ABUNDANCE AND DIVERSITY OF TOP SOIL EARTHWORMS IN RELATION TO CHEMICAL USE IN GOLDEN ROSE AGROFARM, TEFKI AREA, ETHIOPIA

By

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Dedication

This work is dedicated to my father who let the ground for my current educational careers, but not alive today to see the finals

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ABSTRACT

Despite the current environmental controversies, flower farms are becoming hot areas of investment in Ethiopia. Golden Rose Agro Farm is one of the largest, and the pioneer, flower industries in the country. To study the abundance and distribution of top soil earthworms in relation to chemical use in the farm, a total of 208 1mx1 m quadrates were marked on the ground in 10 randomly selected greenhouses. Similarly, 208 quadrates were marked in the outside chemical-free farm for comparison. Soil was then dug to a depth of 15 cm in all of the marked quadrates and hand-sorted to collect top soil earthworms. Eight plastic buckets, each filled with soil at 15 cm depth, were taken and 15 adult worms originally from chemical-free soil were introduced in to each and all were put in the greenhouses, some under chemical treatment and some free for comparison. Worms from the rose farm were also transferred to chemical-free soil and changes were observed after 20 days. Earthworms were taxonomically identified to genus level using taxonomic keys. There was strong statistically significant variation in abundance of both adult and juvenile top soil earthworms between the two farms ($P < 0.01$, $a=0.05$). Out of the 75 adult worms introduced into the chemical-treated buckets, 98.7% (74) were dead, and in the Chemical-free buckets, out of the 45 worms originally introduced, about 95.6% (38 adult and 5 juveniles) were recaptured. About four genera of earthworms were identified in the study area, but majority of them belong to the genus Eiseniella and Dendrobaena. Seasonal variation in number and distribution of earthworms was also observed in the study. Chemical use in Golden Rose Agrofarm strongly affected abundance and distribution of top soil earthworms in the area

1. INTRODUCTION

1.1. Background

In many cultures fresh cut flowers are deeply symbolic of nature's beauty. As a gift they embody a universal desire for connection to other people, to the beauty of nature, to God. Many people like to celebrate major holidays with lavish bouquets of cut flowers. As a result, the flower industry is booming, shipping hundreds of tons of cut flowers all over the world for sale in supermarkets and at florists. Consumers spend millions every year purchasing cut flowers and bringing them into their homes. But most consumers do not think about the environmental and social aspects of the cut flower industry. Several studies conducted in the late 1990s suggested that cut flowers had a serious environmental impact, and some advocacy organizations have attempted to raise consumer awareness about the hidden costs of cut flowers.

Most cut flowers are grown in South America, Africa and Southeast Asia in large greenhouse environments staffed by underpaid, non-unionized workers (ICPC, 1998). The greenhouses are carefully climate controlled to yield the best cut flowers, and they are also heavily sprayed with pesticides, fungicides, and herbicides. Because cut flowers are grown in nations with more lax environmental laws, many banned substances including DDT and methyl-bromide are used in flower production. These substances have a profound impact on the health of the workers. Many suffer from health problems such as skin diseases, respiratory problems, impaired vision, and birth defects due to exposure to these chemicals (ICPC, 1998).

Pesticides can cause cancer, birth defects, reproductive and nervous system damage. Floriculture workers are exposed to pesticides at numerous stages of plant growth. Worker exposure is of particular concern in greenhouses, where up to 127 different chemicals are used in enclosed spaces increasing risk of exposure through the skin and by inhalation. Some flower greenhouses use different pesticides, including the persistent organochlorines DDT, aldrin and dieldrin. A study of flower workers in Costa Rica found that over 50% of respondents had at least one symptom of pesticide poisoning, such as headache, dizziness, nausea, diarrhea, skin eruptions or fainting (IFC, 1999). In Ecuador, nearly 60% of workers surveyed showed poisoning symptoms, including headaches, dizziness, hand-trembling and blurred vision. Reproductive problems are also a concern; studies of the largely female workforce in Colombia found moderate increases in miscarriages and birth defects (IFC, 1999).

In addition to human health hazards, these chemicals are also extremely harmful for the environment. Methyl-bromide has been linked with destruction of the ozone layer, for example, while DDT usage worldwide led to serious problems for many animal and bird populations. Most greenhouses, which produce cut flowers, spray`` chemicals on their crops in large amounts. These chemicals later enter the bodies of workers, the flowers, soil and the ground water. Water and soil pollution around commercial greenhouses harm animal and fish populations and also has an impact on human life as well, by reducing the amount of drinkable water (IFC, 1999).

Environmental health perspectives also report disturbing environmental impacts. For example, after intensive water use by floriculture, the water table has dropped under the savanna surrounding Bogota (ICPC, 1998). In most cases, pesticide residues are directly discharged into waterways, pesticide equipment is washed into streams and rivers, and runoff is allowed to enter important, acquifer recharge areas.

International development agencies also push floriculture as an exportable alternative to traditional crops, but increased competition for water and croplands near transportation centers has created conflicts with indigenous farmers. In rural economies where food shortages are routine, the large-scale production of resource-intensive, non-edible crops like roses does not contribute to food security (ICPC, 1998). Currently, cut flower industries are highly expanding as hot areas of investment in many countries of Africa, including Ethiopia, under very poor and not properly stated environmental policies. Therefore, it is very likely to face all the environmental as well as social implications stated above.

Recent developments that have led to an increased understanding of the structure and function of soil ecosystems have raised the possibility of using community parameters to measure the impact of such pollutants. Of the groups available for monitoring, particular attention has been paid to the macro-invertebrates, because these communities frequently consist of a large number of species that differ in their niche preferences, life histories and sensitivity to pollutants (Lagadic and Caquet, 1998; Spurgeon and Hopkin, 1999). Additionally, soil macro-invertebrates are present over a range of diverse habitats, are relatively easy to sample, can be simple to identify when good keys are available, and frequently have low mobility, which means they are representative of the habitat being sampled (Spurgeon and Hopkin, 1999).

The suitability of macro-invertebrates for monitoring has resulted in the development of procedures for assessing the impact of environmental stress from changes in community structure. Many authors agree that the simplest form of monitoring environmental stress on community is to use abundance data for single species, and hence, any system developed to monitor pollutants from their impacts on community structure must account for temporal variation in abundance (Wharfe, 2004).

Earthworms are excellent bio-indicators of the relative health of soil ecosystems and possess a number of qualities that predispose them for use in monitoring terrestrial ecosystems (Vandecasteele *et al.*, 2004). They are large, less mobile relative to many soil invertebrates, numerous, easy to sample and easily identified. They are also in full contact with the substrate in which they live and consume large volumes of this substrate. Earthworms are not only killed by toxic chemicals but their growth, fecundity and behaviour are also affected. They also accumulate some chemicals in their tissues at higher levels than that of the substrate in which they live, and all these properties make them preferable for surveys concerning environmental contamination.

Earthworms have a considerable influence on the physical structure of the soil by their active burrowing and ingestion of the soil. This results in mixing of the surface and sub-surface soils and enhances nutrient dynamics, because by reducing organic matter to its constituents, they liberate nutrients usable by other organisms (Lee, 1987; Werner, 1996). Their presence or absence in any soil, and the overall species composition, may also reflect environmental changes that are not easily recognised using physical or chemical means. This provides a sensitive measure of soil pollution.

1. 2. Problem Statement

Currently flower farms are blooming in Ethiopia under poor environmental policies and regulations. Even though flower production can generate a wide range of employment, income, foreign exchange and other cash contributing effects, the damage to the ecosystem in general seems to outweigh the benefits (IFC, 1999). Since earthworms are excellent ecological indicators for environmental contamination as a result of hazardous chemicals, this study focuses on their abundance and distribution in relation to chemical use in one of the flower farms in Ethiopia (Golden Rose Agro Farm).

1.3. Objectives

General Objective

To assess abundance and distribution of top soil earthworms in relation to chemical use in Golden Rose Agro Farm by comparing their numbers in chemical-treated soil under flower production and the surrounding chemical-free farm.

Specific Objectives

- To identify the major taxonomic groups of top soil dwelling earthworms in Golden Rose Agro Farm and the surrounding chemical-free farm
- To compare abundances of adult and juveniles of top soil earthworms in chemical-treated soil under the rose farm and the surrounding chemical-free farm
- To measure and compare pH and organic carbon content of the soil under the rose farm and the outside chemical-free farm.

2. LITERATURE REVIEW

2.1. The structure of earthworm communities

2.1.1. Major ecological groups

Several schemes have been proposed to classify earthworm species into major ecological categories, which are basically mainly on differences among species in the burrowing and feeding activities, and vertical stratification in soil. These major ecological groups present functional adaptations to the soil environment that allow different species of earthworms to coexist by exploiting different food resources and habitat (Edwards and Bohlen, 1996; Mclean and Parkingson, 2000; Hale and Host, 2005; Marhan and Scheu, 2005). Earthworm communities nearly always include species that pursue different ecological strategies, and a familiarity with these strategies is essential to an understanding of the structure of earthworm communities.

Earthworm species vary in how they get food, and thus inhabit different parts of the world and have somewhat different effects on the soil environment. They fall into three distinct ecological groups based on feeding and burrowing habits (Lavelle *et al.*, 1994; Beare *et al.*, 1995). Epigeic (litter dwelling) earthworms live and feed in surface litter where they move horizontally through leaf litter or compost with little ingestion of or burrowing into the soil (USDA, 2001). These worms are characteristically small and are not found in low organic matter soils. They are small-bodied (size usually less than 7 cm) and often reddish brown in colour (Hale and Host, 2005; McLean and Parkingson, 2000). *Dendrobaena octaedra, Lumbricus rubellus* and *Dendrodrilus rubidus* are examples of epigeic species.

Endogeic (shallow-dwelling) earthworms are active in mineral top soil layer and associated organic matter. They are moderately sized (5-12 cm) and gray, blue or whitish in colour. They live in the soil mineral horizon (up to 35 cm depth) and are usually not noticed, except after a heavy rain when they come to the surface (Brown, 1995 cited in Gezaheng Degefe, 2006). They feed by ingesting a mixture of mineral soil and soil organic matter, including partially decomposed surface litter, but do not appear to consume unaltered surface litter (Hale and Host, 2005). Endogeic earthworms play an important role in mobilization and stabilization of carbon and nitrogen in forest and arable soils (Marhan and Scheu, 2005). The genus *Apporrectodea* and *Diplocaridae* have endogeic life habits.

Anecic (deep burrowing) earthworms live in permanent, nearly vertical burrows that may extend several feets into the soil. They feed on surface residues and pull them into their burrows for food. These worms are very large bodied (8-15 cm in length) with highly pronounced clitellum, and are reddish brown in color. The familiar bait worm or night crowler (e.g. *Lumbricus terrestris*) is an example of anecic species (Edwards and Bohlen, 1996)

2.1.2. Species Diversity

The number of species in a given earthworm community, which is the simplest measure of species diversity (Edwards and Bohlen, 1996) ranges from 1 to 15 (Spurgeon and Hopkin, 1999). Most earthworm communities are found to contain a limited number of species with a remarkable degree of consistency among different habitats and different geographic regions. The diversity of the earthworm community at a given locality is influenced by such factors as the characteristics of the soil, climate and organic resources of the locality, as well as its history of land use and soil disturbance. Lee (1985) also stated that the species-poor communities of earthworms are those characterized by extreme soil conditions, such as low pH, or fertility, low quality litter or a high degree of soil disturbance. The previous agricultural history of a grass or arable field is also an important factor in determining which species are present in the area (Edwards and Bohlen, 1996).

