92**
Error	36	32.90	67.95	56.73	1.60	0.07

DF= degree freedom; Chem = insecticides; haa = hours after treatment application; ** = highly significant ($p < 0.001$); * = Significant ($P < 0.05$); ns = non significant

8. BIOGRAPHICAL SKETCH

The author, Sisay Kidanu, was born on 17th June 1989 G.C in East Shoa zone of Oromia regional state, Ethiopia. He attended his elementary education at Amanuel Baptist and Tede school from 1996-2003 and his high school education attended at Hawas secondary and preparatory school in Adama from 2004-2007. From 2008-2010 he joined and attended at Jimma University and graduated BSc in Plant Science. After graduation, he joined the Ethiopian Institute of Agricultural Research, Jimma research center in 2011 and worked to 2016. He joined the school of graduate studies of Hawassa University in 2016 to study Degree of Master of Science in Crop Protection.