

BOTSWANA UNIVERSITY OF AGRICULTURE AND NATURAL RESOURCES



**ABUNDANCE AND DIVERSITY OF SAFFLOWER (*CARTHAMUS
TINCTORIUS* L.) INSECTS IN BOTSWANA**

A dissertation submitted to the Department of Crop and Soil Sciences in partial fulfillment of the requirements of the award of the Master of Science Degree in Crop Science (Crop Protection)

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DECLARATION

I hereby declare that this submission is my original work and has not been presented for another Degree in this or any other university.

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TO GOD BE THE GLORY!!!

DEDICATION

I dedicate this work to my loving mother, Moreimang Basiame, for all the sacrifices you made to ensure that I become a responsible woman and mother to my son, Theo. Being a single mother that you are, thank you for being there when I felt pressures of the world. I will always be grateful.

ABSTRACT

Water scarcity has become a major challenge for Botswana farmers to increase their production despite various development initiatives introduced by the government. Safflower (*Carthamus tinctorius* L.) is a drought tolerant crop, with potential to serve as an alternative seed oil crop in Botswana. The plant is new in the country and still awaiting adoption by farmers. Literature has shown that safflower is vulnerable to some insect pests, hence hindering its maximum production. This study was carried out to provide a checklist and investigate the abundance and diversity of insect pests among safflower genotypes in Botswana. Insects survey was carried out in two seasons; summer and winter, on five different safflower genotypes; Gila, PI-537636, Kenya-9819, Turkey and Sina. Collection of data and specimens was done once a week and identification was carried out at the Botswana University of Agriculture and Natural Resources, Entomology Laboratory. Data collected were analysed using descriptive statistics (frequencies and percentages). Variation in insects abundance between genotypes and plant growth stages was determined by analysis of variance (ANOVA). Diversity indices were computed using Shannon diversity index, Sorensen's index and Margalef's richness index. Correlation analysis was used to establish relationships between population of insects, weather parameters and yield. Fifteen insect species belonging to fourteen families and eight orders were observed on safflower. Of these, 10 species were pests while the rest five, comprising four predators and one pollinator were beneficial. Order Hemiptera had the highest number of species in both seasons. *Thrips tabaci* and *Amrasca biguttula biguttula* were the most abundant insect species in summer and winter. *Helicoverpa armigera* and Aphididae species were identified as the most destructive pests of safflower in the current study. Insect pest populations fluctuated along safflower growth stages, but the most populated stage was flowering. The pests fed on all the upper parts (shoots) of safflower plants in the field, with leaves and capitula being the most affected parts of the crop. Even though insects

were recorded in abundance, generally the impact of the pests did not significantly ($P>0.05$) differ between safflower yield from all the five genotypes. This was attributed to compensation ability of safflower plants. The highest diversity index in summer was recorded on genotype Sina ($H'=1.47$) and the lowest was recorded on PI-537636 ($H'=1.32$), while in winter the highest diversity was recorded on PI-537636 and Turkey ($H'=0.94$) and the lowest was $H'=0.72$ on Sina. The values suggested a non-significant difference in diversity of insects between genotypes. Sorensen similarity index also confirmed the similarity between genotypes as Sorensen similarity coefficient varied from 96% to 100% in summer and varied from 80% to 100% in winter. These findings agreed with the hypothesis of this study that there is no significant difference in abundance and diversity of insects between safflower genotypes. The overall impact of weather parameters on total population of insects were non-significant. Temperature indicated non-significant quadratic correlation with total population of insects in winter but positive and non-significant linear correlation with rainfall and relative humidity. In summer, there was a positive and non-significant linear correlation between temperature and total population of insects, a non-significant quadratic correlation with rainfall and positive curvilinear relationship with relative humidity.

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CHAPTER 1

1.0 INTRODUCTION

Safflower (*Carthamus tinctorius L.*) is an annual oil seed crop belonging to the Asteraceae family from genus *Carthamus* (Abd El-Lattief, 2012; Saeidi et al., 2011). Safflower is one of humanity's oldest crops having been first cultivated almost 4,000 years ago, and the plant is native to parts of Asia and Africa, central India through the Middle East to the upper reaches of the Nile River. Safflower is mostly known as 'Kusum' in India and Pakistan, 'Honghua' in China, 'Suff' in Ethiopia, 'Le carthame' in France, 'Saflor' in German and 'Aspir' in Turkey (Emongor, 2010). The plant is characterized as herbaceous, thistle - like with several branches, usually with many long sharp spines on the leaves. Plants can reach 30- 210 cm in height with globular flower heads called capitula which are yellow, orange, red, white in colour (Emongor, 2010). Each plant produces variable capitula up to 167 with each capitulum containing 13-91 achenes (seeds) depending on genotype, climatic conditions, agronomic practices, and growing season (Emongor & Oagile, 2017; Moatshe and Emongor, 2019; Moatshe et al., 2020). It has an extensive root system with a strong fleshy taproot reaching 2-3 m in depth and thin lateral roots which enables it to thrive in dry climates (Emongor & Oagile, 2017).

Currently, safflower is commercially grown in around 60 countries, occupying more than one million hectares of agricultural land, and producing over 850,000 tonnes of seed (FAO, 2020). The top ten leading countries in the world in order of production of safflower seed include Kazakhstan, USA, Russia, Mexico, China, India, Argentina, Turkey, Tanzania, and Australia, respectively (FAO, 2020).

Major factors contributing to low safflower seed yield include drought, low soil fertility, low soil moisture, insect pests and diseases (Weiss, 2000). Insect pests contribute a major share to the reduction in safflower yield across the world. Some insect pests of safflower are widespread, while others are confined to certain regions and climatic conditions (Weiss, 2000).

This study was undertaken to investigate diversity and abundance of different insects associated with safflower in Botswana.

1.1 Justification of study

Botswana is a country in southern Africa which depends mainly on agriculture-arable farming as its main source of food to support rural incomes and livelihoods. Despite its dependence on crop production, there are some challenges that hinder economic transformation of local livelihoods through agriculture. Most food is imported, and local agriculture does not contribute to the bulk of food in the country. Statistics Botswana Report of 2019 on Botswana Environment Statistics and IPCC (2007) classified Botswana as arid to semi-arid, and is drought prone, with erratic rainfall that ranges from 250 mm in the southwest to around 650 mm in the north. Most of the rainfall is received between October and March, which is also the period of warmer temperatures. IPCC (2007) further categorized Botswana as a potential hotspot of climate change, with increased frequency of heat and increases in temperature and drying. Under the semi-arid conditions of Botswana, farmers have difficulty in increasing crop productivity and diversity in crop rotations due to environmental stresses such as high and cold temperatures, inadequate rainfall and very high evapotranspiration rate, and saline soils in some parts of the country, despite initiatives introduced by the government that aim at improving food security through provision of free inputs. Environmental stresses inhibit plants to perform to their best genetic potential (Zhu, 2002). In such conditions, safflower appears a promising alternative crop, because it tolerates environmental stresses which help it to thrive in different ecogeographical climates and still achieve economic yields (Hussain *et al.*, 2015). Several studies have classified safflower as a drought and heat tolerant crop; hence its production all over the world is mainly confined to areas with limited water (Emongor, 2010). The plant has demonstrated drought resistance with a slight decrease in crop yield and significant stability in water use efficiency (Emongor *et al.*, 2015; Marouf *et al.*, 2014). Drought is a very

unpredictable abiotic factor in terms of occurrence, severity, and duration; therefore, growing a crop such as safflower has potential for cultivation to mitigate the effects of climate change as well as promote food security and household income. A study by Emongor et al. (2017) in which safflower genotypes were evaluated under the semi-arid conditions of Botswana, revealed that safflower yielded 888-3113 kg of seed/ha, depending on genotype.

In Botswana research studies on safflower have evaluated genotypes for adaptability, plant density and fatty acid composition of the oil (Emongor et al., 2017; Moatshe 2018; Oarabile *et al.* 2016). Since the identification of insect pests and their natural enemies can give crucial information for safflower production and pest management strategies, this thesis investigated the pre- harvest arthropods of safflower in Botswana. This study focused on collecting, identifying, and comparing the insect fauna of safflower in the field. The information gained through the study will be utilized in developing Integrated Pest Management (IPM) strategies against harmful insects in safflower agro-ecosystem to insure enhanced production in Botswana. In addition, the information will also be vital for safflower adoption since this is still a new crop in Botswana under investigation.

1.2 Objectives

1.2.1 General objective

This study determined the abundance and diversity of arthropods on different safflower genotypes under field conditions.

1.2.2 Specific objectives

1. To assess diversity and abundance of insects on different safflower genotypes at different growth stages
2. To assess and identify the predominant and major insects of safflower
3. To assess and identify parts of safflower plant attacked by insects
4. To establish the relationship between safflower insects and weather parameters
5. To establish the relationship between safflower pests and safflower seed yield

1.3 Research hypothesis

Hypothesis 1

Ho: Insects of safflower genotypes do not differ in diversity and abundance.

Ha: Insects of safflower genotypes differ in diversity and abundance.

Hypothesis 2

Ho: The species abundance do not differ in abundance in summer and winter

Ha: The species abundance differ in abundance in summer and winter

Hypothesis 3

Ho: The species diversity do not differ in summer and winter

Ha: The species diversity differ in summer and winter

CHAPTER 2

2.1 Taxonomy and Distribution of safflower

Cultivated safflower (*Carthamus tinctorius* L.) is an annual oilseed crop belonging to the Asteraceae (Compositae) family, tribe Cardueae (thistles) and subtribe Centaureinae (El-Lattief, 2012). Asteraceae is recognised as the largest family of flowering plants and contains more than 1500 genera and 22,000 species ranging from annual herbs to woody shrubs. Safflower originates native to parts of Asia and Africa, from central India through the Middle East to the upper reaches of the Nile River and into Ethiopia. Today, cultivation of safflower occurs in arid and semi-arid conditions wherever the crops have established tolerance to hot and dry conditions. United States of America (USA) is the world's largest producer of safflower oil followed by India and Mexico, respectively (NationMaster, 2021). In Botswana, under irrigated cultivation, safflower yields ranges from 1500-4400 kg ha⁻¹ and 900- 2900 kg ha⁻¹ in winter and summer, respectively, depending on plant density (Emongor et al., 2013).

2.2 Uses of safflower

Safflower (*Carthamus tinctorius* L.), is a multifunctional crop. Oil obtained from the safflower seed is the chief modern use of the plant, but was traditionally grown for the extraction of textiles and food dyes throughout southern and central Asia and the Mediterranean (Oshima et al., 2020). The plant has shown potential value in medical, pharmaceutical, food, livestock feed, and cosmetic applications worldwide (Emongor, 2010; Mündel & Bergman, 2010). Safflower is currently farmed mostly for edible oil, which is considered one of the healthy oils for human consumption due to its high content of polyunsaturated essential fatty acid linoleic acid (70-87%) and monounsaturated fatty acid oleic acid (11-80%) (Moatshe, 2019; Moatshe et al., 2020). Linoleic acid has been shown to offer nutritional and therapeutic benefits such as the prevention of coronary heart disease, arteriosclerosis, high blood pressure, and hyper lipaemia (Li et al., 2016; Liao et al., 2019). The seeds of safflower are also a rich source of minerals (Zn, Cu, Mn, and Fe), vitamins (thiamine

and β -carotene), and tocopherols α , β , and γ (Velasco et al., 2005). The leaves are rich in carotene, riboflavin, and vitamin C; hence, young seedlings are used as a boiled vegetable side dish with curry rice in India, Pakistan and Burma (Asqarpanah and Kazemivash, 2013). In Ethiopia, boiled and finely pounded safflower kernels are mixed with water and the supernatants is used to prepare the so called 'fitfit', which is used as fasting food (Wodajo and Tesfaye 2015).