2.1.3 Species Associations

Some earthworm species tend to be associated with one another. Such associations usually result from some characteristics of the worms and the habitat. Edwards and Bohlen (1996) stated that *Lumbricus terrestris, Allobophora longa* and *Allobophora calignosa* are characteristic pasture species, but commonly occur together in association with *Lumbricus rubellus*, *Allobophora Chlorotica* and other related species in arable fields. *Dendrobaena octaedra* and *Bimastos eseni* usually live together in the litter layer of the soil, whereas *Allobophora rosa*, *Allobophora longa, Allobophora caliginosa*, *Allobophora chlorotica*, *Lumbricus castaneus*, *L. terrestris* and *L. rubellus* are commonly found together in many soils.

Philipsen *et al.* (1976) also studied species associations among earthworm communities and reported considerable overlap between species associations in different communities. Many authors attribute these associations to similarity in ecological requirements between species. However, Philipson *et al.* (1976) pointed out that the co-occurrence of different earthworm species does not necessarily mean that those species have similar ecological requirements, and even some of the associations can be due to casual events.

2. 2. Effects of agricultural practices and chemicals on abundance and distribution of earthworms

It is now clear that earthworms are important in providing soil fertility and improving soil structure. Consequently, it is important to know how earthworm populations are affected adversely or favorably by different agricultural practices. The four main agricultural practices mentioned by many authors (Edwards and lofty, 1977; Edwards and Bohlen, 1996; USDA, 2001; Kladivko, 2003) to have influence on earthworm populations are cultivation or cropping, fertilizers, chemicals and heavy metals or acid depositions.

2. 2. 1. Cultivation, Cropping, Chemicals and Fertilizers

In 1881 Darwin considered the earthworms to be nature's plough, but he did not consider how ploughing affected earthworm populations. There is good evidence that many natural ecosystems tend to contain more earthworms than ploughed agricultural systems (Edward and Bohlen, 1996; Kladivko, 2003). Many researchers agree nowadays that grasslands contain more earthworms than arable land in most parts of the world. The decreased number of earthworms that occur in cultivated arable land could be due to the loss of the insulating layer of vegetation, to a decreased supply of food as the organic matter content gradually decreases with subsequent cultivations, or to predation by birds when the worms are brought to the surface during cultivating.

When old grassland is ploughed, the number of earthworms in it decreases steadily with time after ploughing with repeated cultivations, and some earthworm researchers have considered that these differences in earthworm populations are due mainly to mechanical damage during cultivation. However, it is unlikely that mechanical damage was a primary cause of the decreased number of worms (Edwards and Bohlen, 1996; Smeaton *et al.*, 2003), because the plough merely turns the soil over and probably has little effect on those earthworm species with deep burrows, and also earthworm populations could easily recover due to their high regeneration power. As a result, some authors attribute this decrease in number of earthworms in agricultural lands to predation after ploughing.

Edwards and Lofty (1977) compared earthworm populations in plots that were ploughed and cultivated with others that were left unploughed for many years and confirmed that cultivation of grass plots has severe effects on earthworm populations. In their experiment, repeated cultivation over successive seasons depressed the earthworm populations progressively.

Many researchers also suggested that the type of crop in a given farm land could affect the number of earthworm population. Hop and Hopkin (1996) in their experiment reported that alfalfa grass cropped plots contained more earthworms than lespedeza grass cropped plots. Probably, this is because of the proportion of the plant material that is returned to the soil after harvest (Edwards and Bohlen, 1996).

Also in one of the few investigations on the influence of cropping on earthworm populations, the supply of organic matter to the soil was the most important factor favoring the buildup of earthworm populations (Edwards and Bohlen, 1996). Straw residues ploughed into the soil and the growing of short term hay crops increased earthworm populations greatly. In general, the inclusion of crops such as cereals, that leave considerable residues, encourage the build up of earthworm populations much more than growing crops like soybeans and other legumes, which decompose quite rapidly and leave little residue (Edwards, 1984).

Agricultural chemicals that reach the soils include pesticides, heavy metals, poly chlorinated biphenyls and acid precipitations. The degree of exposure of earthworms to such chemicals in soils depends upon many factors that may be associated not only with the chemicals, the route of exposure, and the soil type, but also the environmental conditions and the species and behavior of the earthworms (Baveco and Roose, 1996). Several other factors also affect the influence of chemicals on earthworms. These include the route of exposure, water solubility, volatility, adsorption capacity, moisture content, proportions and amount of clay and organic matter in the soil, pH, temperature and persistence in soils.

Several aspects of the behavior of earthworms can also affect the toxicity of chemicals to their communities. Different species of earthworms can be exposed to chemicals to quite different degrees and in very different ways (Delahaut and Koval, 2006). For instance, *L. terrestris* is often exposed to a high concentration of pesticides because this species moves over and feeds at the soil surface. *A. caliginosa* also lives in the superficial layers of the soil and the adults may move over the soil surface, thereby becoming particularly vulnerable to surface pesticide residues. *Aporrectodea longa,* on the other hand, seems to be less susceptible to pesticides than many other species of earthworm because it can burrow deep into the soil and also usually enters an obligatory diapause in adverse conditions (Edwards and Lofty, 1977; Edwards and Bohlen, 1996).

There is an extensive literature on the effects of pesticides on earthworms (Thompson and Edwards, 1974; Dean-Ross, 1983; Edwards and Neuhauser, 1989; Lofs-holmin and Bostrom, 1988), but the effects of other chemicals are not well known (Potter *et al.*, 1990; Edwards and Bohlen, 1996). Workers such as Haque and Ebbing (1983); Edwards

(1984) and Roberts and Dorough (1984) have tested a range of pesticides and other chemicals for their toxicity to earthworms and concluded that different chemicals and pesticides vary greatly in their degree of toxicity to the worms.

Most of the inorganic chemicals such as lead arsenate and copper sulfate commonly used as pesticides before the Second World War are moderately toxic to earthworms, but are potentially harmful if the soil contains large residues of them (Edwards and Bohlen, 1996). These chemicals are very persistent, and their build up in soil can lead to long term exposure of earthworms. Most of the organochlorine insecticides are also persistent in soils (Perfect, 1980; Delahaut and Koval, 2006). Chlordane and endrin were reported by several workers to be extremely toxic to earthworms. Thompson (1971) also mentioned that chlordane, heptachlor, aldrin, dieldrin, telodrin, DDT and carbaryl are toxic to earthworms.

In general, many researchers agree that organophosphate insecticides such as chlorfenvinphos, disulfoton and dyfonate are toxic to earthworms. Parathion, ethopropos and fonfos are all proved to be moderately toxic to earthworms. Phorate was extremely toxic to earthworms and has almost eliminated earthworms from many soils, even at normal agricultural rates (Edwards and Bohlen, 1996).

Many carbamate insecticides and fungicides strongly affect populations of earthworms. Heimbach and Edwards (1983) mentioned that of the other carbamate insecticides tested for toxicity to earthworms, high toxicity has been reported for aminocarb, methiocarb, oxamyl and promecarb, and very high toxicity for aldicarb, bufencarb, carbaryl, carbofuran, methomyl, propoxur and thiofanox. Dean-Ross (1983); Haque and Ebbing (1983) and Roberts and Dorough (1984) also suggested that it is reasonable to predict that the majority of the carbamate pesticides are toxic to earthworms.

Fumigant nematicides and fungicides such as D-D mixture, metham sodium and methyl bromide are normally applied to the soil to control pathogens and nematodes. However, most of these chemicals are broad-spectrum biocides and penetrate the soil as vapors and kill most of the earthworms, even those that live in deep burrows (Edwards and Bohlen, 1996). Chloropicrin and the contact nematicide methomyl are also very toxic to earthworms, and it seems there is little doubt that the majority of fumigant and contact nematicides are toxic to earthworms.

None of the fungicides that have been tested were toxic to earthworms (Leemput *et al.*, 1989; Anton *et al.,* 1990) with the exception of the carbamate fungicides such as benomyl, which is very toxic, and carbendazim, which is moderately toxic (Stringer and Wright, 1976). Very few herbicides are also directly toxic to earthworms (Edwards, 1989). However, it is probable that they may exert considerable indirect effects due to their influence on weeds as a source of supply of organic matter on which earthworms feed in soil. Reports from many research works show that chloroform, profam, dinoseb and triazine herbicides such as simazine have moderate effects on earthworm populations. Reinecke and Nash (1984) and Haque *et al.* (1982) also reported take up and metabolism of some herbicides by earthworms.

Environmental pollution by heavy metals is also increasing rapidly. The most important pollutants include cadmium (Cd), lead (Pb), copper (Cu), mercury (Hg), zinc (Zn), nickel (Ni), antimony (Sb) and bismuth (Bi) though many other metals can also cause pollution. Relatively few data are available on the toxicity of heavy metals to earthworms, and much more research is required (Pietz *et al.*, 1984). Copper toxicity resulting from long-term use of copper-based fungicides in orchards completely eradicated earthworms where copper levels in the soil were greater than 80 ppm (Didden, 2001).

Chromium is reported by Soni and Abbasi (1981) to be extremely toxic to many earthworm species, but the authors found that mercury was about 20 times more toxic than chromium to the worms. It has been reported that copper affected earthworm populations at concentrations more than 287ppm, cadmium at 33ppm and lead at 4800ppm, but Bengtsson *et al.* (1983) also provided comparable data for significant effects on earthworm populations at 78ppm for copper, 171ppm for zinc and 36ppm for lead. Therefore, Lee (1985) commented that the differences between the relative toxicity of the compounds tested may explain some of the conflicting data in the literature on the concentrations that have deleterious effects on earthworms, and clearly there is a need for much further testing of the toxic levels of the different chemicals to earthworms.

It has clearly been established that organic fertilizers affect number of earthworms in soil, but there had been fewer studies of the influence of inorganic fertilizers on earthworm populations. The effect of fertilizers on earthworms may be direct, for instance, by changing the acidity of the soil, or indirectly, by changing the form and quantity of the vegetation that ultimately provides food for worms. For instance, application of superphosphate and lime to pastures caused a dense clover sward to develop and this in turn increased the number of earthworms in soil about four-fold (Edwards and Bohlen, 1996).

There is good evidence that nitrogenous fertilizers favor the build up of large number of earthworms. Large amount of nitro chalk applied to many pasture soils, for example, indirectly increased the earthworm population due to greatly increased grass production (Edwards and Lofty, 1977). They also reported increased number of earthworms after application of different forms of nitrogenous fertilizers; in one of their studies they estimated that there were 128 worms per meter square in plots without nitrogen, and 176 per meter square in plots to which nitrogenous fertilizers had been added. Applications of nitro chalk and nitrate of soda to grassland also resulted in increased earthworm populations. Lime also seems beneficial to earthworms, and this is probably because most species of earthworms tend to avoid acidic soils, or probably because they may have a need for calcium (Hale and Host, 2005).

Many workers agree that superphosphate is very beneficial to earthworms, but Gerard and Hay (1997) reported this fertilizer decreased the number of earthworms in grass plots. Therefore, the effect of inorganic superphosphate fertilizers on abundance and distribution of earthworms seems controversial.

2. 3. Modes of toxicity of chemicals to earthworms

Many chronic toxicity symptoms of earthworms exposed to chemicals have been recorded in the literature (Venter and Reinecke, 1988; Edwards and Bohlen, 1996), some of which are serious and short term, whereas others are minor but involve long term effects on the earthworms and their functions. The following are some of the chronic and sub-lethal toxicity effects of chemicals to earthworms (Edwards and Bohlen, 1996):

2. 3. 1. Malformations, effects on nervous and physiological functions, reproduction, growth and activity

Several studies have reported different types of malformations of earthworms in response to exposure to chemicals. For instance, Zoran *et al.* (1986) pointed out that the fungicide benomyl had teratogenic effects on the posterior segment regeneration of the earthworm *Eisenia fetida.* The effects also include an increased frequency of segmental groove anomalies and many monstrosities. Anton *et al.* (1990) exposed earthworms to carbamates and observed that carbamates can cause tumors and swellings along the earthworm's body.