2.3 Factors affecting growth and yield of safflower

2.3.1 Abiotic factors

Safflower has been reported to do well under most abiotic factors such as saline soils, drought, low and high temperatures. Safflower seed yield is less affected by soil or irrigation water salinity and tolerates salt more than other commonly grown oilseed crops such as groundnut, sunflower, soybean and sesame (Gengmao et al., 2015). Its salt tolerance is an asset as the area affected by some degree of salinity increases world-wide (Emongor & Oagile, 2017). Safflower also tolerates a wide range of temperatures from -7°C to 40°C during the elongation and flowering stages of growth and development, provided there is no frost (Dhopte, 2017, Emongor et al., 2017). However, when flower buds are being formed or flowering has just commenced, temperatures below 0°C may cause considerable damage in the form of sterile heads. At seedling stage, the crop can tolerate much lower temperatures of -15°C to -10°C , while at the rosette stage it can withstand a temperature of -7°C (Carapetian, 2001; Emongor & Oagile, 2017). The extensive and strong tap root system of safflower benefits it by being able to extract soil moisture at deeper depths that are not available to most other crops (Sampaio et al., 2016). Weeds that compete with safflower include grass and broadleaf weeds. Later in the season many weeds can outgrow safflower in height and the resulting shading can reduce crop yields significantly (Oshima et al., 2020)

2.3.2 Biotic factors

The biotic stresses involve diseases and insect pest.

2.3.2.1 Diseases of safflower

Leaf blight, caused by the fungus *A. carthami*, is a major disease for safflower grown in India and Australia, having the potential to cause significant seed yield losses in the range of 10-50% (Taware et al., 2014). Wilt, a seed-borne disease caused by the fungi *Fusarium proliferatum* and *F. oxysporum*, has been identified as a serious disease for safflower crops grown in India, affecting 40 to 80% of the annual crops (Singh and Kapoor, 2018).

2.3.2.2 Pests of safflower

According to Weiss (2000), insect pests contribute a major share in the reduction of safflower yield. Around the world, a total of 101 pests have been reported to attack safflower at different stages of crop growth and development (Koul et al., 2014). Safflower has been reported to be most susceptible to insect damage during establishment and between budding and harvest when chewing and sucking insects directly feed on the developing buds causing seed shrinkage and blasting of flower heads. A field survey conducted by Saeidi et al. (2015) showed that 36 species of insect pests feed on safflower plant in India. Of these, safflower aphid, *Uroleucon compositae* (Theobald), leaf eating caterpillar, *Perigea capensis* (Walker) and capsule borer, *Helicoverpa armigera* (Hubner) were the major pests. Amongst these major pests, safflower aphid, *Uroleucon compositae* (Theobald) was found to be the most destructive because in high infestations it can destroy the crop.

Twenty arthropods were recorded in a survey of insect pests of safflower in the Iranian Province of Kohgiluyeh and Boyerahmad. Among these insect pests, safflower fly (*Chaetorellia carthame*), and silver- Y-moth (*Helicoverpa peltigera*) were found to cause considerable damage to safflower plants, while others were not at economic levels (Saeidi & Adam, 2011). The most serious safflower pest in Asia and Europe was reported as the safflower

fly (*Acanthiophilus helianthi* Rossi- Tephritidae), sometimes known as the shoot fly or capsule fly (Talpur et al., 1995). Safflower fly devastates most safflower production in Iraq (Saeidi et al., 2012), Pakistan (Talpur et al., 1995), and India (Vaishampayan & Kapoor, 1970; Verma et al., 1974). Seed-yield loss caused by safflower fly in Iraq was estimated at 30-70% for different safflower cultivars (Sabzalian et al., 2010). The safflower fly is a polyphagous insect belonging to the Tephritidae family (Saeidi et al., 2015). Adult flies lay eggs on the inner side of involucre bracts of safflower green capitula (Ashri & Knowles, 1960). Heavy infestations of safflower fly occur during the reproductive phase of the plant, and the fly prefers to lay its eggs inside developing heads throughout the flowering stage (Talpur et al., 1995). Larvae hatch from eggs, penetrate the capitula bracts, and feed on receptacle tissue or the whole seed (Faure et al., 2004). Larval feeding on seeds causes significant losses in seed weight, yield, and seed marketability (Ashri, 1971). Javed et al. (2013) conducted field surveys to determine population dynamics of insect pests of safflower in Islamabad, Afghanistan. Several injurious insect pests causing economic losses on various parts of safflower plants were recorded and safflower aphid (*Uroleucon carthami*) was found to be the predominant pest. Selim (1977) identified 23 species of insects in Mosul, northern Iraq, comprising 11 species of Coleoptera, 6 of Hemiptera, 2 of Diptera, 2 of Thysanoptera, 1 of Lepidoptera and 1 of Isoptera.

According to Smith et al. (2006), safflower production in the Mediterranean region is limited by pests including *Acanthiophilus helianthi* (Rossi), *Heliothis peltigera* SchiV. (Lepidoptera: Noctuidae), *Chaetorellia carthami* Stackelberg, *ch. Jaceae* R.D., *Terellialuteola* Wiedemann, *Urophora mauritanica* Macquart (Diptera: Tephritidae), *Larinus grisescens* Gyll., *Larinus syriacus* Gyll., *Larinus orientalis* Cap., *Larinus ovaliformis* Cap., (Coleoptera: Curculionidae) on the flower heads; and *Lixus speciosus* Mill. (Curculionidae), *Agapanthia* sp. (Coleoptera: Cerambycidae) four *Chloridea* spp., *Plusia gamma* L. (Noctuidae), *Pyrameis*

cardui L. (Lepidoptera: Nymphalidae) and *Cassida palaestina* Reiche (Coleoptera: Chrysomelidae) on vegetative parts.

2.4 Insect pests of related oil crops

Insect pests that are associated with oil seed crops such as soybean, sunflower, sesame and groundnut cause substantial economic and quality losses to the products (Sinha et al., 2018). Around the world, soybean harbours more than 300 insects (Gaur & Mogalapu, 2018). Amongst them only a few attain major pest status. Insect pests attack soybean from seedling stage to maturity and cause nearly 25% reduction of yield. In India, soybean has been reported to be attacked by 275 species of insect pests. Out of these pests, six species were found to cause a major damage, these includes Bihar hairy caterpillar (*Spilarctia obliqua*), stemfly (*Melanagro myzasojae*), aphids, jassids (*Pheliona maculosa*) and white fly (*Bemisia tabaci*) (Patel & Rahul, 2020). Soybean Stem Fly (*Melanagromyza sojae*) is regarded as one of the most important pests in soybean fields of Asia (e.g., China, India), North East Africa (e.g., Egypt), parts of Russia, and South East Asia (Arnemann et al., 2016). Cultivated sunflowers host a variety of insects and most of these insects do not cause economic loss (Du Plessis, 2014). Sunflower is subject to insect damage from planting onwards to drying of seeds on the heads. The pests that attack sunflower are largely polyphagous and attack a variety of crops and wild host plants. The most important species attacking the sunflower crop are whitefly (*Bemisia tabaci* Gennad.), plant hopper (*Empoasca* spp), cabbage semilooper (*Thysanoplusia orichalcea* F.), hairy caterpillar (*Diacretia obliqua* Walk.), green stink bug (*Nezara viridula* L.), boll worm (*Helicoverpa armigera* (Hubner)) and dusky bug (*Nysius inconspicuus* Distant) (Kakakhel et al., 2000). Insect pests cause yield losses ranging from 10-60% under different climatic conditions contributing to low productivity of the sesame (Langham, 2019). The foliage feeders are the dominant group of insects, occupying 54% of total number of pests of sesame, comprising of both sucking pests (*Orosius albicinctus*, *Nesidiocoris tenuis*, *Bemisia tabaci* and

Aphis gossypi) and defoliators (*Antigastra catalaunalis*, *Spilosoma obliqua*, *Acherontia lachesis* and *A. styx*). The pod feeding bugs comprises of *Elasmolomus sordidus*, *Clavigralla gibbosa*, *Nezara viridula* and *Dolycoris indicus* (Natarajan et al., 2019). The other pollinator fauna of sesame included as many as 7 species. African monarch butterfly (*Danaus chrysippus*), Cabbage butterfly (*Pieris rapae*), Lady beetle (*Coccinella undecimpunctata*), House fly (*Musca domestica*), Bean butterfly (*Cosmolyce baeticus*), Flesh fly (*Sarcophaga sp.*) and Drone fly (*Eristalis sp*) (Farag Mahmoud, 2012). African monarch butterfly is the most dominant. The main piercing-sucking insect associated with leaves and pods of sesame are leafhopper (*Empoasca lybica*), which is the most dominant, followed by Tobacco whitefly (*Bemesia tabaci*) Green peach aphid (*Myzus persicae*). Green stink bug (*Nezara viridula*) is associated with flowers and leaves of sesame (Farag Mahmoud, 2012).

Production of groundnut is largely constrained by insect pests by direct damage or as vectors of virus diseases (Bajia et al., 2017). The greatest yield loss caused by insect pests at any crop stage of groundnut was 31.4% in 1988 and 23% in 1989 (Singh & Sachan, 1992). Damage occurring during the bloom and vegetative stages resulted in maximum yield loss. Aphid (*Aphis craccivora* Koch.), Leaf miner (*Aproarema modicella* Deventer), Jassids (*Empoasca kerri* Pruthi), Thrips (*Scirithithrips dorsalis* Hood), White grub (*Holotrichia consanguinea* Blanchard) ,Bihar Hairy Caterpillar, Gram Pod Borer, Red Hairy Caterpillar and Wire Worm are some of the major pests of groundnut (Wightman & Amin, 1988).

2.5 Damage and losses caused by safflower pests

Biradarpatil & Jagginavar (2018) estimated 62.8% maximum safflower yield loss due to *Helicoverpa armigera*. The findings from their study indicated that *H. armigera* density had a significant effect on number of seeds per capsule, 100-seed weight, and yield per plant. Plants that were infested with 10 larvae of *H.armigera* recorded the minimum number of seeds per capsule, 100-seed weight and seed yield per plant were 8.4, 2.5 g and 2.5 g, respectively and

maximum recordings were obtained from the control plot; when no larvae was introduced, 36.7, 6.8 g and 13.5 g, respectively were achieved. Saeidi et al. (2011) exposed that 90% of damage caused by *H. armigera* on safflower was caused by the third instar, or older larvae. Fifth and sixth instar larvae were the most damaging stages.

Hanumantharaya et al.(2008) revealed that aphids reduced safflower seed yield by 30 to 80% depending on the species density and weather conditions. In damaged seed, oil content decreased by up to 32% and seed weight by 50.6% (Dambal & Patil, 2016). Apart from loss in seed oil yield, aphids attack petals which decrease the quality of the value-added product of safflower. Safflower aphid makes first appearance on safflower in elongation phase of growth and reach a peak population at flowering stage (Akashe et al., 2010; Hanumantharaya et al., 2008; Kamath & Hugar, 2001). Subsequently, the population declined due to maturity of the plant.

Caterpillars of *Perigea capensis* (Walker) have been reported to be the main defoliator of safflower, and the pest attacks all stages of vegetative development (Esfahani et al., 2012; Mehdi et al., 2012). The larvae feed on the leaves and sometimes on capitula, bracts and flower heads. Sekhar & Rai (1991) reported that excessive foliage feeding by *P. capensis* caused yield loss of 62.6-100%. Results of the study showed that, control plants (no infestation) recorded 23.3 g seed weight, which was similar in weight (20.3 g) from a safflower infested by 1 larva per plant. Yield loss of 62.6% was recorded from treatment of three larvae per plant when compared to the control and 100% yield loss was recorded from the treatment with eight larvae per plant (Sekhar & Rai, 1991). Sabzalian et al. (2010) reported that percentage of seed yield loss due to *Acanthiophilus helianthi* infestation was more drastic in cultivated safflower than the wild accessions. Cultivated safflower yield loss was estimated at 29.0–72.8% as compared to 0.0–21.4% for wild accessions (Sabzalian et al., 2010). From a study on comparison of different methods to control *A. helianthi* in safflower, it was evident that seed damage of 39.4%

was obtained where there were no interventions compared to the acceptable level of 5% damage under the integrated management method (Saeidi et al., 2013). Newly hatched larvae fed on the soft parts of the capsules and later instars fed on the soft part within the capsule resulting in disturbed plant development due to reduction in flower buds which ultimately cause significant losses in seed weight and seed yield (Saeidi et al., 2013). Damage inflicted by first and fourth generation instars were insignificant while the second and the third generation caused significant damage to the flower heads (Saeidi et al., 2013).

Carlson (1964) reported that dense populations of thrips and lygus bugs must be present to cause economic loss of safflower seeds and significant bud blasting. Economically significant seed losses were obtained when 60 or more lygus bug per sweep were present (equivalent to 1 lygus bug per 4 buds) while 25 to 30 lygus bugs per sweep (a ratio of 1 lygus bug per 8 buds) did not cause economic damage (Carlson, 1964). Bronzing and blasting of developing safflower buds was also caused by western flower thrips and most of the injury was caused by the feeding of the nymphs under the bracts of the buds (Carlson, 1964).

2.6 Insects of safflower on various plant parts

Mehdi et al. (2012a) identified three species of fruit flies (*Acanthophilus helianthin*, *Chaetorellia carthame* and *Trellia luteola*), *Larinus flavescens*, *Larinus liliputanus* and *Helicoverpa peltigera* as insects pests feeding inside the flower heads of safflower. Saeidi & Adam (2011) showed that *A. helianthi* (Rossi) spent its entire life inside the safflower head and heavy infestations occur during the reproductive phase of the safflower plant and then declined as the plant matures. Two species of Hemiptera: Lygaeidae from the genus *Oxycarenus*: *O. palens* and *O. hyalipennis*, aphids, thrips and dermestid beetles were recorded as insect pests feeding outside the flower heads of safflower (Saeidi & Adam, 2011). Lygaeidae caused latex to leak from the flower head resulting in darkening of internal seeds and sideway leaning of the infested flower heads (Saeidi & Adam, 2011). It was also found that Lygaeidae

infestation was higher in summer than spring, and higher in the early periods of spring than in the late periods (Saeidi & Adam, 2011). The dermestid beetles appear at maturity when the flower heads become ripe and dry (Saeidi & Adam., 2011). On average, 40% of the flower heads damage was caused by pests feeding on the outside of the flower head. *Uroleucon compositae*, *Pleotrichophorus glandulosus*, *Brachycaudus helichrysi*, *Neoliturus fenestratus*, *Euscelis alsius*, *Macrosteles laevis*, *Psammotettix striatus*, *Circulifer haematoceps*, *Thrips tabaci*, *Aeolothrips collaris*, *Haplothrips sp*, *Helicoverpa peltigera* were recorded as pest insects feeding on the other parts of safflower plant (Saeidi & Adam, 2011). Saeidi et al. (2015) confirmed that larvae of safflower flies including *Acanthiophilus helianthi*, *Chaetorellia carthami* and *Terellia luteola*, are the most important pests attacking the flower heads and seeds of safflower. Lygus species, *Oxycarenus palens*, *O. hyalipennis*, aphids and thrips were only found on flower heads of safflower (Saeidi et al., 2015).

The literature reviewed above showed that no research has been carried out in the response of safflower germplasm to the abundance and diversity of arthropods in Africa and SADC. Therefore, the current study seeks to generate information on the abundance, diversity of insects, and potential insect pests of safflower in Botswana and other semi-arid regions within SADC for optimum safflower production to design an integrated pest management system which allows maximum expression of genetic potential of safflower resulting with high seed yield.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Experimental site

The study was conducted on the farm around Molepolole village in Kweneng district of Botswana (latitude 24° 24' 24'S and longitude 25° 29' 42'E elevated at 1149m above sea level). The climate is classified as semi-arid with summer rainfall, which rains during the period of October to March. The mean annual rainfall received at this site varies from 500 mm to 600 mm. The mean daily temperature in summer varies from 25-32.6⁰C and in winter from 15-20⁰C but at night it could go below 10⁰C (Makhabu et al., 2019). The agro- meteorological data (temperature, relative humidity, and rainfall) of the site during the trial is presented in appendix.

3.2 Experimental design

The experiment was a randomized complete block design with five treatments and three replications. The treatments were five safflower genotypes Sina, PI-537636, Turkey, Kenya-9819 and Gila. Genotypes were randomly allocated randomly to experimental units within each block. The experimental field was 17 m x 15 m with each experimental unit measuring 3 m x 3 m.

3.3 Agronomic practices

3.3.1 Land preparation and sowing

The land was cleared, ploughed using mould board followed by disc harrowing to a fine soil tith. Safflower seeds were sown manually on the 9th October 2020 in the first trial of the experiment (summer) and 29th April 2021 in the second trial of the experiment (winter). Two seeds were sown per hill at a spacing of 0.25 m spacing between plants and 1 m between rows (drip lines) and two weeks after emergence the seedlings were thinned leaving one per hill giving a population of 40 000 plants/ha.

3.3.2 Irrigation

Supplemental irrigation was delivered using drip irrigation system to meet the crop water requirements as related to reference evapotranspiration. The plants were irrigated when there was no rain twice a week at 6 mm per irrigation interval. The average water recommendation for safflower ranges between 600 - 1200 mm depending on climate and length of plant growth period (FAO, 2011).

3.3.3 Soil fertility and pests management

The fertilizer, Super phosphate, was incorporated into the soil at a rate of 42 kg P₂O₅/ ha. Manual weeding was done to keep the experimental plots weed-free. The experimental units were subjected to natural infestation hence no insect control was done.

3.3.4 Harvesting and processing

Ten plants were randomly selected and hand harvested to determine seed yield. The capitula were threshed and winnowed to separate the seeds from the chaff. Weighing the seeds was accomplished with a Mettler PM 400 digital balance.

3.4 Data collection

3.4.1 Insects identification

Data was collected from standing plants on the plant canopy as well as close as possible to basal region of the plants. Ten plants were randomly selected and tagged for data collection. Visual counts and collection of insects were done once per week (Mondays) between 8:00-11:00 am, from seedling emergence until physiological maturity (plants started drying after grain filling). Plant parts infested and the growth stage of the plants were recorded, along with temperature, relative humidity, and rainfall amounts.

Insects specimens were collected by hand-picking and sweep netting from the plant canopy. The specimens were killed in ethyl acetate, pinned, and dried in the oven at 40°C for 72 hours. Collected larvae were reared to adult stage before being identified. General morphological descriptions of insect pests was done under the microscope and magnifying glass, using the

procedure of Zehnder (2010). The samples were identified using a dichotomous key and books (Picker et al., 2019). Feeding behavior of pests was also identified in the field.

3.4.2 Seed yield determination

Seed yield (kg ha^{-1}) was determined as described by Beyyavaş et al., 2011.

Seed yield per hectare was worked out with the help of the following formula

$$\text{seed yield} \left(\frac{\text{kg}}{\text{ha}} \right) = \frac{\text{total seed yield (kg)}}{10} * 40\ 000$$

3.4.3 Assessment of damage

Assessment of damage caused by insect pests and their feeding behaviour was done on 10 randomly selected plants in each plot. For each insect pest, different plant parts were assessed for damage and they were rated either as affected or not affected.

3.5 Data analysis

Data collected were subjected to SAS (SAS Institute, Version 9.4) for analysis of variance (ANOVA) and the Least Significant Difference (LSD) test at $P= 0.05$ was used to determine variation in insects abundance between genotypes and plant growth stages. Relative abundance was calculated to measure the percentage of individuals of a particular insects order over all the individuals in the community. Paired Student's t-test in SAS (SAS Institute, version 9.4) was used to compare insect species between summer and winter season. In order to study the influence of key abiotic factors on the pest incidence, simple correlations were worked out between the pest incidence and meteorological factors for the two seasons. Correlation analysis was also used to determine the relationship between safflower pests and safflower seed yield. The Shannon-Wiener Index (H'), Species Richness, Species Evenness and Margalef's Richness Index were computed to determine insects diversity between seasons. Sorensen's Index was used to calculate similarity coefficient to determine species similarity between safflower genotypes.

3.5.1 Relative abundance

$$RA = \frac{n_i}{N} * 100$$

Where RA is relative abundance, n_i is the total number of individuals in the particular insects order, and N is the total population of all the individuals in the sample community.

3.5.2 The Shannon- Wiener Index (H')

$$H' = \sum_{i=1}^s \frac{n_i}{n} \log \frac{n_i}{n}$$

Where H' is Shannon- Wiener Index, S is the number of species in a sample; n_i is the number of individuals belonging to species i and n is the number of individuals in a sample from a population.

3.5.3 Species Richness

$$R = \frac{S - 1}{\log N}$$

Where R is species richness, S is number of species in the community, and N is total population of all the species.

3.5.4 Species Evenness

$$J = \frac{H'}{\log S}$$

where J is species evenness, H' is Shannon-Wiener Index and S is number of species in the community.

3.5.5 Sorensen's Index

$$S_s = \frac{2a}{2a + b + c}$$

where S_s is Sorensen's similarity coefficient, a is number of species in sample A and sample B (joint occurrences), b is number of species in sample B but not in sample A, c is number of species in sample A but not in sample B.

CHAPTER 4

4.0 RESULTS

4.1 General pattern of species abundance and diversity

The average seasonal trends in terms of the numbers of insects observed during the study are shown in Table 1. In total, fifteen (15) insects species belonging to eight (8) orders and fourteen (14) families were found to infest safflower at different stages of growth, from rosette stage to maturity stage, during summer and winter season (Table 1). These eight orders consisted of Hemiptera, Coleoptera, Hymenoptera, Orthoptera, Diptera, Lepidoptera, Thysanoptera and Araneae (Table 1). Ten (10) species of these orders were found to be pests, four species were predators, and one was a pollinator. When data were pooled for all the arthropod groups observed during the study period, it was discovered that 87.5% of the all the orders comprised of insects with the remaining part comprising of Arachnida (Spiders).