Haque and Ebbing (1983) reviewed the symptoms caused by 23 pesticides to earthworms and reported that the most common reaction to chemicals was coiling of the body and longitudinal muscle contraction, after which the body became rigid and sometimes swellings appeared on the body surface. The swellings often burst creating bleeding sores (Tomlin and Gore, 1974). Stenersen *et al.* (1973) also mentioned the same symptoms occurred when the earthworms are treated with propoxur, methidathion, endosulfan, triazophos, carbofuran, terbufos and methamidophos. Aldicarb, endosulfan, benomyl and calcium cyanide also caused constrictions of the body to occur, according to the authors.

There is a growing body of evidence that chemicals can have various drastic effects on the nervous systems of earthworms. Drewes *et al.* (1987); Callahan (1988) and Nadeau *et al.* (2001) mentioned that earthworms exposed to benomyl by contact showed sub-lethal neurotoxic effects. Both carbamates and organophosphates have been shown to inhibit acetylcholinesterase activity (Edwards and Fisher, 1991) though carbamates are found to inhibit acetylcholinesterase more than did organophosphates (Niklas, 1979). The organophosphate insecticides phosphamidon, monocrotophos and dichlorvos all inhibited acetylcholinesterase activity in earthworms (Bharathi and Rao, 1984). However, carbamates are demonstrated more toxic to *Lumbricus terrestris* than organophosphates, and this leads to the suggestion that carbamates may affect additional target sites in the earthworms (Stenersen *et al.,* 1973; Roberts and Dorough, 1984).

There is relatively good study on effects of chemicals on reproduction of earthworms. Cocoon production and hatching of *E. fetida* were influenced by pentachlorophenol in soil (Gestel and Dis, 1989). Neuhauser *et al.* (1984) also tested the effects of five heavy metals on cocoon production in *E. fetida* and found that cocoon production decreased with increasing concentration of all the metals. In another study by Bouwman and Reinecke (1987), clitellum development and cocoon production were completely inhibited in earthworms exposed to doses of carbofuran. Cadmium and zinc also inhibited cocoon production in *E. fetida* at concentration of 2.5ppm or greater (Neuhauser *et al.,* 1984). The broad-spectrum drug, ivermectin, also caused a strong decline in cocoon production in earthworms (Gunn and Sadd, 1994). Some chemicals also adversely affect hatching success of cocoons and elongate incubation period (Reinecke and Venter, 1985).

Earthworm species differ greatly in their growth patterns and any effect on growth is usually most common in rapidly growing species. For instance, loss of weight or slowing of growth as a result of chemicals has been reported much more often for the fast growing *E. fetida* than for the slow growing *L. terrestris* (Edwards and Bohlen, 1996). Neuhauser *et al.* (1984) tested the effects of five different heavy metals (mixed with manure) on the growth of *E. fetida* and observed that all metals reduced growth relative to that in the controls.

2. 4. Uptake and bio-accumulation of chemicals into earthworms

Lipophilic agricultural chemicals, due to their lipid solubility and affinity for their soil associated organic matter, become sorbed preferentially to the organic matter in soil and stream sediments. As a result, biota associated with the soil or sediment is exposed to relatively high concentrations of pesticides and may bioaccumulate such materials. Earthworms, being the most important soil organisms, have been accepted as the test animal to predict terrestrial hazards (Hoque and Ebbing, 1988). They may accumulate many hazardous chemicals to levels higher than those in the surrounding soil, and residues may in turn be ingested by birds and other animals feeding on them.

Because earthworms are on the lower level of the food chain, contamination and accumulation of toxic substances in them could result in toxicity to their predators (Beyer and Gish, 1980; Roberts and Dorough, 1985; Gish, 1970). In an extensive survey of pesticide residues in soil and earthworms, Roberts and Dorough (1984) sampled agricultural fields in eight states and analyzed for DDT, aldrin, heptachlor and their metabolites, and insecticide residues in soils averaged 1.5 ppm, whereas residues in earthworms averaged 13.8 ppm, a nine-fold concentration of chemicals in the worms over the soil. In another study on persistence of organochlorine insecticide residues in soil and earthworms they also demonstrated that pesticide residues in earthworms remained high enough to be considered to be toxic to some sensitive species of birds that prey on earthworms.

Insecticide residues of fensulfothion, chlorpyrifos and trichlornate were also found in earthworms three weeks after these chemicals were applied to the soil at recommended rates (Edwards, 1984; Edwards and Bohlen, 1996). The authors also added that diazion was highly accumulated in earthworms taken from soil that have been treated with the insecticide. Diazon soil residues of 4ppm increased to 200ppm in the worms within one month after application of the chemical. Several investigations on the bioaccumulation and persistence of chemical residues in earthworms and other soil invertebrates also suggest that the use of insecticides may pose serious risks to non-targeted species that feed on earthworms. Bayer and Krynisky (1989) reported that earthworms can accumulate persistent soil-borne pesticides and are important sources of contamination of terrestrial wildlife.

Earthworms also appear to accumulate other toxic materials, particularly heavy metals such as cadmium, copper and lead from soils, which constitute an additional hazard to animals that prey on them (Neuhauser *et al.,* 1984; Roberts and Dorough, 1984). Some earthworms had accumulated up to 330ppm lead and 670ppm zinc, indicating that animals feeding on them along road sides would be chronically exposed to toxic doses of heavy metals. Curl *et al.* (1987) also stated that when earthworms are maintained in soil containing (C^{14}) cypermethrin they accumulate radioactive residues, and these residues are not eliminated even when the worms are transferred to untreated soil.

Therefore, many field surveys recommended that hazardous pollutants in the environment could be monitored by analyzing earthworms from soils suspected of being contaminated with persistent insecticides, heavy metals, polychlorinated and polybrominated biphenyls or dioxins (Inglesfield, 1984; Roberts and Dorough, 1984; Gestel *et al.*, 1989).

2. 5. The influence of environmental factors on abundance and distribution of earthworms

Earthworms are thin-skinned invertebrates with little protection against the changes in physical and chemical conditions of soil (Edwards and Bohlen, 1996). Several environmental factors affect their activity, population structure, abundance and distribution in the soil. The most critical and frequently mentioned factors include organic matter content of the soil, type of soil, moisture content, aeration and carbon dioxide and pH and temperature (Wood, 1972; Lee, 1985; Werner *et al.*, 2005). Moreover, Werner *et al.* (2005) added that climatic and biotic factors strongly affect abundance and distribution of earthworms. Baker (1983) also mentioned soil depth; water table depth, ash content and dry bulk density affect distribution, abundance and species associations of earthworms.

2. 5. 1. Organic matter and soil type

The amount of organic matter in the soil strongly influences abundance and distribution of earthworms, and soils that are poor in organic matter do not usually support large number of earthworms. Several workers have reported strong positive correlations between earthworm numbers and biomass and organic matter content of the soil. Schmidt *et al.* (2004) reported in their extensive works on Egyptian agricultural soils that an increase in organic carbon content was always associated with increased numbers and biomass of earthworms. Stehouer *et al.*, 1994 and Edwards and Bohlen, 1996 also pointed that large amounts of dead roots and other organic matter in pasture usually coincide with large number of earthworms, and it is probably the gradual decrease in soil organic matter, when pasture is ploughed and used for arable crops, that leads to a corresponding decrease in earthworm populations.

Soil type and texture also influence earthworm populations, though there are only very few studies on this aspect (Edwards and Bohlen, 1996). There were differences both in total and relative numbers of each species of earthworms in soil of different textural compositions. Earthworm populations show highest preference to highest and medium textured loam soils, and smaller populations occur in poorly drained clay soils and coarse sandy soils (Table 1). Backman (2005) cited in Gezahegn Degefe (2006); Muys *et al*. (1992) and Baker *et al.* (1992) attribute this variation in differences in distribution and abundance of earthworms with soil types to the organic content, aeration, and other essential soil properties.

Table 1 Relationship between soil type and earthworm populations (From Edwards and Bohlen, 1996)

Soil type	Population	No/m^2	No of species
Light sandy	232.2	57	10
Gravelly loam	146.8	36	9
Light loam	256.8	63	8
Medium loam	226.1	56	9
Clay	163.8	40	9
Alluvium	179.8	44	9
Peaty acid soil	56.6	14	6
Shallow acid soil	24.6	6	5

2. 5. 2. Moisture, pH, temperature and aeration

Earthworms generally require the presence of adequate moisture for growth and survival, and also their activity is strongly determined by moisture content of the soil. This is mainly because they breathe through their epidermis (moist skin) and the blood capillaries on the surface should get sufficient moisture to perform their respiratory activity (Eckert and Randal, 1988). As a result, most earthworms are more active in moist soils than dry ones, and prevention of desiccation or water loss is the major factor in earthworm survival (Mary, 1982; Kretzschmar and Bruchou, 1991). However, not all species of earthworms have the same moisture requirements.

Different earthworm species have adopted different strategies to cope with dry soil conditions. Some (e.g. *L. terrestris, A. longa, E. fetida*) migrate to deeper soil when the surface soil is too dry, some diapause (e.g. *A. calignosa, Aporrectodea spp.*), some produce drought-resistant cocoons even under extreme drought conditions (Kretzschmar and Bruchou, 1991). If they cannot avoid dry soil by other means, the earthworms can still survive the loss of a large part of the total water content of their bodies. Edwards and Bohlen (1996) stated that *L. terristris* can lose 70% and *A. chlorotica* 75% of their total body water and still survive. But many workers agree that prolonged drought strongly affects earthworms.

Despite the fact that earthworms require adequate moisture for survival, excessive moisture or heavy rainfall can have an effect on them. This is because too much moisture takes the place of dissolved oxygen in the soil, which may cause the worms to crawl to the soil surface where they can be exposed to ultra violet radiation and predation (http: //www. naturewatch cited in Gezahegn Degefe, 2006).

Low pH values are usually unfavorable for many species of earthworms and often cause a decline in species numbers, except for few acid-tolerant species that can harbor acidic soils (Werner *et al.,* 2005). Several workers have stated most species of earthworms prefer soils with neutral pH and a slight drop in pH may lead to consequent decline in the number of earthworms (Edwards and Bohlen, 1996). However, *L. terrestris* can occur in soils with a pH of 3.5; *A .calignosa* in soils with pH of 5.2-5.4, and few species can even occur in soils of pH lower than 4.3 (Edwards (1988) also reported *E. fetida* could tolerate a pH range from 4.0-7.0, but Rivero-Hernandez (1991) pointed that the worms prefer soils with a pH between 7.0 and 8.0.

Edwards and Bohlen (1996) took soil samples with different pH values (4.0, 4.1, 4.4, 5.0, 5.1, 5.6, 5.8, 6.9 and 7.0) and placed mature individuals of earthworms on the surface of each and compared their reaction as well as time they took to burrow down. And he observed worms in the three most acidic soils showing a violent avoiding reaction, twisting, jerking convulsively and exuding coelomic fluids from their dorsal pores. They then extended to their full length and crawled about the soil surface intermittently raising and waving the anterior segments. Then the activity gradually became sporadic and they lay motionless and became flaccid, and finally, 58 out of 60 worms exposed to pH below 4.4 died within 21 hours. However, too high pH values also reduce earthworm activity (Werner *et al.*, 2005).

Like for every living organisms, the activity, metabolism, growth, respiration and reproduction of earthworms are greatly influenced by temperature. Earthworms can be killed by temperatures outside their survival limits. It has been suggested that earthworm populations in soils can be destroyed by frost in the absence of ground cover, and high surface temperature and dry soils are also much more limiting to them than low temperatures and waterlogged soils (Edwards and Bohlen, 1996).