4.2 Species abundance and diversity in summer and winter

During the summer season, a total of 3374 specimens belonging to 13 species under eight orders were collected from five different safflower genotypes for 13 weeks. Order Hemiptera had the highest number of species collected during summer season (Table1). The four (4) Hemipteran species collected were *Amrasca biguttula biguttula*, *Calidea panaethiopica*, *Elasmucha grisea* and *Spilostethus pandurus*, *Amrasca biguttula biguttula* (n=674) was the most collected while the least collected was *Elasmucha grisea* (n=39). Order Coleoptera consisted of two species namely: *Cheilomenes lunata* and Curculionidae species. The most abundant coleopteran species was Curculionidae species (n=26) and the least abundant was *Cheilomenes lunata* (n=4). Order Hymenoptera consisted of two species, that is: *Apis mellifera* and Formicidae species. The most abundant hymenopteran species was Formicidae species (n= 47) and the lowest was *Apis mellifera* (n= 21). Order Orthoptera consisted of only *Zonocerus elegans* (n= 19). Similarly, order Diptera consisted of only Tephritidae species (n= 65). *Helicoverpa armigera* (n= 160) was the only Lepidopteran species observed during summer.

Order Thysanoptera also had only *Thrips tabaci* (n= 1914) observed during summer. The only Araneae member seen during summer was Spider (n= 12). Numerically, Thysanoptera was the most abundant order during summer season, comprising of 56.73% of the total population, followed by Hemiptera with 32.78%, Lepidoptera (4.74%), Hymenoptera (2.02%), Diptera (1.93%), Coleoptera (0.89%), Orthoptera (0.56%) and lastly order Araneae with 0.36%.

During winter season, a total of 5459 specimens belonging to 13 arthropod species under seven orders were collected from five different safflower genotypes for 23 weeks. Order Hemiptera consisted of four species, which were, *Amrasca biguttula biguttula*, *Elasmucha grisea*, *Spilostethus pandurus* and Aphididae species. The most collected Hemipteran species from the safflower plants was *Amrasca biguttula biguttula* (n=634) and the least collected was *Elasmucha grisea* (n=12). Order Coleoptera consisted of three species, namely, *Cheilomenes lunata*, Curculionidae species and *Exochomus flavipes*. The most abundant coleopteran species was *Exochomus flavipes* (n=26) and the least abundant was Curculionidae species (n=9). Order Hymenoptera consisted of two species, that is *Apis mellifera* and Formicidae species. The most abundant hymenopteran species was Formicidae species (n=236) and the lowest was *Apis mellifera* (n=35). Order Diptera consisted of only Tephritidae species (n=13). Similarly, order Lepidoptera had only *Helicoverpa armigera* (n=13) during winter. *Thrips tabaci* (n= 4240) was the only member of the order Thysanoptera during winter. The only one Araneae member recorded in winter was Spider (n=22). Thysanoptera was the most abundant order during winter season, occupying 77.67% of the total population, followed by Hemiptera with 15.08%, Hymenoptera (4.96%), Coleoptera (1.41%), Araneae (0.40%), and lastly order Lepidoptera and Diptera each occupying 0.24%.

Table 1: Seasonal incidence of the insects of safflower during experimental period

| Insect species | Mean number of individuals collected per plant | | | | | | | | | | | |
|------------------------------------|--|------|--------|-------|------|--------------|---------------|------|--------|-------|------|--------------|
| | Summer season | | | | | | Winter season | | | | | |
| | PI | Sina | Turkey | Kenya | Gila | Total | PI | Sina | Turkey | Kenya | Gila | Total |
| COLEOPTERA | | | | | | | | | | | | |
| Coccinellidae | | | | | | | | | | | | |
| <i>Cheilomenes lunata</i> | 0.00 | 0.06 | 0.17 | 0.00 | 0.00 | 0.23 | 0.00 | 0.67 | 0.11 | 0.00 | 0.00 | 0.78 |
| <i>Exochomus flavipes</i> | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.78 | 0.50 | 0.44 | 0.61 | 0.67 | 3.00 |
| Curculionidae | | | | | | | | | | | | |
| <i>Curculionnidae sp.</i> | 0.44 | 0.11 | 0.44 | 0.16 | 0.28 | 1.43 | 0.11 | 0.00 | 0.11 | 0.28 | 0.00 | 0.50 |
| ORTHOPTERA | | | | | | | | | | | | |
| Pyrgomorphidae | | | | | | | | | | | | |
| <i>Zonocerus elegans</i> | 0.22 | 0.11 | 0.33 | 0.17 | 0.22 | 1.05 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| DIPTERA | | | | | | | | | | | | |
| Tephritidae | | | | | | | | | | | | |
| <i>Tephritidae sp.</i> | 0.94 | 0.67 | 0.39 | 0.39 | 1.22 | 3.61 | 0.17 | 0.17 | 0.17 | 0.17 | 0.06 | 0.74 |
| HEMIPTERA | | | | | | | | | | | | |
| Cecadellidae | | | | | | | | | | | | |
| <i>Amrasca biguttula biguttula</i> | 6.83 | 7.94 | 7.67 | 6.89 | 8.11 | 37.44 | 8.44 | 4.83 | 7.06 | 8.94 | 5.94 | 35.21 |
| Scutelleridae | | | | | | | | | | | | |
| <i>Calidea pannaethiopica</i> | 2.06 | 5.61 | 3.83 | 1.78 | 6.28 | 19.56 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Acanthosomatidae | | | | | | | | | | | | |
| <i>Elasmucha grisea</i> | 0.39 | 0.44 | 0.17 | 0.56 | 0.61 | 2.17 | 0.06 | 0.06 | 0.44 | 0.11 | 0.00 | 0.67 |
| Lygidae | | | | | | | | | | | | |
| <i>Spilostethus pandurus</i> | 0.44 | 0.56 | 0.39 | 0.39 | 0.50 | 2.28 | 0.17 | 0.17 | 0.50 | 0.44 | 0.06 | 1.34 |
| Aphididae | | | | | | | | | | | | |
| <i>Aphididae sp.</i> | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2.33 | 0.61 | 0.89 | 3.33 | 1.33 | 8.49 |
| HYMENOPTERA | | | | | | | | | | | | |
| Apidae | | | | | | | | | | | | |
| <i>Apis mellifera</i> | 0.28 | 0.22 | 0.39 | 0.11 | 0.17 | 1.17 | 0.06 | 0.50 | 0.28 | 0.83 | 0.28 | 1.95 |
| Formicidae | | | | | | | | | | | | |

| | | | | | | | | | | | | |
|--|-------------|--------------|-------------|-------------|--------------|-----------------|--------------|--------------|--------------|--------------|-------------|-----------------|
| <i>Formicidae sp.</i> | 0.44 | 0.67 | 0.39 | 0.72 | 0.39 | 2.61 | 4.67 | 2.00 | 0.67 | 2.28 | 3.50 | 13.12 |
| LEPIDOPTERA | | | | | | | | | | | | |
| Noctuidae | | | | | | | | | | | | |
| <i>Helicoverpa armigera</i> | 1.72 | 1.83 | 1.94 | 1.89 | 1.50 | 8.88 | 0.11 | 0.17 | 0.17 | 0.28 | 0.00 | 0.73 |
| THYSANOPTERA | | | | | | | | | | | | |
| Thripidae | | | | | | | | | | | | |
| <i>Thrips tabaci</i> | 21.78 | 18.28 | 22.33 | 20.56 | 23.39 | 106.34 | 50.72 | 47.89 | 31.06 | 56.56 | 49.33 | 235.56 |
| ARANEAE | | | | | | | | | | | | |
| Spider (non- insect) | 0.06 | 0.11 | 0.16 | 0.28 | 0.06 | 0.67 | 0.72 | 0.00 | 0.06 | 0.11 | 0.33 | 1.22 |
| Seasonal mean number of individuals | 35.6 | 36.61 | 38.6 | 33.9 | 42.73 | (187.44) | 68.34 | 57.57 | 41.96 | 73.94 | 61.5 | (303.31) |

Table 2: Mean population of insects species collected from safflower

| Insect species | Mean population | |
|------------------------------------|--------------------------|---------------------------|
| | Summer | Winter |
| <i>Thrips tabaci</i> | 21.27±4.50 ^{aB} | 47.11± 6.88 ^{aA} |
| <i>Amrasca biguttula biguttula</i> | 7.49±0.63 ^{bA} | 7.04±0.86 ^{bA} |
| <i>Calidea panaethiopica</i> | 3.91±1.13 ^{bc} | - |
| <i>Helicoverpa armigera</i> | 1.78±0.21 ^{bcA} | 0.14±0.04 ^{bB} |
| <i>Tephritidae sp.</i> | 0.72±0.12 ^{cA} | 0.14±0.05 ^{bB} |
| <i>Formicidae sp.</i> | 0.52±0.17 ^{cB} | 2.62±0.79 ^{bA} |
| <i>Spilosthethus pandurus</i> | 0.46±0.09 ^{cA} | 0.27±0.06 ^{bA} |
| <i>Elasmucha grisea</i> | 0.43±0.10 ^{cA} | 0.11±0.05 ^{bB} |
| <i>Curculionidae sp.</i> | 0.29±0.07 ^{cA} | 0.10±0.05 ^{bB} |
| <i>Apis mellifera</i> | 0.23±0.08 ^{cA} | 0.39±0.18 ^{bA} |
| <i>Zonocerus elegans</i> | 0.21±0.0 ^c | - |
| <i>Spider</i> | 0.13±0.04 ^{cA} | 0.24±0.11 ^{bA} |
| <i>Cheilomenes lunata</i> | 0.04±0.03 ^{cA} | 0.16±0.13 ^{bA} |
| <i>Aphididae sp.</i> | - | 1.70±0.38 ^b |
| <i>Exochomus flavipes</i> | - | 0.60±0.13 ^b |

Means followed by the same small letter within a column are not significantly different ($P \leq 0.05$), Tukey's Honest Significant Different test. Means followed by the same capital letter within a row are not significantly different (Student's T-test, $P > 0.05$).

When data was pooled for all the insect groups observed during the study period, population of individual species differed significantly ($F_{2,1} = 10.38$, $P = 0.0013$) between seasons (Table 2). Mean population of insects recorded in winter was significantly ($P < 0.05$) higher (4.6197 ± 0.4892) than the mean population of insects recorded in summer (2.3500 ± 0.4892) (Table 2). Population of *Amrasca biguttula biguttula*, *Spilosthethus pandurus*, Spider, *Cheilomenes lunata* and *Apis mellifera* did not significantly differ between summer and winter season (Table 2). Formicidae species, *Helicoverpa armigera*, Tephritidae species, Curculinidae species, *Thrips tabaci* and *Elasmucha grisea* differed significantly between summer and winter season (Table

2). *Thrips tabaci* was significantly ($P < 0.05$) more abundant than any other insect species in both seasons (Table 2).

During summer, population of individual species differed significantly ($F_{2,12}=21.89$, $P=0.001$) (Table 2) between species. *Thrips tabaci* had significantly higher population than other insect species, followed by *Amarasca biguttula biguttula*, *Calidea panaethiopica* and *Helicoverpa armigera*, respectively, in order of abundance (Table 2). Other species were recorded in the descending order as Tephritidae species > Formicidae species > *Spilostethus pandurus* > *Elasmucha grisea* > Curculionidae species > *Apis mellifera* > *Zonocerus elegans* > Spider > *Cheilomenes lunata* (Table 2). Aphididae species and *Exochomus flavipes* were not present in summer season.

Similarly, during winter season, population of individual species differed significantly ($F_{2,12}=46.31$, $P=0.0001$) (Table 2). *Thrips tabaci* recorded significantly ($P < 0.05$) higher population than *Amarasca biguttula biguttula* and Formicidae species (Table 2). Other species recorded in the descending order were Aphid > *Exochomus flavipes* > *Apis mellifera* > *Spilostethus pandurus* > Spider > *Cheilomenes lunata* > *Helicoverpa armigera* > Tephritidae species > *Elasmucha grisea* > Curculionidae species. *Zonocerus elegans* and *Calidea panaethiopica* were not present in winter.

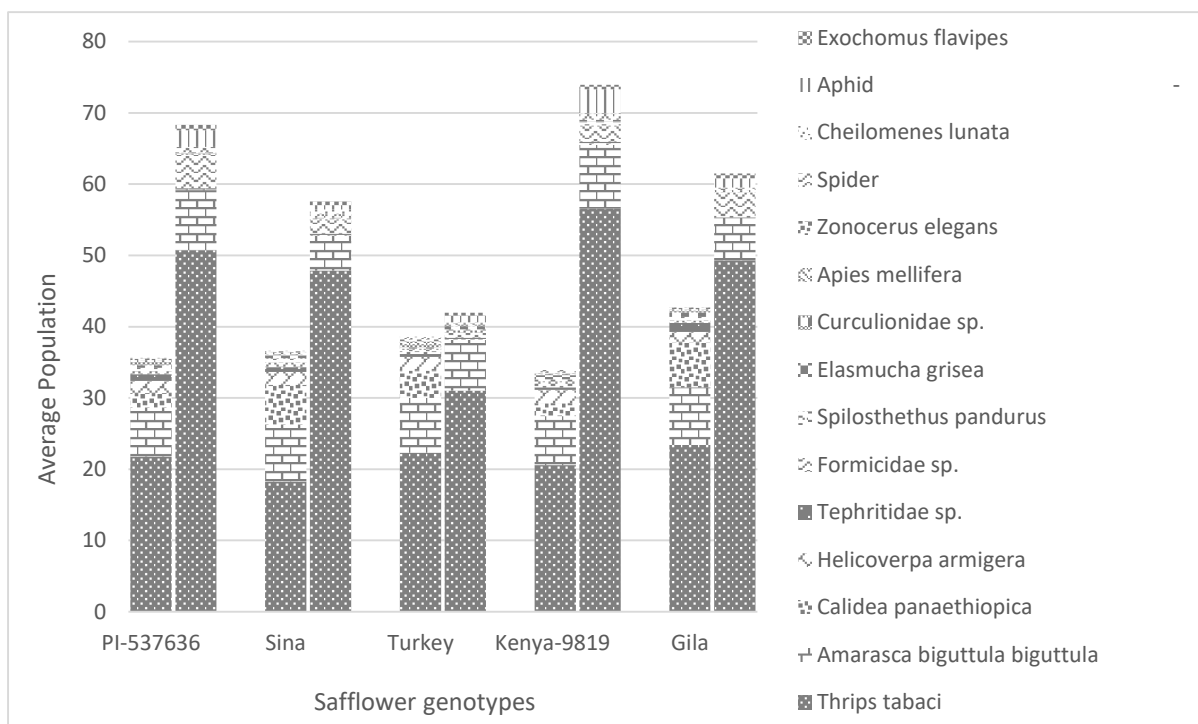


Figure 1 shows the proportional abundance and diversity of different insect species collected from different safflower genotypes during summer and winter season. Population of insect species among genotypes did not differ significantly ($F_{2,1} = 1.88$, $P = 0.1711$) in both summer and winter grown safflower (Figure 1). In summer, the highest mean population was recorded in genotype Gila, followed by Turkey, Sina, PI-537636 and Kenya-9819, respectively (Figure 1). The Kenya-9819 recorded the highest population mean of insect species in winter, followed by PI-537636, Gila, Sina and lastly Turkey, respectively.

4.3 Species similarity between safflower genotypes

Table 3: Values of Sorensen similarity coefficient applied to the insects' species collected from safflower genotypes during summer season

| Genotype | PI- 537636 | Sina | Turkey | Kenya-9819 | Gila |
|-------------------|-------------------|-------------|---------------|-------------------|-------------|
| PI 537696 | - | 96% | 96% | 100% | 100% |
| Sina | - | - | 100% | 96% | 96% |
| Turkey | - | - | - | 96% | 96% |
| Kenya-9819 | - | - | - | - | 100% |
| Gila | - | - | - | - | - |

Table 3 shows values of Sorensen similarity coefficient applied to the insect species collected from Safflower genotypes during summer season. The highest species similarity was noted between Kenya-9819 and PI- 537696 (100%), Gila and PI- 537696 (100%), Turkey and Sina (100%) and Gila and Kenya-9819 (100%). Moreover, the similarity coefficient between other genotypes was also observed to be very high (96%).

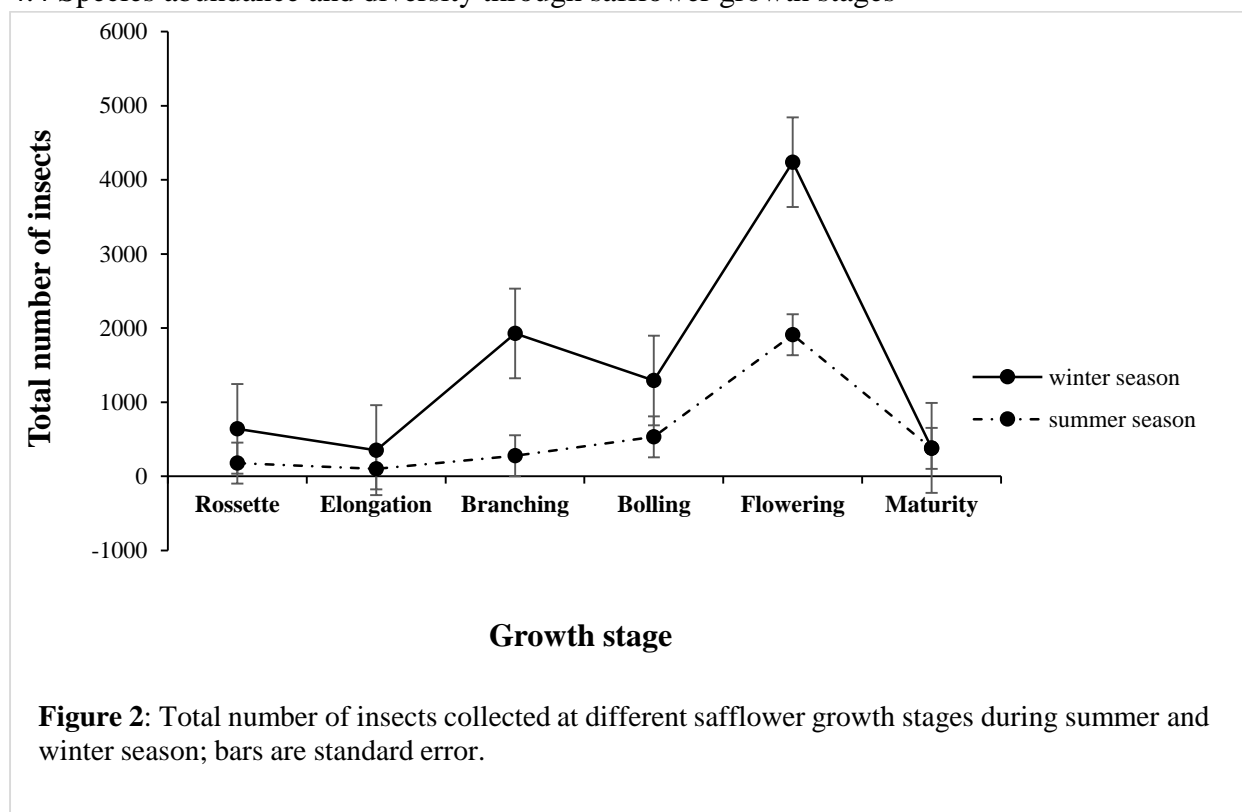
Table 4: Values of Sorensen similarity coefficient applied to the insects species collected from safflower during winter season

| Genotype | PI 537636 | Sina | Turkey | Kenya-9819 | Gila |
|-------------------|------------------|-------------|---------------|-------------------|-------------|
| PI537696 | - | 86.96% | 96% | 100% | 85.71% |
| Sina | - | - | 91.67% | 86.96% | 80% |
| Turkey | - | - | - | 96% | 81.82% |
| Kenya-9819 | - | - | - | - | 85.71% |
| Gila | - | - | - | - | - |

Table 4 depicts values of Sorensen similarity coefficient applied to the insect species collected from safflower genotypes during winter season. During winter, the highest similarity coefficient of above 90% was noted between Sina-Turkey (96%), PI-537696- Kenya-9819 (100%), PI-537696- Turkey (96%) and Turkey-Kenya-9819 (96%) (Table 6). The lowest

Similarity coefficient below 90 % were found between PI-537696- Sina (86.96%), PI-537696- Gila (85.71%), Sina-Kenya-9819 (86.96), Gila-Sina (80%), Kenya-9819-Gila (85.71) and Gila-Turkey (81.82%) (Table 4). The lowest similarity, 81.82%, coefficient during winter season was noted between Turkey and Gila (Table 4).

4.4 Species abundance and diversity through safflower growth stages



The total number of insects collected at different safflower growth stages during summer and winter season are shown in Figure 2. In summer, the phenological stages of safflower significantly ($F_{2,5}=16.33$, $P=0.0001$) differed in insect species abundance (Figure 2). The flowering stage had significantly ($P < 0.05$) higher insect population than other growth stages of safflower (Figure 2). The bolling, maturity, branching, rosette, and elongation stages did not significantly ($P > 0.05$) differ in insect population abundance (Figure 2). However, in summer the elongation stage of safflower had the least insect species population (Figure 2). Similarly, population of insects differed significantly ($F_{2,5}= 12.80$, $P= 0.0001$) between safflower growth stages in the winter season (Figure 2). The insect species were significantly ($P < 0.05$) more

abundant during flowering stage than other phenological stages of safflower (Figure 2). This was followed by branching, bolling, rosette, elongation and maturity stage in order decreasing abundance (Figure 2).

Population of insect species at rosette, elongation and maturity stages differed significantly ($P < 0.05$) between seasons (Figure 2). Rosette and elongation stages had significantly ($P < 0.05$) higher population of insect species in winter than summer (Figure 2). While maturity stage had significantly ($P < 0.05$) higher population of insect species in summer than in winter (Figure 2). The insect species population at branching, bolling and flowering stages did not differ significantly ($P > 0.05$) between seasons (Figure 2).

Table 5 : Mean species composition of safflower growth stages

| Growth stages | Mean species composition | |
|-------------------|---------------------------|--------------------------|
| | Summer | Winter |
| Flowering | 10.33± 0.34 ^{aA} | 9.33±1.77 ^{aA} |
| Rosette | 5.00±0.58 ^{bB} | 7.67±0.34 ^{abA} |
| Bolling | 9.00±0.58 ^{aA} | 7.33±0.34 ^{abA} |
| Branching | 5.67±0.88 ^{bA} | 6.33±0.34 ^{abA} |
| Elongation | 4.33±0.88 ^{bA} | 5.00±0.00 ^{bcA} |
| Maturity | 5.33±0.34 ^{bA} | 2.00±0.00 ^{cB} |

Means followed by the same small letter within a column are not significantly different ($P \leq 0.05$), Tukey's Honestly Significant Different test, \pm standard error. Means followed by the same capital letter within a row are not significantly different (Student's T-test, $P > 0.05$).