Some workers studied the upper and lower lethal temperatures for earthworms and found that the upper lethal temperature for earthworms is lower than for many other invertebrates, although there is considerable variation in estimates of these temperatures by different workers. Edwards and Bohlen (1996) reported median upper lethal temperatures of 37.0-37.5°C for *Pheretima californica* and 39.55-40.75°C for *A. calignosa*, 25°C for *E. fetida* and 29.7°C for *A. rosea.* Temperature also affects fecundity, duration of cocoon incubation time and the growth period from hatching to sexual maturity in earthworms (Holmastrup *et al.*, 1991).

Experimental evidences on the effects of soil oxygen tension on the distribution and abundance of earthworms in soil are very rare. However, the distribution of some species appeared to be limited by the minimum oxygen tensions occurring at certain seasons. Edwards and Bohlen (1996) stated that there was some correlation between numbers of some earthworm species and oxidation-reduction potentials of the soil. He also related the extremely low numbers of earthworms in saturated depressions in the soil to the low oxygen content of the soil. *E. fetida* could not survive in aged sewage sludge and retreats from layers of organic matter when they become anaerobic (Kaplan *et al.*, 1980; Edwards, 1988). Though earthworms can live at a relatively low oxygen levels, in the complete absence of it, they may be adversely affected or even die (Mary, 1982).

3. MATERIALS AND METHODS

3. 1. Description of the study area

Golden Rose Agro Farm is located in South west Shoa Zone, Oromiya Region at about 48 kms southwest of Addis Ababa, near the town of Tefki. It is located on the main road crossing from Addis Ababa to Jima at an elevation of about 2060 m. The company is part of a family group whose headquarters are located in London and is fully equipped with imported technology for flower production. The rose farm contains 21 greenhouses (Fig.1) that cover a total land area of about 50 ha currently under rose production. In each of the greenhouses, there are beds constructed for flower production. The beds are frequently watered and chemicals and fertilizers are also provided for each bed in the form of spray as well as driplines.

Figure 1. Some of the greenhouses in Golden Rose Agro Farm (Dec. 02/2006)

The farm produces 35 commercial varieties of roses of the family Rosaceae (*Rosa spp*) for export, each having their own commercial names. These are: Appricort, Arena, Attracta, Ballet, Charmer, Circus, Duo-unique, Duett, Eltro, Esperance, Golden gate, Gold sphinx, Grand prix, High boury, High fantacy, High society, Hollywood, Jupiter, Kalhari, Kerio, King fisher, King spride, Kiwi, Orange unique, Pasha, Passion, Red champ, Red one, Renee, Shanti, Sunbern, Tropical amazon, Tombola, Ranacula and Wow. Golden Rose Agrofarms Ltd. exports shipments to Europe using Lufthansa and Ethiopian Airlines services, and it hopes to broaden its target market within Europe and to enter the United States as well in the near future.

Golden Rose Agro Farm is the first and largest of all flower farms in the country with the maximum flower yield of about 28,800,000 flowers per year. The Agro Farm started exporting roses in February 2000. The Farm makes extensive use of chemicals (pesticides, fertilizers, wetting agents and acids) for proper production of good quality roses (Fig. 2). More than 116 types of commercial chemicals and fertilizers are currently being used for different purposes. The farm makes extensive use of chemicals to control agricultural pests and diseases, to maintain good flower quality, to maintain proper soil pH for flower production, to provide nutrients for the flowers in the form of drip lines and to maintain proper blooming periods. The most commonly used chemicals in the rose farm are summarized in Appendix 1.

Figure 2. Workers spraying chemicals in the greenhouses (Dec. 02/2006)

The control site is the area surrounding the Golden Rose Agro Farm. It covers the outside agricultural land where there is no chemical use (only fertilizers such as DAP and urea are rarely used). The minimum and maximum average temperatures in the area vary from 12-15^{o}C to 29-30^{o}C respectively. The dominant crops in the area during the wet season are cheak pea (*Cicer arietinum*), lentil (*Lens culinaris)*, cowpea (*Vigna unguiculata*), teff (*Erogrotis tef)*, common bea (*Phaseolus vulgaris*), wheat (*Triticum sativum)* and some grass species. Trees such as *Cordia africana* and *Acacia spare* are also found in the area. In the dry season, however, since all the crops were harvested, no live vegetation was seen except few plant species and the little grass vegetation that were found scattered on the ground (Fig. 3). Since the area is usually covered by accumulated surface water during the rainy season, the soil remains wet till the end of November and this might have created suitable condition for the earthworms. Map of the study area showing Golden Rose Agrofarm and the surrounding chemical-free farm is described in Fig. 4.

Figure 3. The chemical-free farm in the dry season
(Feb. 10/ 2007)

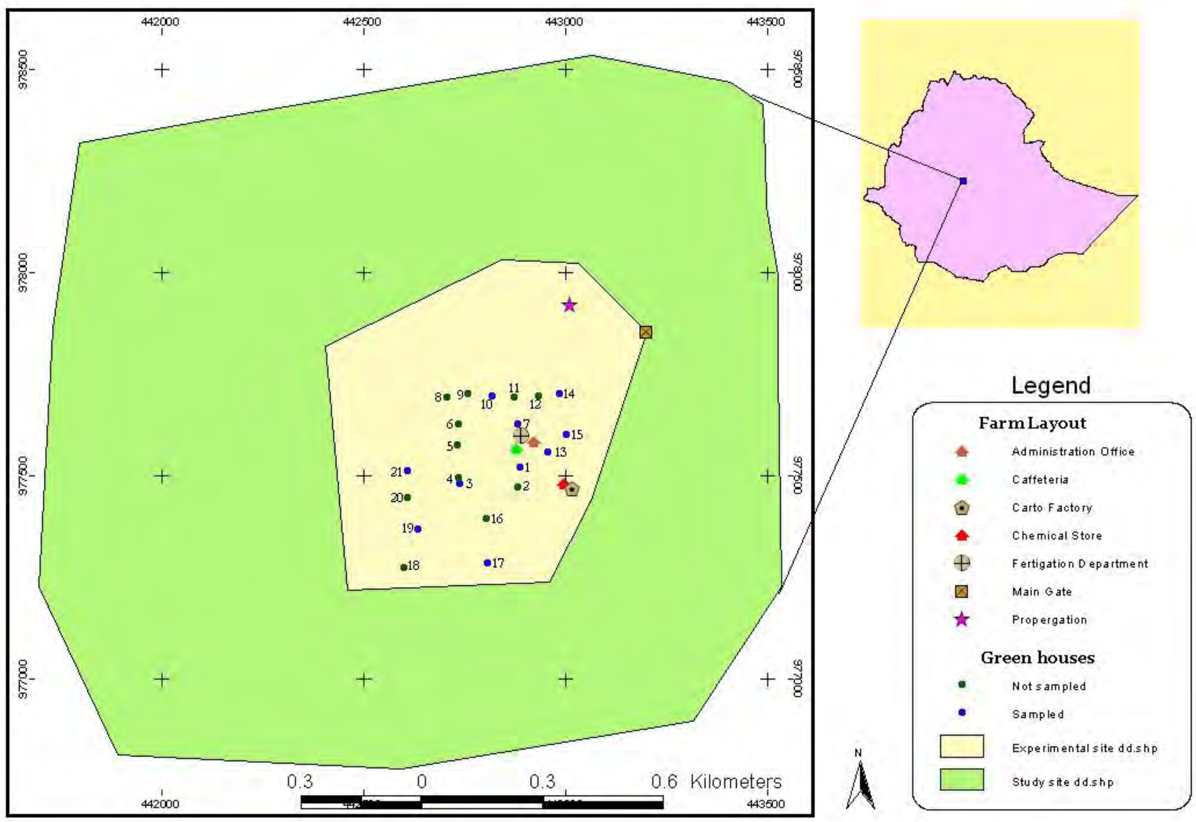

Figure 4. Map of the study area

3. 2. Sampling techniques

The study area consisted of two nearby but functionally independent sites: the land occupied by the Golden Rose Agro Farm, which is currently under extensive chemical use, and the surrounding agricultural land, which is almost free from chemicals. The total twenty-one greenhouses in the Golden Rose Agro Farm were systematically grouped into old and new phases (old phase greenhouses are relatively older ones and have an age of more than five years, whereas the new phase greenhouses are younger than five years). Then ten greenhouses were selected for sampling by lottery method (seven greenhouses were selected from the old phase and three were selected from the new phase).

More samples were taken from the old phase because relatively more chemical effect was expected in areas where chemicals were used over longer duration (Loke and Gester, 1988).

In each of the randomly selected greenhouses, four 1mx1m ($1m^2$) quadrats were marked on the ground at about equal intervals (the approximate distance between the quadrats was 50 m) on each sampling occasion. However, for the new phase greenhouses, the number of sample quadrats marked per greenhouse was extended to eight. This was because the greenhouses in the new phase are almost twice as large as the old ones in size and more samples had to be taken accordingly to get accurate estimation of the worms.

On each sampling occasion, fifty-two 1mx1m quadrats were marked on the soil surface (twice in the dry season and twice in the wet season), which accounts to a total of 208 quadrats marked in the rose farm. The total sample quadrats marked in the greenhouses were expected to be good representatives for the whole area of the farm under extensive chemical use. DeJong-Hughes (2005) also stated that the number of samples taken at any site in earthworm estimation should be a good compromise for the correct prediction of earthworm population size. However, sampling in the greenhouses was not as conducive as in the outside farm due to dense flower covers and their roots on each flower bed in the greenhouses.

The second site was the area surrounding Golden Rose Agro Farm, which has similar climatic conditions and soil type, but without chemical treatment unlike that of the rose farm. The area selected for sampling approximately covers about $1km^2$ (100 ha) land surrounding the rose farm. Considering the rose farm as the central reference point, the outside farm was roughly divided into four sampling stations: the regions at the north, south, east and west of the rose farm. For each sampling station, 13 quadrats were randomly marked on the ground per 50 m of land during each sampling occasion (twice in the dry season and twice in the wet season), which accounts 104 quadrats per season and then a total of 208 quadrats on the chemical-free farm during the four sampling occasions.

While taking samples from this area, sampling from plots under teff (*Erogrotis tef*) cultivations was intentionally avoided. This is because some farmers in the locality use weed killers to control weeds in the tef farms and this may add chemical residues to the soil and influence population of the surface dwelling earthworms. Edwards and Bohlen (1996) also mentioned that many agricultural chemicals can influence the number of earthworms in the nearby soil environment.

At both the flower farm and outside chemical-free farm, soil was dug from each marked quadrats to a depth of 15 cm, hand sorted on site and all the earthworms present were carefully removed and transferred to 70% ethanol and preserved for further population estimation (Fig. 5). The worms were grouped to the three ecological groups (epigeic, endogeic and anecic ones) and only epigeic (top soil dwelling) ones were considered for further work in this study. The sampling technique used (free hand sorting) is considered to be an efficient way to sample epigeic (top soil dwelling) species (Spurgeon and Hopkin, 1999; Whalen, 2004; Hale and host, 2005; Gezahegn Degefe, 2006). They also stated that hand sorting is particularly relevant when assessing populations of earthworm species that live close to the soil surface. Therefore, it is assumed to be the best method to sample top soil earthworms for population estimation and comparison works.

 

Figure 5. Hand sorting top soil earthworms (Oct. 12/ 2006)

In all the sampling sites, during each sampling session, worms were easily sorted as adults and juveniles based on the presence and absence of fully developed clitellum. Adult earthworms have a uniquely colored, swollen band that looks like a collar along their body (Fig. 6). In cases when the clitellum was not clearly visible by the naked eye, magnifying lens was used to sort the worms to adult or juvenile. Most of the sorting process was done at the sampling sites during the sampling processes while the worms were still alive, or within few days of collection. This is because when some adult worms die, their body shrinks and this may to some extent mislead the sorting process if the worms were not sorted alive.

Figure 6. Adult earthworm with matured clitellum (the swollen part is the clitellum)

3. 3. pH and Organic carbon content

Since pH and organic matter content of the soil commonly affect abundance and distribution of the earthworm populations in any habitat, soil samples were taken from both farms at a depth of 15 cm and measured for the two parameters for comparison. To measure pH, 10 soil samples (each weighing approximately 100 g) were taken from each of the two sites, mixed with 100 ml of distilled water and the solution was measured using digital pH meter, and then the mean pH values of the two farms were compared. To measure organic carbon content, 4 soil samples (each weighing 1Kg) were taken from each site, tested for organic carbon content using Walkley-Black method (the organic carbon of the soil was oxidized by potassium dichromate in sulfuric acid solution) and the mean values of results from the two sites were again compared.

3. 4. Mortality test

To exclude the effect of greenhouse (to see whether the chemical was really the main factor influencing abundance and distribution of the worms in the rose farm), eight plastic buckets were taken and soil was filled into each up to 15 cm depth. The soil used for this purpose was taken from the chemical-free area. Fifteen adult worms (a total of about 120 worms) were introduced into each bucket and then the buckets were put in the greenhouses (Fig. 7). To avoid any escape of worms, all the buckets were sealed with transparent plastic sheets punctured with small holes to allow exchange of air (Loke and Gestel, 1998). Five of the eight specimens were put under chemical treatment and three were left free for control. In the five buckets, soil was mixed uniformly with agricultural doses of the mixture of chemicals commonly used in the rose farm (such as diazion, dursban, confidor, spidermecc, tedion, pentac, bctin, metatoxin, acrobat, mondizon, meltatox, sporakill and others). The chemicals were then constantly applied to each at weekly intervals (10 ml of the mixed chemicals were applied each time). All the necessary requirements were equally provided for both groups except that one was put under chemical treatment and the other was left free. Finally, the numbers of worms in each bucket were carefully counted after twenty days (Loke and Gestel, 1989) and results were compared.

Figure 7. Introducing worms into the greenhouses (Oct. 20/2006)

3. 5. Taxonomy

To distinguish the major taxonomic groups of earthworms collected during the sampling periods, their taxonomy was identified to genus level using taxonomic keys. For convenience recent as well as old keys were referred and information from both sources was integrated. Mainly the taxonomic keys of Great Lakes Worm Watch (www.naturewatch.ca/english/wormwatch/programs/inv2.html), Worm Watch (2002) and taxonomic key of Edwards and Lofty (1977) were used. Furthermore, the taxonomic results found were again compared and cross checked against the profiles of different groups of earthworm taxas provided in the literature. Since identifying juvenile earthworms is commonly difficult and requires more experience (Baker, 1983; Worm

Watch, 2002; Hale and Host, 2005), only adult worms (with clitellum) were considered for identification. Moreover, worms with poor sample quality (as a result of such factors as mechanical damage and missed parts of the body) were not considered for taxonomic purpose.

Coloration (pigmentation), setal arrangements, space between the setae, position of the clitellum in relation to body segments, location of the male and female pores in relation to body segments, the total number of segments, and shapes of the paired ovaries are some of the morphological and anatomical characters considered for identification purpose. Dissecting microscope and hand lens were used to confirm body structures that could not easily be seen with naked eye during the identification process.

Color plays an important role in identifying earthworms (Worm Watch, 2002). However, there was frequent and extensive decoloration of the worms following treatments with formalin (1-2%) and ethanol (70%) during the sampling process (ethanol and formalin were used to preserve worms for further population estimation). As a result, live specimen was prepared and used for taxonomic purpose. During various sampling sessions, sufficient numbers of adult worms were collected and transferred to plastic buckets containing soil with good organic matter and moisture content. To maintain good organic matter content, finely ground cattle manure and other organic remains of plants were spread on the soil surface (Lokke and Gestel, 1989). The water level in the bucket was also adjusted to moderate level since saturation of the soil with water can affect aeration (Ausden *et al.*, 2001). The mouth of the bucket was covered by thin plastic sheet to avoid escape of worms since earthworms can crawl over the surface and leave out. To allow aeration, minute perforations were made on the surface of the plastic sheet covers. The specimens were then put in the laboratory (at about 25^{o}C) under good follow up till worms were taken out and used for identification purpose. Out of the total living worms kept in the laboratory, 20 adult worms were taken and identified to genus level.

3. 6. Statistical Analysis

The difference in mean abundance of adult and juvenile top soil earthworms between the rose farm and the chemical-free farm, and changes in their abundance in relation to wet and dry season were analyzed by independent t-test. Taking the mean population in the sampled areas minimizes biases resulting from the unequal sample quadrats as well as inaccuracy during counting the worms. The statistical probability $P < 0.05$ was considered significant.

Population density and the total population of top soil earthworms in the two farms were estimated by dividing the mean number of worms collected from all quadrats by total number of quadrats and multiplying the sample mean by the total area in each of the farms respectively. Statistical Package for Social Science (SPSS) software version 13.01 was used to run the statistical comparisons.

4. RESULT AND DISCUSSION

4. 1. Taxonomy

Out of the adult worms randomly picked for identification, about four genera are identified. The two most dominant genera encountered in the area were *Eiseniella* and *Dendrobaena*. These two genera together comprise 85% of the total taxonomic groups identified in the study area. The remaining two genera (*Allelobophora* and *Aporrectodea*) consist of not more than 15% of the total groups identified. Worms that belong to the genus *Aporrectodea* were very few in number (consist only 5% of the total genera identified in the area) (Table 2). All of the worms collected from the soil under the rose farm belong to the genus *Eiseniella* and the other three genera identified in the chemical-free farm were absent in the rose farm.

All of the worms identified in this study belong to the same family, *Lumbricidae*. It seems that only few taxonomic groups cluster in large numbers and taxonomic diversity is low in the area. Gezahegn Degefe (2006) also identified about five genera of earthworms that belong to the family *Lumbricidae* in his comparative study at Assela Biofarm and Herero State Farm. This might indicate that earthworms in many parts of Ethiopia live in cluster of only few species. However, studies concerning earthworms are very rare in the country and more extensive works are required. It has also been often stated that the *Lumbricidae* have an ability to adapt to new environments more than any other oligochaets, and that once introduced into an area cause the disappearance of the endemic earthworm fauna (Edwards and Bohlen, 1996). The authors also mentioned that this group of earthworms have the ability to live in those environments that are constantly disturbed by the cultural and agricultural activities of man.

Table 2. The common genera of earthworms identified in the study area and their percentage proportions

Genus	Number Identified	Percentage (%)
Eiseniella	10	50
Dendrobaena	7	35
Allelobophora	2	10
Aporrectodea	1	5
Total	20	100

Most of the worms identified in this study belong to epigeic ecological group probably due to the sampling procedure. Most workers involved in earthworm research (e.g. Baker, 1983; Sporgeon and Hopkin, 1999; Hale and Host, 2005) also reported large proportion of epigeic ecological groups to the others. Gezahegn Degefe (2006), however, reported more endogeic earthworms in his comparative study on different ecological groups of earthworms in Assela Biofarm and Herero State Farm. Moreover, the sampling technique in this study attempted only to capture top soil dwelling species (at not more than 15 cm depth) and this was promising to capture epigeic (surface dwelling) earthworm species.

Earthworms of the genera *Eiseniella* and *Dendrobaena* were dominant in this study may be because they had good adaptations to the soil conditions (such as pH, quantity and quality of organic matter, moisture, temperature etc.) of the area relative to the others. Lee (1985); Beare *et al.* (1995) and Edwards and Bohlen (1996) also pointed that diversity and abundance of earthworm species in a given area depend on their habits as well as habitat requirements. Different earthworm species usually have different ecological requirements (Philipson *et al.*, 1976) and adaptations to different soil types, and this might have favored the relative abundance and distribution of the two dominant genera (*Eiseniella* and *Dendrobaena*) and affected that of the others in the study area. Edwards and Bohlen (1996) also reported that species of *Lumbricidae*, particularly the genera *Lumbricus*, *Eiseniella*, *Dendrobaena* and *Bimastos* to be dominant in agricultural lands and gardens throughout the world.

All of the worms collected from the rose farm belong to the genus *Eiseniella* and the other three genera identified in the chemical-free farm were absent in the rose farm probably because the genus *Eiseniella* has developed good resistance to chemicals than all the other three genera found in the study area. Different earthworm species have different degrees of tolerance to chemical treatments in agricultural fields (Spurgeon and Hopkin, 1999), which in turn depends on the behavioral activities of the species (Delahaut and Koval, 2006). The concentration doses at which different chemicals affect different earthworm species as well as the frequency of exposure of each species to the chemicals also vary depending on the habit and activity of each group (Edwards and Lofty, 1977; Edwards and Bohlen, 1996), and this might have favored the genus *Eiseniella* than the other genera in the chemical-treated soil under the rose farm.

4. 2. Abundance of adult and juvenile top soil earthworms in the chemical-free farm

Majority of the earthworms collected in the area ecologically belong to the epigeic groups (Table 3).

Table 3. Numbers of the three ecological groups of earthworms encountered in the study area

Site	Number of worms found		Ecological groups encountered		
	Adult	Juvenile	Epigeic	Endogeic	Anicic
Chemical-free farm	670	49	679	27	13
Rose farm	9	0	9	0	0
Total	679	49	688	27	13

Large number of adult top soil earthworms were collected relative to juvenile ones. In the wet season, a total of about 666 adult and 49 juvenile worms were recorded (Table 4). The mean numbers of earthworms captured (per meter square area) in the wet months were 6.4 adults and 0.5 juveniles respectively.

Table 4. Number of adult and juvenile top soil earthworms collected in the wet season in the chemical-free farm

Season	Number of quadrats sorted	Number of worms collected		
		Adult	Juvenile	Total
Wet	104	666	49	715
Dry	104	4	0	4

In the dry season, however, the number of top soil earthworms collected was very small. Out of the total 104 quadrats sorted, only 4 adult worms were collected (all were from sample quadrats associated with moist areas such as accumulated cow dung and domestic wastes), and no juvenile worm was encountered at all. The mean numbers of worms collected in the chemical-free farm (per m^2) is shown in Fig. 8.

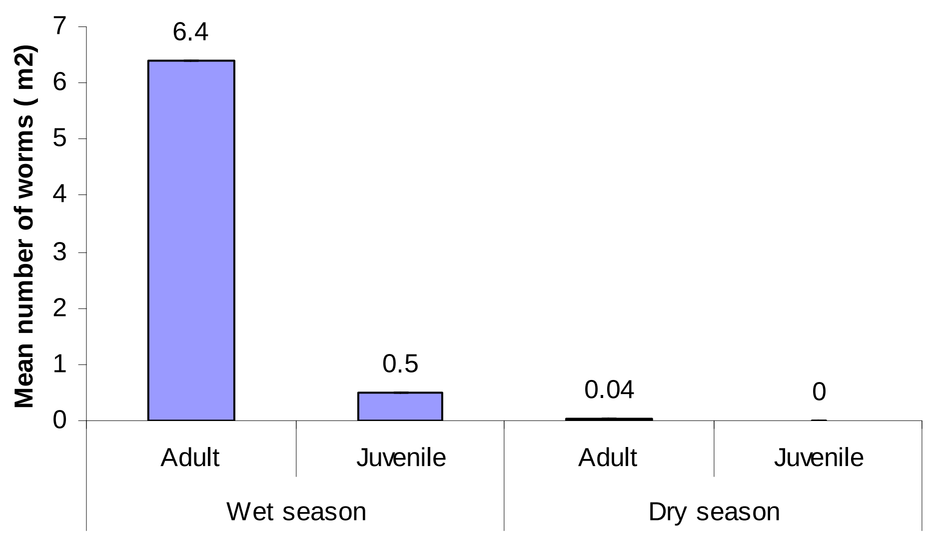

Figure 8. Mean number/m^2 of adult and juvenile top soil earthworms collected in the chemical-free farm

Therefore, at α=0.05 the data shows strong statistically significant variation in the number of adult and juvenile worms (P= 0.000 and 0.000 respectively) between the wet and dry season (appendix 2).