Table 5 illustrates the mean species composition of insects collected from different safflower growth stages during summer and winter season. During summer, the number of insect species differed significantly ($F_{2,5}=13.55$, $P= 0.0003$) between growth stages (Table 5). Statistically, flowering and bolling stages were not significantly different in number of species (Table 5). Maturity, rosette, and elongation recorded the lowest mean number of species and were statistically similar in number of insects species (Table 5).

Similarly in winter, the average number of species differed significantly ($F_{2,5}=13.02$, $P=0.0004$) between growth stages (Table 5). Flowering had significantly high mean number of species (Table 5). Average number of species at rosette, bolling and branching did not differ significantly from one another. Elongation and maturity stages recorded lower number of species than other phenological stages, with maturity stage recording statistically the lowest mean number of species in winter (Table 5).

The number of species at rosette and maturity stages differed significantly ($P < 0.05$) between seasons (Table 5). Rosette had significantly ($P < 0.05$) high and low number of species during winter and summer, respectively (Table 5). While maturity stage had significantly ($P < 0.05$) higher number of species during summer than winter (Table 5). The phenological stages elongation, branching, bolling and flowering recorded statistically similar number of species between seasons (Table 5).

4.5 Most abundant species collected from safflower

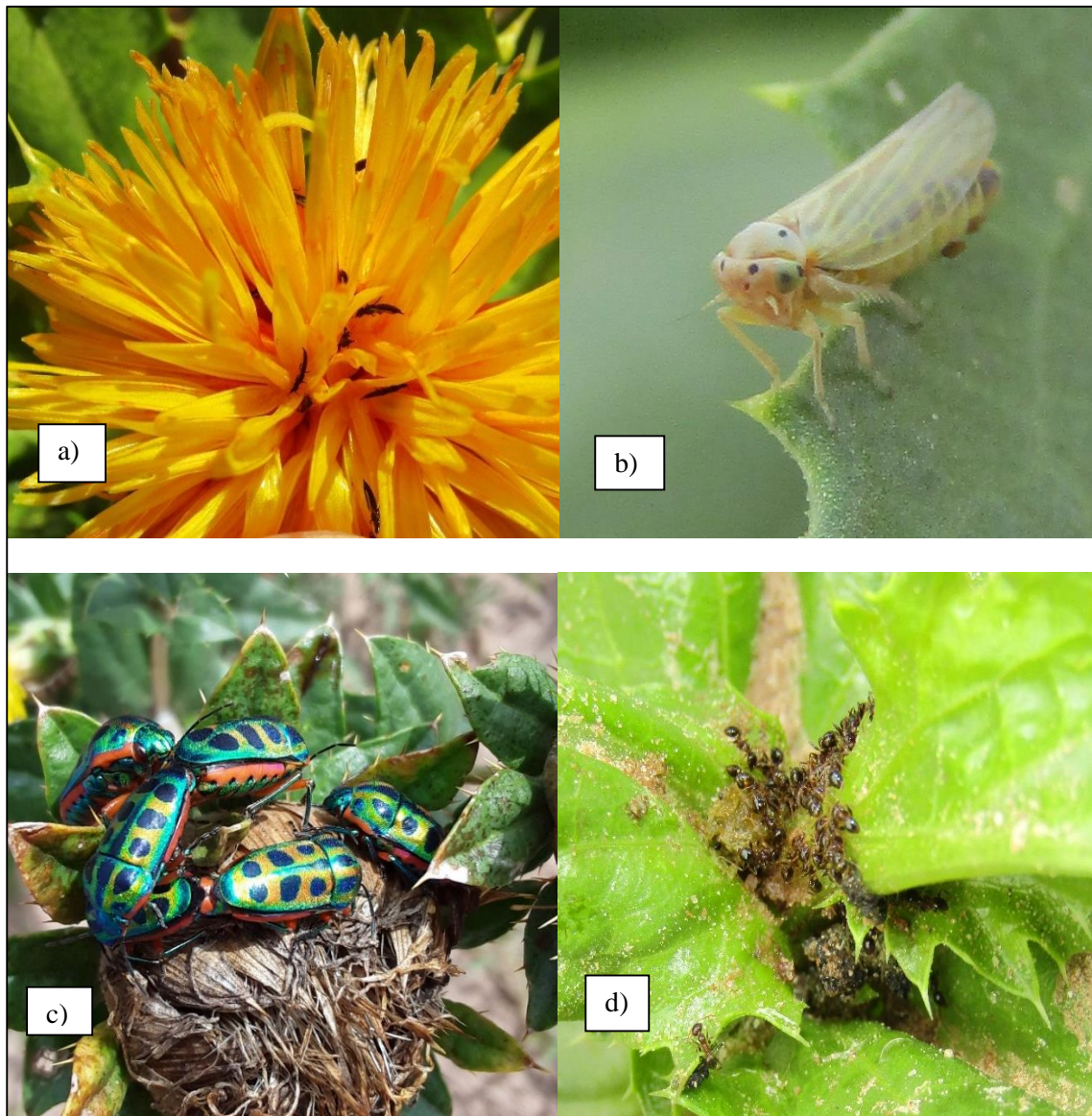


Figure 3: Most abundant species of safflower a) *Thrips tabaci* b) *Amaraca biguttula biguttula* c) *Calidea panaethiopica* d) *Formicidae* species

Figures 3 present pictures of the most abundant insect species collected from five different safflower genotypes during summer and winter season. The most abundant species during summer were *Thrips tabaci* > *Amrasca biguttula biguttula* > *Calidea panaethiopica*. In winter the most abundant species were *Thrips tabaci* > *Amrasca biguttula biguttula* > *Formicidae* species (Table 2).

4.5.1 *Thrips tabaci*

Thrips tabaci was present in both summer and winter season (Table 2). The population of *Thrips tabaci* was significantly different ($F_{2,1}=26.24$, $P=0.0001$) between seasons. The highest mean (47.11 ± 6.88) was recorded in winter while in summer the population mean was (21.27 ± 4.50) (Table 2). During summer season, the population of *Thrips tabaci* did not differ significantly ($F_{2,4}=0.24$, $P=0.9174$) among safflower genotypes but differed significantly ($F_{2,5}=93.97$, $P=0.0001$) with growth stages (Figure 4a,5). The highest population mean of *Thrips tabaci* was recorded in genotype Gila, followed by Turkey, PI-537636, Kenya-9819 and the lowest population was recorded in Sina (Figure 4a). The population of *Thrips tabaci* started to build up 3 weeks after plant emergence (elongation stage) and continued to increase up to the 7th week (Figure 5). Thereafter, the population declined and increased again from week 9 up to week 10 (flowering stage) (Figure 5). From week 11, the population dropped to zero with crop gaining physiological maturity (Figure 5). Highest peak during summer season was reached at week 10, flowering stage (Figure 5).

In winter season, the population of *Thrips tabaci* was not significantly different ($F_{2,4}=1.09$, $P=0.3674$) among safflower genotypes but significantly different ($F_{2,5}=33.55$, $P=0.0001$) between growth stages (Figure 4b, 5). The highest mean population was recorded in the genotype Kenya- 9819, followed by PI-537636, Gila, Sina and lastly Turkey (Figure 4b). *Thrips tabaci* started to be noticed in safflower 5 weeks after plant emergence (Rosette stage), the population increased gradually up to week 7 (Elongation stage) (Figure 5). From week 8 up to week 11, the population of *Thrips tabaci* recorded was zero (Figure 5). The pest started to appear again at week 12 (branching stage), increasing gradually up to week 14. Again, from week 15 to week 17, the population recorded was zero (Figure 5). From week 18, the pest resurfaced, and the population kept fluctuating up to week 21 (Flowering stage) (Figure 5). At week 22 and 23,

safflower plant was at maturity stage and no population was recorded during these two weeks.

The highest peak was recorded at week 19 (Flowering stage) during winter season (Figure 5).

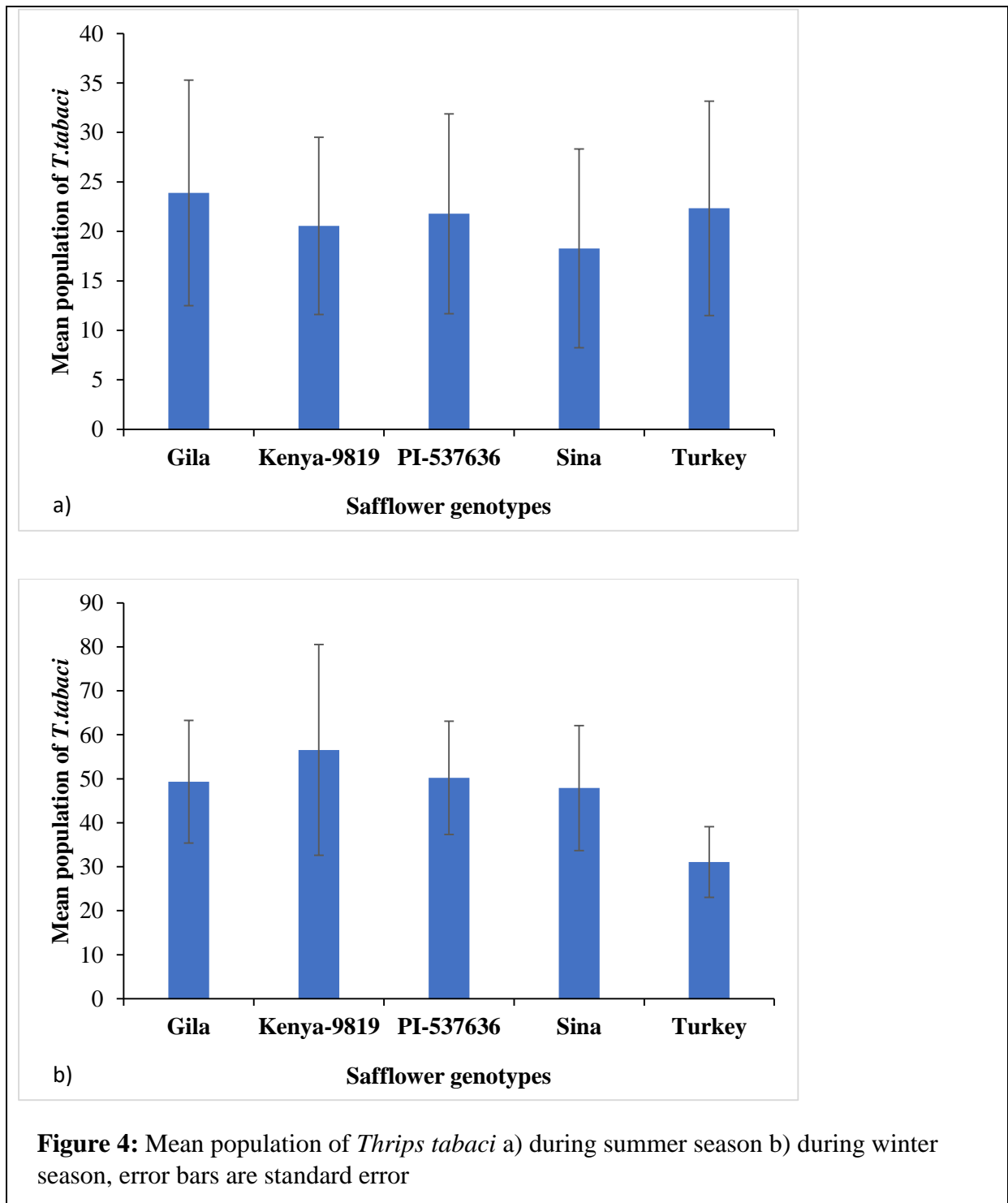
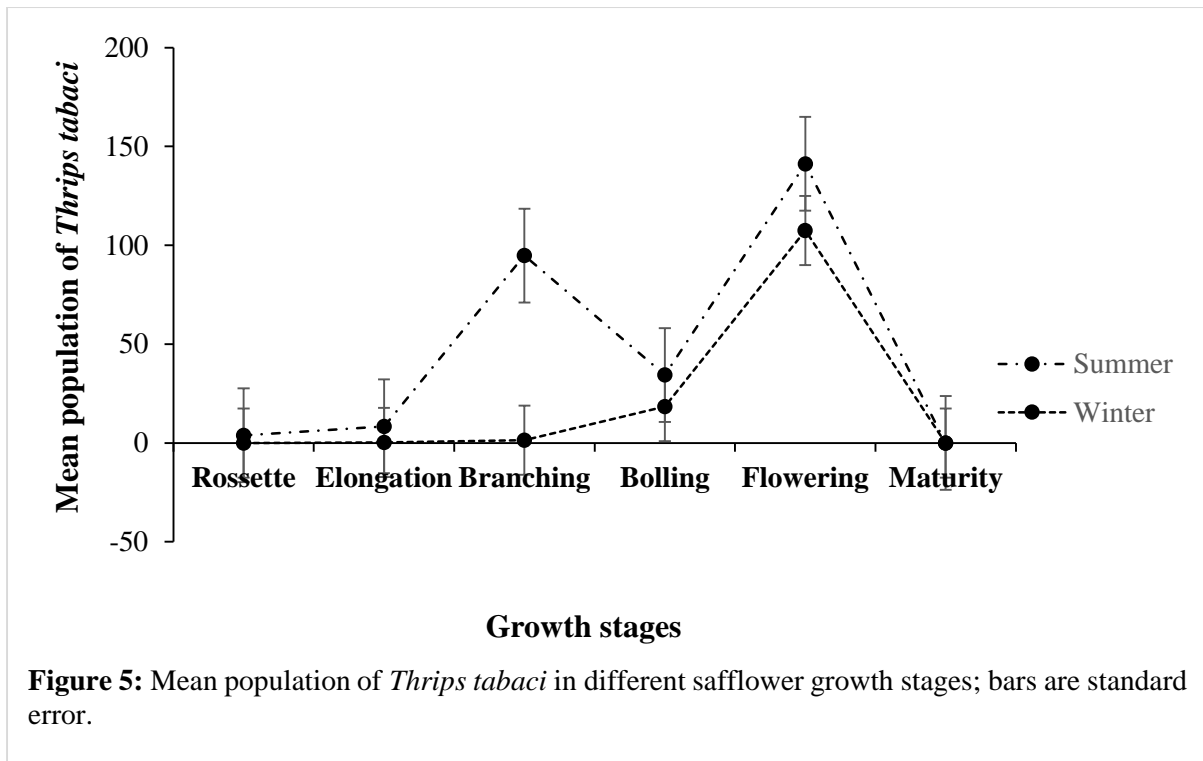


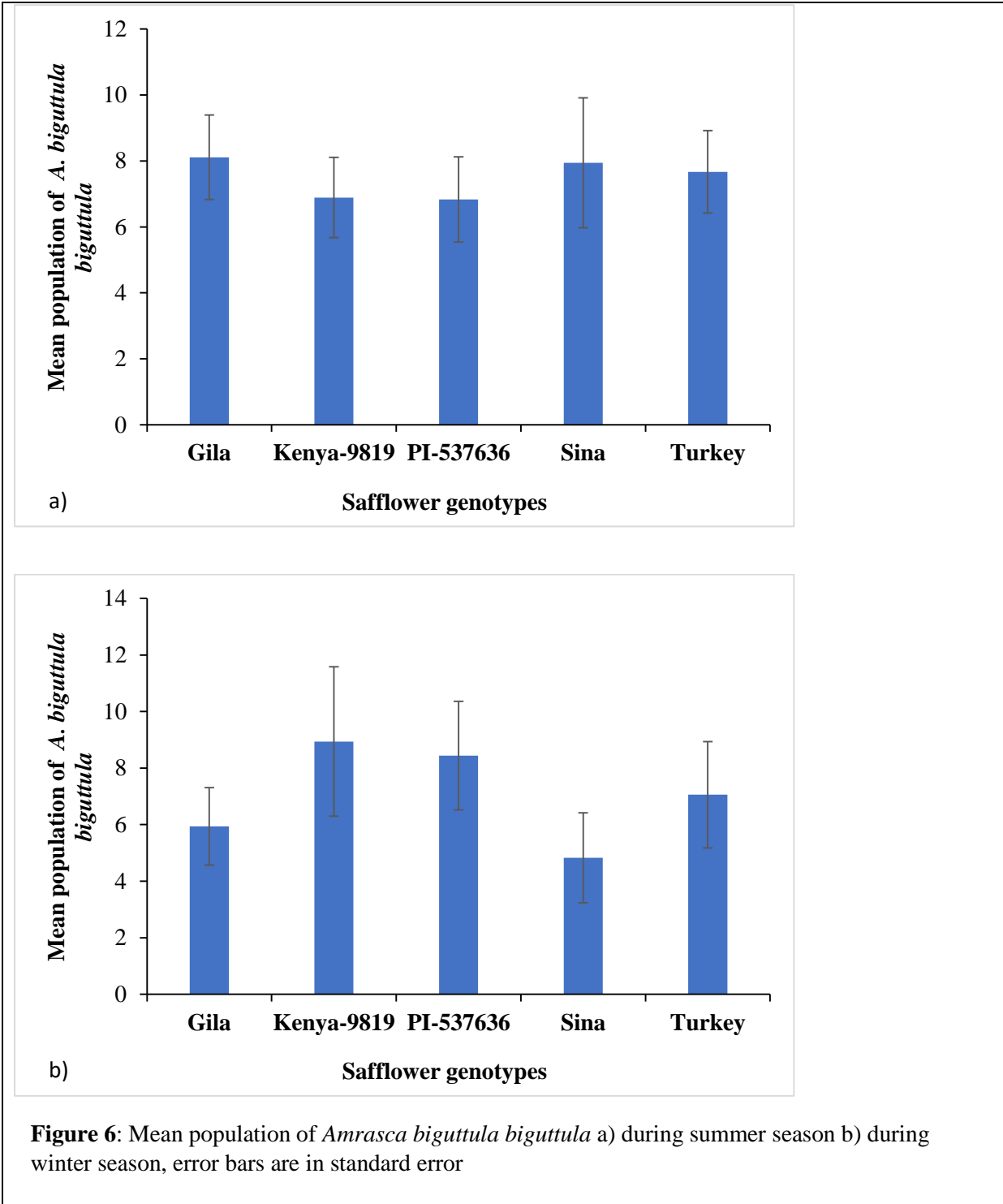
Figure 4: Mean population of *Thrips tabaci* a) during summer season b) during winter season, error bars are standard error

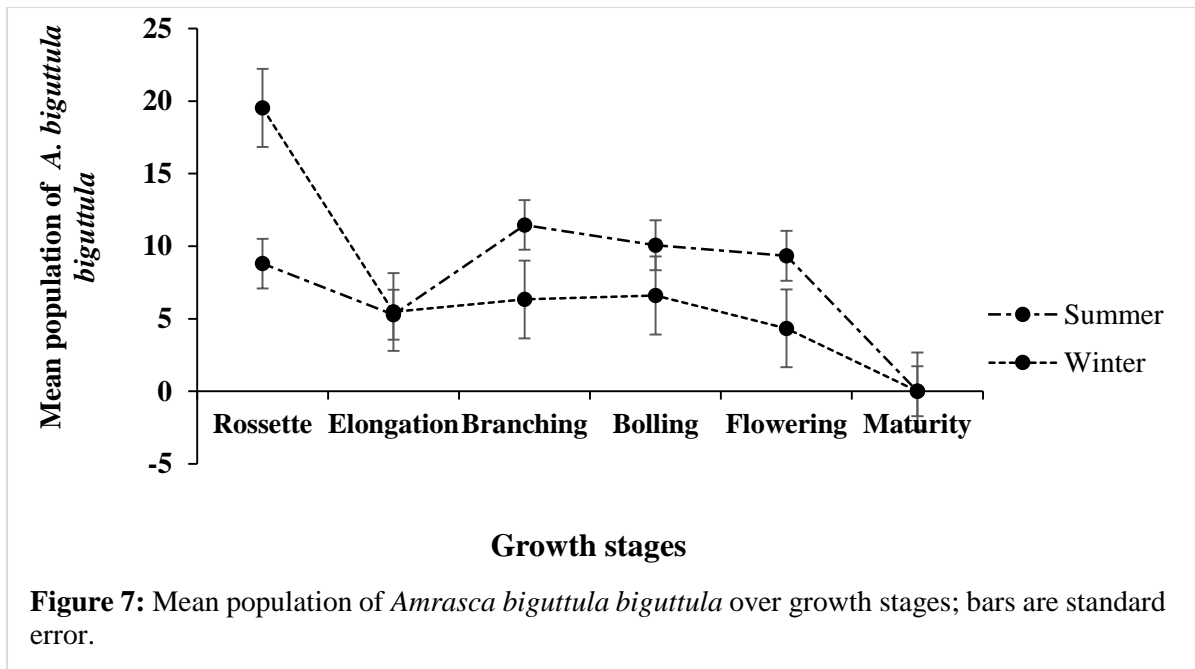


4.5.2 *Amrasca biguttula biguttula*

Amrasca biguttula biguttula was present in both summer and winter season (Table 2). The population of *Amrasca biguttula biguttula* did not differ significantly ($F_{2,1}=0.26$, $P=0.6107$) between summer and winter. The highest mean population was recorded in summer (7.49 ± 0.63) and lowest population was in winter (7.04 ± 0.86). During summer, the mean population of *Amrasca biguttula biguttula* did not significantly differ ($F_{2,4}= 0.29$, $P= 0.8818$) among safflower genotypes but significantly differed ($F_{2,5}=12.55$, $P=0.0001$) among growth stages (Figure 6a, 7). The mean population of *Amrasca biguttula biguttula* insect species was highest in the genotype Gila followed by Sina, Turkey, Kenya-9819 and lastly PI537636 (Figure 8a). *Amrasca biguttula biguttula* were observed from the 1st week after plant emergence (Rosette stage), the numbers kept fluctuating until week 10 (Flowering stage) in a decreasing manner (Figure 7). Thereafter, the numbers suddenly declined to zero until week 13 (Maturity stage). *Amrasca biguttula biguttula* reached their highest peak at week 4 (Bolling stage) in summer (Figure 7).