The significant variation in the mean abundance of both adult and juvenile worms between the wet and dry season in the area obviously shows the role of seasonality in determining the earthworm population. The strong decline in the number of top soil earthworms in the dry season relative to the wet season was may be due to the dry soil condition since large number of them were collected from the same area in the wet season. During the wet season, when there was sufficient moisture as a result of rain and surface accumulated water, large number of worms (about 666 adults and 49 juveniles) were captured from the same area, but relatively no worm (only 4 adult worms) was encountered in the same area using equal number of quadrats during the dry season. This may indicates the influence of moisture content of the soil on the earthworm population.

Gezahegn Degefe (2006) in his study also reported negligible number of earthworms in the dry season relative to the wet season, and suggested strong effects of moisture on earthworms. Mary (1982); Kretzschmar and Bruchou (1991) and Potter *et al*. (1994) also underlined that a drop in moisture content of the soil strongly affects number and distribution of earthworms in any type of soil. Similarly, Holmstrup (2000) stated that under dry soil conditions and low moisture contents, the earthworms either migrate down to the deeper soil profiles where they can get moisture, or produce encysted (resistant) cocoons and disappear. Fernando *et al*. (2006) also indicated seasonal population dynamics among earthworms, though different species of earthworms have different degrees of resistance to moisture loss of the soil.

In addition to moisture, temperature might have also affected abundance and distribution of top soil earthworms in the dry season. This is evident because the average temperature of the area which was in the range of 12-15^{o}C during the wet months (September and October, 2006) increased to about 29-30^{o}C in the dry months (December and February, 2007). It is also well studied that a slight increase or drop in soil temperature affects abundance and distribution of earthworms (Edwards and Lofty, 1977; Holmastrup *et al.*, 1991; Edwards and Bohlen, 1996; Sandifer and Hopkin, 1996). Therefore, the few adult worms encountered in the area in the dry season were probably the ones that had good adaptations to temperature and water loss of the dry season.

4. 3. Abundance of adult and juvenile top soil earthworms in the rose farm

In the rose farm, only a total of 9 adult worms were collected from all the quadrats sorted over the whole sampling period, and no juvenile earthworm was encountered in any of the sampled greenhouses (Table 5).

The mean numbers of adult and juvenile worms collected (per m^2) in the rose farm were then 0.04 and 0 worms respectively (Fig. 9).

Table 5. Number of adult and juvenile top soil earthworms collected in the rose farm

Season	Number of Quadrats sorted	Number of worms collected		
		Adult	Juvenile	Total
Wet	104	5	0	5
Dry	104	4	0	4

Even though the terms 'wet and dry' season can describe the chemical-free farm, it is impossible (and of course not logical) to determine wet and dry season in the greenhouses, due to the extensive watering throughout the year. Consequently, this study did not consider seasonal variation in the number of worms (rather considered the total numbers) while comparing the number (abundance) of worms in between the two farms.

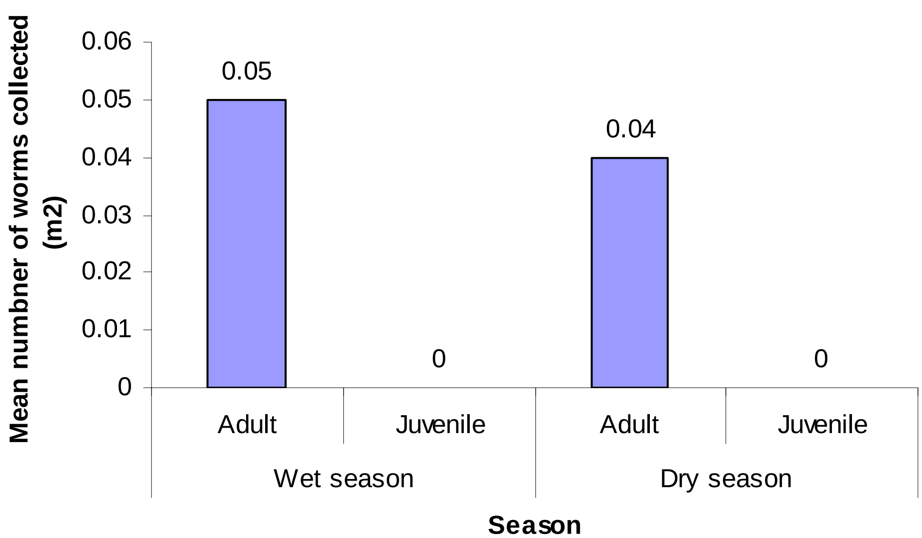

Figure 9. Mean number of adult and juvenile top soil earthworms (per m^2) observed in the rose farm

In the study, large number of top soil earthworms were encountered in the chemical-free farm relative to the rose farm (Fig. 10). Therefore, at α=0.05, comparison of the mean number of adult and juvenile worms between the two farms shows strong statistically significant variation (P= 0.000 and 0.000 respectively) (see appendix 2)

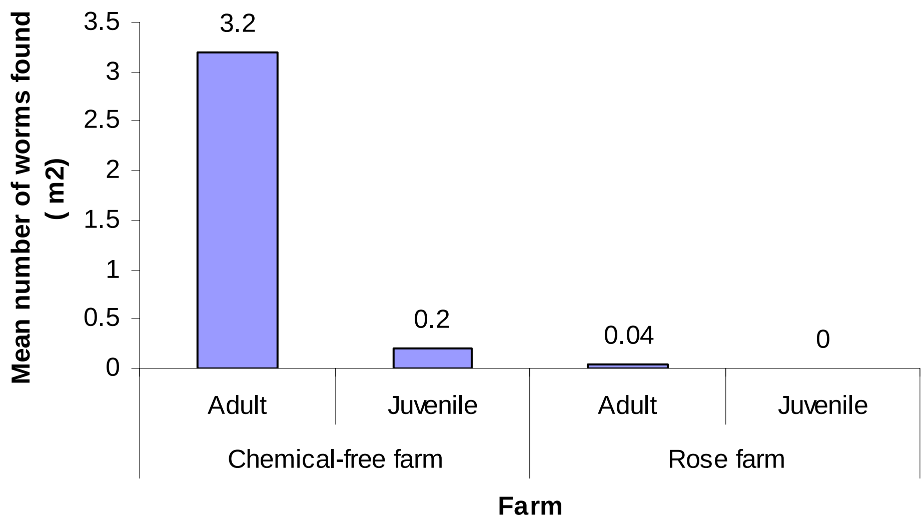

Figure 10. Comparison of mean numbers of top soil earthworms (per m^2) collected in the two farms

One may attribute the difference in the mean number of both adult and juvenile worms between the two farms to such factors as mechanical damage due to human activities and disturbances related to agricultural works. However, Edwards and Lofty (1977); Ramsay and Hill (1978); Tuker (1992) and Edwards and Bohlen (1996) strongly argued that mechanical damage and disturbances related to human activities in agricultural fields have only insignificant effects on earthworm populations. They also stated that earthworm populations do not strongly decline from mechanical damages during tillage operations, but rather from a reduction in the organic matter content of the soil. Most earthworm species also have regeneration power and in most cases can revive from mechanical damages as a result of agricultural activities (Tian *et al.*, 2000). Earthworms are often called "friends of the agriculturalist" and friendly agricultural activities do not harm their population, but agricultural activities related with chemicals and loss of organic matter can adversely affect their abundance and distribution in soil (Ezemonye *et al.*, 2006).

In fact, the most important factor that is known to influence abundance and distribution of earthworms in such disturbed agricultural fields is removal of surface litter and plant material due to human activities (Edwards, 1984; Edwards and Bohlen, 1996; Hop and Hopkin, 1996). Large number of worms also appeared to happen in the chemical-free farm relative to the rose farm, though mechanical damage and disturbances due to agricultural activities were common for both farms. Therefore, human activities due to agricultural practices other than chemicals can not be significant reasons for the strong decline in the earthworm population in the rose farm. Large number of chemicals (more than 116 types) are currently in use in Golden Rose Agro Farm as pesticides, fertilizers, buffering agents, wetting agents and additives, and this might have influenced the abundance and distribution of earthworms in the rose farm relative to the chemical-free one.

Without considering the presence of chemicals, the situation in the rose farm seems suitable for the existence of earthworms. The soil under flower production was originally the same type with that of the chemical-free farm, though it is currently under extensive use of nitrogenous and sulfate fertilizers. These fertilizers, however, are reported to positively influence the number of earthworms in agricultural soils. For instance, Edwards and Lofty (1977); Edwards and Bohlen (1996) and Gerard and Hay (1997) reported that nitrogenous and sulfate fertilizers (except $CuS0_4$) favor the build up of earthworm population.

The soil in the greenhouses had also moisture level suitable for the existence of earthworms (in range of 13-15%). The moisture level at which earthworms can survive best is recommended to be 10-17% (Edwards and Lofty, 1977 as cited in Gezaheng Degefe, 2006). Since greenhouses had also no significant effect on earthworms in this study (see section 5.5), the extensive chemical use was the main factor accounted for the strong decline in abundance and distribution of top soil earthworms in the rose farm.

Therefore, the few worms that were encountered in the greenhouses might have either developed resistance to chemicals through time, or were recently introduced from the outside together with fresh soil during bed construction in the greenhouses.

4. 4. pH and Carbon content

There was no much difference in the pH of the soil between the two farms. The pH of the soil in the rose farm and the chemical-free farm was 6.2 and 6.5 respectively. More organic carbon content was also recorded for the soil under the rose farm than the chemical-free one. The respective carbon content measured for the soil under the rose farm and the outside chemical-free site was 2.1% and 1.2% (Table 6).

Table 6. pH and organic carbon content recorded in the two farms

Parameter	Rose farm	Chemical-free site
pH	6.2	6.5
Organic Carbon	2.1%	1.2%

The pH of the soil in the rose farm (6.2) was slightly lower than that of the outside farm (6.5) probably because large number of chemicals that can lower soil pH, including sulfuric acid, phosphoric acid and nitric acid are highly used in the rose farm. It has long been demonstrated that earthworms are very sensitive to the hydrogen ion concentration of aqueous solutions, so it is not surprising that soil pH limits their abundance, distribution and species present (Singh *et al.*, 2005). However, several earthworm species are known to live in soils with much less pH values than 6.2 (Edwards, 1988; Werner *et al.*, 2005).

Therefore, pH, though may influence abundance and distribution of earthworms, can not be the case for the strong decline (almost absence) of earthworms in the rose farm. Moreover, quite large number of worms were collected from the outside chemical-free farm at about nearly equal soil pH (6.5), indicating that some other factors than pH induced the reduction in the number of earthworms in the rose farm.

Organic matter content is the major factor that influences earthworms in any type of soil. Earthworms use organic matter as a source of food to survive. Therefore, the amount of organic matter available influences not only the abundance and distribution of earthworm population but also the type of species present and their rate of growth and fecundity (Edwards and Lofty, 1977; Edwards, 1984; Edwards and Bohlen, 1996). Lofs-Holmin and Bostrom (1986) also investigated the influence of organic matter on earthworm cocoon production, and clearly observed more cocoon production by worms that were provided with additional decaying organic matter. Schmidt *et al.* (2004) also reported in their extensive works that an increase in organic carbon content was always associated with increased numbers and biomass of earthworms.

Even though the soil under the rose farm had relatively higher carbon content than the surrounding one, the number of top soil earthworms recorded in the rose farm was negligible, and large number of them were instead found in the chemical-free farm.

The rose farm had also good moisture content throughout the year and was also expected to be rich in essential nutrients such as NPK as a result of daily application of fertilizers such as $CaN0_3$, $MgN0_3$, $KN0_3$, K_2S0_4, urea and other inorganic fertilizers to the farm for good flower yield. Therefore, it is quite normal to expect larger number of worms in the rose farm than in the surrounding farm. However, the result of this study shows that worms appear in large number in the soil under the rose farm and large number of them rather aggregated in the outside chemical-free farm, indicating that extensive chemical use is the main factor that affected their abundance and distribution in the rose farm.

Hans *et al.* (1990) also investigated the effects of agricultural chemicals (aldrin, endosulfan, heptachlor and lindane) and stated that all had effect on the growth of the earthworm *E. fetida.* Stenersen *et al.* (1973); Tomlin and Gore (1974); Niklas (1979); Haque and Ebbing (1983); Roberts and Dorough (1984); Neuhauser *et al.* (1984); Zoran *et al.* (1986); Drewers *et al.* (1987); Callahan (1988); Anton *et al.* (1990); Edwards and Fisher (1991) and Nadeau *et al.* (2001) also supported that most agricultural chemicals have effect on the earthworm population.

4. 5. Mortality Test

The result obtained from mortality test is presented in Table 7. Almost in all the five chemical-treated buckets no worm was found (out of the originally introduced 75 adult worms only a single worm with poor body conditions was observed). All the originally introduced worms were almost dead. However, in all the three control buckets, worms were in good life condition and nearly the number originally introduced was recaptured including some juvenile ones, though originally juvenile worms were not introduced.

The mean number of adult worms recovered after 20 days in the experimental group was 0.2 individual whereas those of the control group was 12.7 worms. Therefore, at α=0.05, there is statistically significant variation in the number of adult worms between the two groups (p= 0.039) (appendix 2). On the other hand, the mean numbers of juvenile worms in the experimental and control groups were 0 and 1.67 worms respectively, and at α=0.05 there is statically significant variation between the number of juvenile worms in the experimental and the control groups (P= 0.009) as described in Fig. 11.

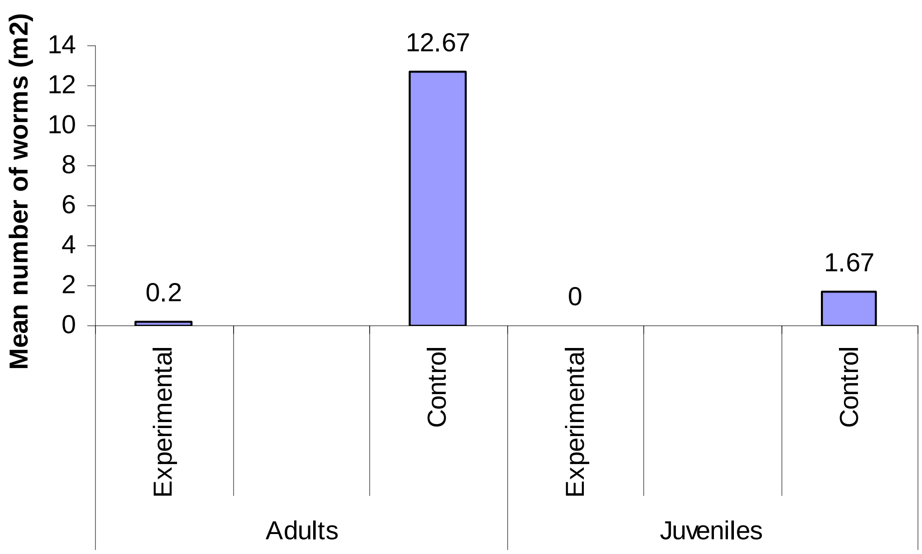

Figure 11. Difference in the mean numbers of worms in the mortality experiment

Since all the resources except chemicals were equally provided for both the experimental and the control groups, the complete absence of worms in the chemical-treated buckets was obviously due to the effect of chemicals. Several chemicals enter into the soil via driplines and large number of them are also daily sprayed in each of the greenhouses (Fig. 12) to assure proper production and good yield of flowers, and residues should have entered into the soil and affected these sensitive soil invertebrates. Gestel *et al.* (1992) and Yoshimura *et al.* (2005) also mentioned that most agricultural chemicals affect earthworm populations even at normal agricultural doses.

Figure12.Workers spraying chemicals in the greenhouses (Feb. 10/2007)

On the other hand, an increase in the number of worms in the control buckets was probably due to reproduction. The worms might have produced cocoons which later hatched to juveniles due to the favorable conditions (mainly absence of chemicals) in the control buckets. However, there was still some probability for fertile cocoons to have been originally introduced into the buckets incidentally together with the soil from chemical-free farm.

The result of mortality test strongly supports that earthworms can live in the greenhouses and even reproduce in the absence of chemical treatments as long as all the required resources are available, and then the most reasonable factor for the strong reduction (almost absence relative to the chemical-free farm) in the number of earthworms in the rose farm was chemical effect.

In the second test where earthworms were collected from the greenhouses and transferred into chemical-free soil, two juveniles were also encountered together with the originally introduced five adult worms. The worms were also relatively active and had good body size and color after 20 days. This probably shows that the worms which were originally under chemical effect in the rose farm could even reproduce when proper life conditions were provided for them. Verhoef and Gestel (1984) and Curl *et al.* (1987) also observed an increase in number of earthworms after transferring them from chemical-treated agricultural soil to relatively chemical-free soil.

The appearance of juveniles after worms were transferred into chemical-free soil strongly suggests that chemicals might also have affected cocoon production, and hence reproduction in earthworms. There is relatively good study on effects of chemicals on reproduction of earthworms. Holmstrup and Martin (2007) strongly supported that chemicals affect cocoon production and then reproduction efficiency in earthworms. Cocoon production and hatching of *E. fetida* were influenced by pentachlorophenol in soil (Gestel *et al.,* 1989). In another study by Bouwman and Reinecke (1987), clitellum development and cocoon production were completely inhibited in earthworms exposed to doses of carbofuran. Cadmium and zinc also inhibited cocoon production in *E. fetida* at concentration of 2.5ppm or greater (Neuhauser *et al.,* 1984). The broad-spectrum drug, ivermectin, also caused a strong decline in cocoon production in earthworms (Gunn and Sadd, 1994). Many agricultural chemicals adversely affected hatching success of cocoons and elongated incubation period (Reinecke and Venter, 1985). Therefore, it sounds that chemicals could strongly influence reproduction success of the earthworms in the rose farm, and probably this is why juveniles were not present in the greenhouses.

4. 6. Population estimation of top soil earthworms in the two farms

In the chemical-free farm, a total of 719 worms (670 adults and 49 juveniles) were collected from the 208 quadrats marked and the mean density of worms was 3.5 worms/m^2. Thus, the total population estimation was about 3,500,000 worms/ 100 ha. In the rose farm, on the other hand, out of the total 208 quadrats marked, only 9 adult worms were captured and no juvenile was observed. Thus, the mean density of worms was 0.04 individuals/m^2 and the total population estimation of top soil earthworms was about 20, 000 worms/ 50 ha (Table 7). Therefore, the occurrence of worms in the rose farm and the chemical-free farm was in the ratio of 1:88 respectively.

Table 7. Summary of mean population density and total population estimation of top soil earthworms in the two farms

Site	Number of worms/ 208 m^2	Mean population density/m^2	Total population estimation/ ha
Chemical-free farm	719	3.5	3, 500, 000 worms/ 100ha
Rose farm	9	0.04	20, 000 worms/50ha

Baker (1983) also hand sorted sample from 24.5 m^2 area and collected about 3425 earthworms, from which he identified a total of 15 species.

Spurgeon and Hopkin (1999) found a mean density of 589.9 earthworms out of 336 (25cmx25cm) quadrats hand sorted. Whalen (2004) also compared distribution of earthworms in cultivated corn field, hay field and deciduous forest in Quebec (Canada) and observed 46-177 earthworms m^{-2} in corn field, 138-224 worms m^{-2} in hay field, and 124-253 worms m^{-2} in the forest. He also identified 10 *Lumbricid* species in his study. Likewise, Hale and Host (2005) sorted 15 (35cmx35cm) quadrats on a total of 500 m^2 land area and found a mean of 54 worms m^{-2}. They also identified about 8 species of earthworms, *Dendrobaena octaedra* and *Aporrectodea* species being the dominant ones. Gezahegn Degefe (2006) also reported means of 5.9 earthworms m^{-2} in the wet season and 1.9 worms m^{-2} in the dry season. Therefore, the number of earthworms observed in this study goes in line with other studies. Especially, the finding of Gezahegn Degefe (2006) strongly supports this study. However, since studies concerning earthworms is so rare in Ethiopia, more extensive works are still required for more accurate comparisons.

In this work, large ratio of adult to juvenile worms (675:49) is revealed during the whole study period, probably indicating that population was not in a faster reproduction rate at the moment (stable population). Brown (2001) also agrees that population can stabilize itself by reducing fertility. However, many former studies, including Gezahegn Degefe (2006) reported more immature earthworms than adult ones in their studies. The complete absence of juveniles in the flower farm may indicate strong decline of reproduction in the greenhouses due to pressure from excessive use of hazardous chemicals.

5. CONCLUSION AND RECOMMENDATIONS

The reduced number of top soil earthworms in Golden Rose Agro Farm relative to the large number of them found in the surrounding chemical-free area with similar climatic conditions and soil type clearly indicates that there is some serious factor that affected their abundance and distribution in the rose farm. Since the soil under the flower farm had pH, organic carbon content and moisture suitable for existence of earthworms and since mechanical damages as a result of agricultural activities are not the ones, the major factor that severely affected abundance and distribution of top soil earthworms in the rose farm was the effect of agricultural chemicals.

The result of mortality test also confirmed that worms can live and even reproduce in the greenhouses in the absence of chemicals, indicating that the presence of greenhouses in the rose farm had no significant effect on abundance and distribution of the worms as long as chemicals are not present. Therefore, the extensive chemical use in the rose farm was the primary factor that affected abundance and distribution of top soil earthworms in Golden Rose Agro Farm.

The presence of large number of earthworms in the wet season and the strong reduction in number during the dry season shows the strong role that seasonality plays in determining the earthworm populations. On the other hand, the presence of large number of adult worms relative to juveniles in the chemical-free farm indicates low rate of reproduction (probably due to population stabilization), whereas the complete absence of juvenile worms in the rose farm was probably due to reduced rate of reproduction as a result of chemical effects.

However, the results of this study need to be supported with further tests such as earthworm tissue analysis for chemical residues and lethal concentration doses of each of the chemicals used in the rose farm to draw more definite conclusions about the effects of chemicals on abundance and distribution of earthworms in the area.

Therefore, based on the results of the present study as well as observations during the study period, the following are recommended:

- Since earthworms are excellent bio-indicators of environmental contaminations as a result of hazardous chemicals, they should be conserved and used in environmental impact assessments. Hazardous pollutants in the environment could be monitored by analyzing earthworms from soils suspected of being contaminated with persistent insecticides, heavy metals, polychlorinated and polybrominated biphenyls or dioxins.
- Earthworms can bio-accumulate hazardous chemical residues in their body at higher levels than their immediate environment (soil) and this can further affect organisms at higher trophic levels such as birds. For example, moles, snakes, rats, mice, frogs, and birds that feed up on them can easily be contaminated of the chemical residues in their tissues, and hence this affects wildlife. Since they are at the first level of detritus food web, this affects the whole ecosystem. Therefore, proper chemical use and management is essential to maintain healthy ecosystem.
- Earthworms are quite useful in any agro ecosystem and can also metabolize some hazardous chemicals in chemical-treated farms. So they can be used as detoxifying machineries as well as in maintaining soil fertility. Since they can facilitate decomposition of excessive wastes in the environment, people can also conserve and use them in environmental waste management.

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Appendix 1

List of Chemicals used in Golden Rose Agro Farm

Table 1. Chemicals used for Powdery mildew (Fungicides)

Chemical	Amount per spray	Method of spray
Aliette	200g/100L (of water)	Top to middle
Anvil	100ml/100L	Top to middle
Baycor	100g/100L	Top to middle
Bayleton	100g/100L	Top to middle
Collis	100ml/100L	Top to middle
Impulse	60ml/100L	Top to middle
Kumulus DF	25ml/100L	Top to middle
Meltatox	25ml/100L	Top to middle
Merpan	25g/100L	Top to middle
Milpan	100g/100L	Top to middle
Nustar	25ml/100L	Top to middle
Neipan	25g/100L	Top to middle
Polar	40g/100L	Top to middle
Rubgy	25ml/100L	Top to middle
Rubigan	25ml/100L	Top to middle
Saprol	15ml/100L	Top to middle
Sporakill	40g/100L	Top to middle
Stroby	25g/100L	Top to middle
Systhane	50ml/100L	Top to middle
Topaz	25ml/100L	Top to middle

Table 2. Chemicals used for Downy mildew (Fungicides)

Chemical	Amount per spray	Method of spray
Acrobat	200g/100L (of water)	Middle to top
Bravo	200g/100L	Middle to top
Canon	300ml/100L	Middle to top
Dynone	250g/100L	Middle to top
Equaton	200ml/100L	Middle to top
Foliogold	150ml/100L	Middle to top
Galben	200g/100L	Middle to top
Helcozeb	250g/100L	Middle to top
Milrazon	259g/100L	Middle to top
Mondizon	250g/100L	Middle to top
Metatoxin	250ml/100L	Middle to top
Pencozeb/Sanc	250g/100L	Middle to top
Previcure	200ml/100L	Middle to top
Proplant	200ml/100L	Middle to top

Polyron	200g/100L	Middle to top
Ridomil/Powder	250g/100L	Middle to top
Ridomil/Ground	200ml/100L	Middle to top
Roval	250ml/100L	Middle to top

Table 3. Chemicals used for red spider mites (Insecticides)

Chemical	**Amount per spray**	**Method of spray**
Appolo	50ml/100L (of water)	Middle to top
Abamactin	50ml/100L	Middle to top
Bactin	50ml/100L	Middle to top
Dynamec	50ml/100L	Middle to top
Ficoramite	50ml/100L	Middle to top
Hiltak	60ml/100L	Middle to top
Mitak/Kiltak	250ml/100L	Bending
Mastte	50ml/100L	Middle to top
Mitigan	50ml/100L	Middle to top
Majster	60ml/100L	Middle to top
Mizirock	60ml/100L	Middle to top
Masar	40g/100L	Middle to top
Neoron	50ml/100L	Middle to top
Nissuron	50ml/100L	Middle to top
Oscar	50ml/100L	Middle to top
Omite CR	250ml/100L	Middle to top
Omite EC	100g/100L	Middle to top
Oberon	60ml/100L	Middle to top
Pentac	120ml/100L	Middle to top
Peropal	45ml/100L	Middle to top
Polystrin	60ml100L	Middle to top
Pride	50ml/100ml	Middle to top
Secure	35ml/100L	Middle to top
Spidermec	25ml/100L	Middle to top
Tedion	355ml/100ml	Middle to top
Talstar	100ml/100ml	Middle to top
Torque	100ml/100ml	Middle to top
Vertimec	25ml/100L	Middle to top

Table 4. Chemicals used for Thrips and Aphids (Insecticides)

Chemical	**Amount per spray**	**Method of spray**
Cascade	100mg/100L (of water)	Top to middle
Centider	50ml/100L	Top to middle
Confidor	100ml/100L	Top to middle
Conflook	80ml/100L	Top to middle
Diazion	200ml/75ml	Top to middle
Divipan EC	100ml/100L	Middle to top

Dursban	50g/100L	Middle to top
Korate	150ml/100L	Middle to top
Lannate	100mg/100L	Middle to top
Marshal	100g/100L	Middle to top
Mavrik	30ml/100L	Middle to top
Metasystox	100ml/100L	Middle to top
Methomex	250ml/100L	Middle to top
Orthene	200ml/100L	Top to middle
Pyrinex	200ml/100L	Top to middle
Rydate	120ml/100L	Top to middle
Suprathion	250ml/100L	Top to middle
Talstar	100ml/100L	Top to middle
Vydate	100ml/100L	Top to middle
Karate	150ml/100L	Top to middle

Table 5. Agro bacteria chemicals/ Crown gall chemicals

Chemical	**Amount per spray**	**Method of spray**
Koelot	Unknown	Bending
Cosider	Unknown	Bending

Table 6. Chemicals used for Botritus (Dieback)

Chemical	**Amount per spray**	**Method of spray**
Benlate	100ml/100L (of water)	Middle to top
Bavistin	100g/100L	Middle to top
Kocide	80ml/100L	Middle to top
Score	100g/100L	Middle to top
Trionex	100ml/100L	Middle to top

Table 7. Chemicals used for Black spot

Chemical	**Amount per spray**	**Method of spray**
Antracol	200ml/100L (of water)	Top to middle
Bravocarb	100ml/100L	Top to middle
Daconcil	150ml/100L	Top to middle
Rova	100ml/100L	Top to middle

Table 8. Chemicals used for Leaf rust

Chemical	**Amount per spray**	**Method of spray**
Antracell	500/100L of water	Bending
Plantvax	200/100L of water	Bending

Table 9. Chemicals used for caterpillar

Chemical	**Amount per spray**	**Method of spray**
Divipan	Unknown	Bending

Pyrinex	Unknown	Bending
Thionex	Unknown	Bending

Table10. Wetting agents/Additives

Chemical	**Amount per spray**	**Method of spray**
Aplord		Top to middle
Aquaright	50ml/100L (of water)	Top to middle
Aquastic	30ml/100L	Top to middle
Aquawet	15ml/100L	Top to middle
Biofilm	30ml/100L	Top to middle
Hygrowet/Aqua	50ml/100L	Top to middle
Shantan	89ml/100L	Top to middle
Supafilm	50ml/100L	Top to middle
Basamid gran	50ml/100L	Top to middle
Ground sulfur	30ml/100L	Top to middle
Nemacur	15ml/100L	Top to middle
Shath	50ml/100L	Top to middle
Rugby	30ml/100L	Top to middle
Wet sulfur/Theovit	30ml/100L	Top to middle
Sulfur/Dust	50mg/100L	Top to middle

Table11. Fertilizers given as drip lines

Fertilizer	**Concentration(gm/m^3 of water)**
$CaN0_3$	550
$MgN0_3$	350
$MgS0_4$	300
$KN0_3$	350
$K2S0_4$	200
$(NH_2)_2C0$, Urea	20
NH_4N0_3	45
$MnS0_4$	2.5
$ZnS0_4$	1.85
$CuS0_4$	0.88
Borax	5

Table12. Acids

Acid	**Concentration (ml/m^3 of water)**
H_2S0_4	10,000
$HN0_3$	80
H_2P0_4	30

Appendix 2

Statistical Calculations for Independent t-test

Table 1. Comparison of mean number of adult top soil earthworms in the chemical-free farm in wet and dry seasons

Group Statistics

Variable	N	Mean	St. Deviation	St. Error mean
Data1 1	104	6.47	23.303	2.296
2	104	0.04	0.194	0.019

Independent Sample test

Data 1	Leven's test for equality of variances		t- test for equality of means						
Equal variance assumed	F 32.269	Sig. .000	t	df	Sig.(2 tailed)	Mean difference	St. error difference	95% confidence interval of the difference	
								Lower	Upper
Equal variance not assumed			2.799	204	.006	6.427	2.296	1.900	10.955
			2.799	102.014	.006	6.427	2.296	1.873	10.982

Table 2. Comparison of mean number of juvenile top soil earthworms in the chemical-free farm in wet and dry seasons

Group Statistics

Variable	N	Mean	St. Deviation	S t. Error mean
Data2 1	104	.448	1.650	.163
2	104	.00	.000	.000

Independent Sample test

Data 2	Leven's test for equality of variances		t- test for equality of means						
Equal variance assumed	F	Sig.	t	df	Sig.(2 tailed)	Mean difference	St. error difference	95% confidence interval of the difference	
	37.323	.000						Lower	Upper
			2.926	204	.004	.476	.163	.155	.796
Equal variance not assumed			2.926	102.000	.004	.476	.163	.153	.798

Table 3. Comparison of mean number adult top soil earthworms between the two farms

Group Statistics

Variable	N	Mean	St. Deviation	S t. Error mean
Data3 1	208	3.25	16.751	1.167
2	208	.04	.267	.019

Independent Sample test

Data 3	Leven's test for equality of variances		t- test for equality of means						
Equal variance assumed	F	Sig.	t	df	Sig.(2 tailed)	Mean difference	St. error difference	95% confidence interval of the difference	
	30.489	.000						Lower	Upper
			2.749	410	.006	3.209	1.167	.914	5.503
Equal variance not assumed			2.749	205.104	.007	3.209	1.167	.907	5.510

Table 4. Comparison of mean number juvenile top soil earthworms between the two farms

Group Statistics

Variable	N	Mean	St. Deviation	S t. Error mean
Data4 1	208	0.24	1.188	.083
2	208	.00	.000	.000

Independent Sample test

Data 4	Leven's test for equality of variances		t- test for equality of means						
Equal variance assumed	F 34.592	Sig. .000	t	df	Sig.(2 tailed)	Mean difference	St. error difference	95% confidence interval of the difference	
								Lower	Upper
			2.874	410	.004	.238	.83	.075	.410
Equal variance not assumed			2.874	205.000	.004	.238	.83	.075	.410

Table 5. Comparison of mean number of adult worms between the experimental and control groups in mortality experiment

Group statistics

Variable	N	Mean	St. Deviation	S t. Error mean
Data5 1	3	12.67	2.517	1.453
2	5	.20	.447	.200

Independent sample test

Data 5	Leven's test for equality of variances		t- test for equality of means						
Equal variance assumed	F 6.883	Sig. .039	t	df	Sig.(2 tailed)	Mean difference	St. error difference	95% confidence interval of the difference	
								Lower	Upper

Equal variance not assumed			11.395 8.500	6 2.076	.000 .012	12.467 12.467	1.094 1.467	9.790 6.373	15.144 18.561

Table 6. Comparison of mean number of juvenile worms between the experimental and control groups in mortality experiment

Group Statistics

Variable		N	Mean	St. Deviation	S t. Error mean
Data6	1	3	1.67	1.528	.882
	2	5	.00	.000	.000

Independent sample test

Data 6	Leven's test for equality of variances		t- test for equality of means						
Equal variance assumed	F 14.423	Sig. .009	t	df	Sig.(2 tailed)	Mean difference	St. error difference	95% confidence interval of the difference	
								Lower	Upper
Equal variance not assumed			2.588 1.890	6 2.000	.041 .199	1.667 1.667	.644 .822	.091 -2.128	3.243 5.461

Declaration

I, the undersigned, declare that this thesis is my original work and that all sources of materials used for the thesis have been correctly acknowledged.

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