In winter, there was no significant difference ($F_{2,4}=1.68$, $P=0.1628$) among safflower genotypes in relation to *Amarasca biguttula biguttula* insect species population, but there was a significant difference ($F_{2,5}=20.76$, $P=0.0001$) between growth stages of safflower (Figure 6b, 7). The highest population mean of *Amarasca biguttula biguttula* insect species were recorded in genotype Kenya-9819, followed by PI-537636, Turkey, Gila and Sina (Figure 6b). Like in summer season, *Amarasca biguttula biguttula* was first observed in the first week after plant emergence (Rosette stage) (Figure 7). The population fluctuated up to week 7 (Elongation stage) (Figure 7). From week 8 to week 11, the population was zero. *Amarasca biguttula biguttula* appeared again on the 12th week and increased in number up to week 14 (Figure 7). From week 14, the population went to zero again. The pest resurfaced from week 18, with population decreasing gradually up to week 21 (Figure 7). At week 22 and 23 (Maturity stage), the population was zero. The population peak of *Amarasca biguttula biguttula* during winter grown safflower was recorded in week 18 (Flowering stage) (Figure 7).





4.5.3 *Calidea panaethiopica*

Calidea panaethiopica was present only in summer season, no population was recorded during winter (Table 2). In summer, the population of *Calidea panaethiopica* did not significantly differ ($F_{2,4} = 1.50$, $P = 0.2089$) among safflower genotypes but significantly differed ($F_{2,5} = 24.11$, $P = 0.0001$) between plant growth stages (Figure 8, 9). Mean population of *Calidea panaethiopica* was highest on the genotype Gila followed by Sina, Turkey, PI537636 and Kenya-9819. *Calidea panaethiopica* started to appear 6 weeks (bolling stage) after plant emergence and continued, until week 8 (Figure 9). From week 8 the population gradually declined up to week 10 (Figure 9). *Calidea panaethiopica* showed an increasing trend from week 11 until week 13 (Maturity stage) (Figure 9). The population of *Calidea panaethiopica* reached its peak at week 12 (Maturity stage) (Figure 9).

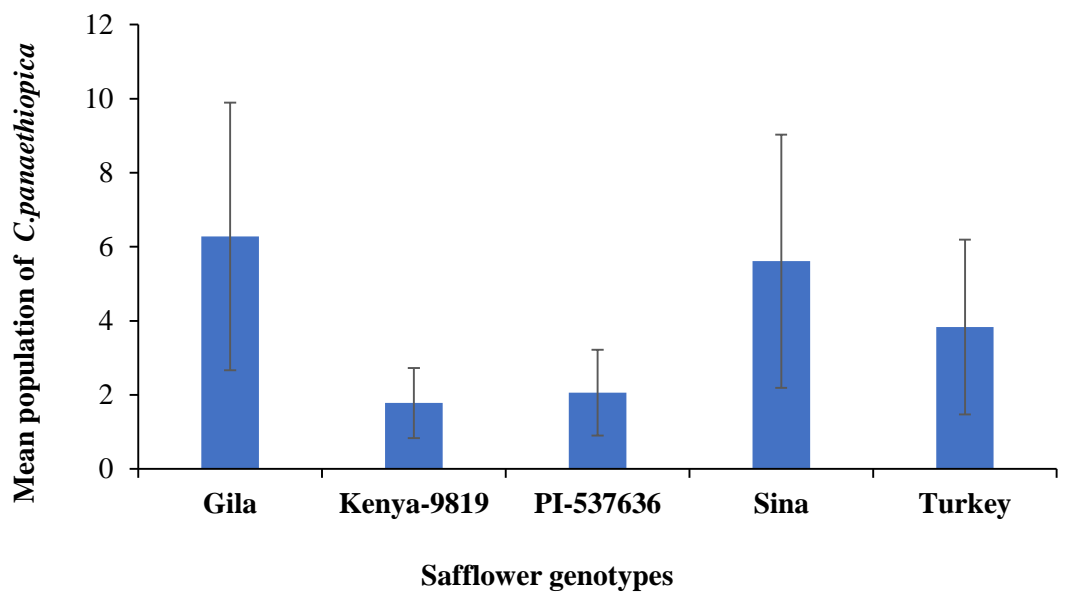


Figure 8: Mean population of *Calidea panaethiopica* during summer season , error bars are in standard error.

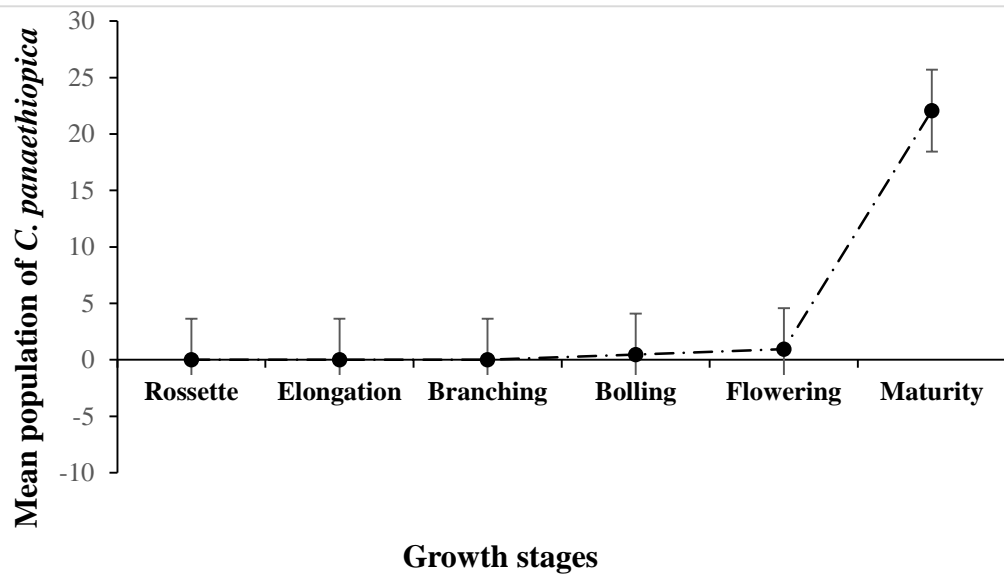
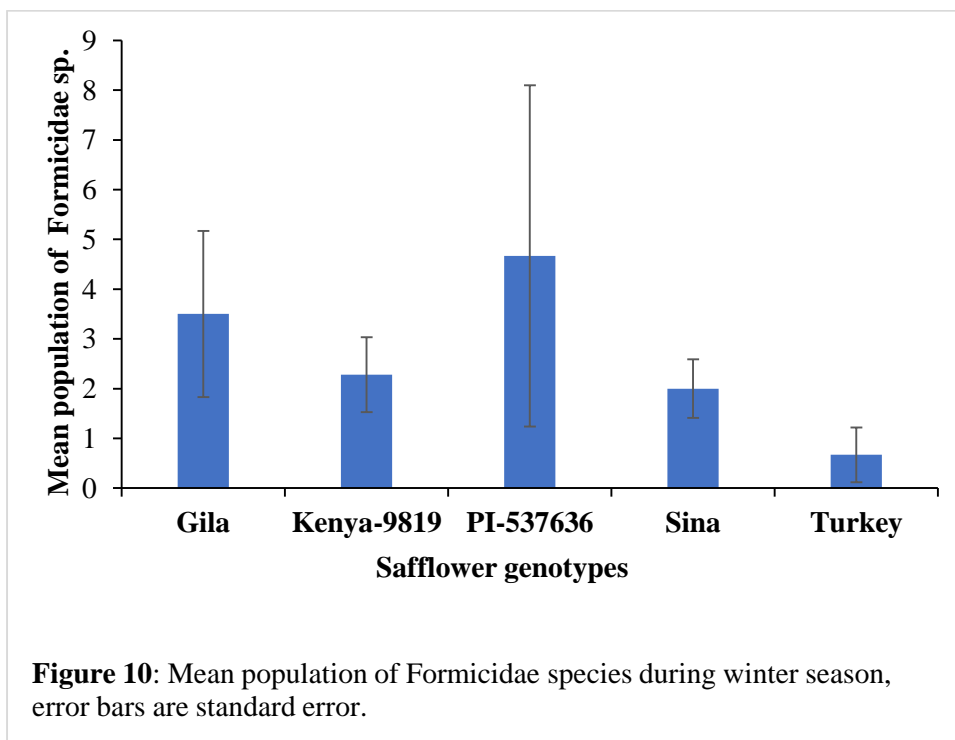
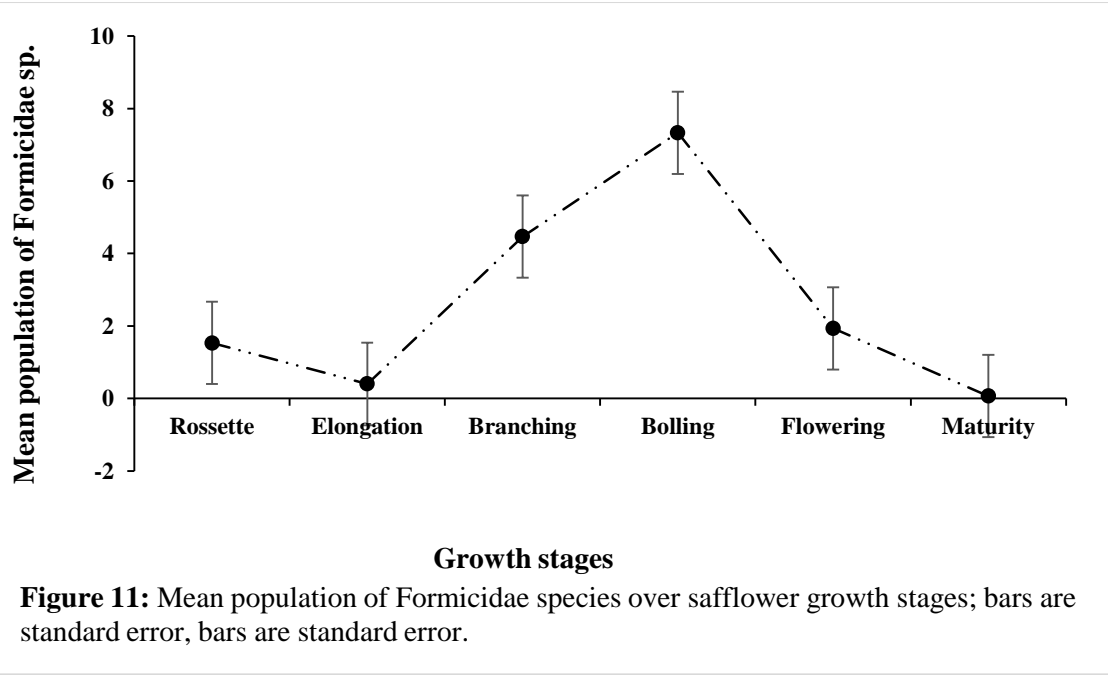


Figure 9: Mean population of *Calidea panaethiopica* over safflower growth stages, bars are standard error.

4.5.4 Formicidae species

Formicidae insect species were present in summer and winter grown safflower (Table 2). The species were one of the most abundant species in winter and summer, however, they were among the least abundant (Table 2). During winter, the population of Formicidae insect species did not differ significantly ($F_{2,4}= 0.80$, $P= 0.5311$) among safflower genotypes and growth stages ($F_{2,5}=2.22$, $P=0.0610$) (Figure 10, 11). Formicidae insect species were more abundant in genotype PI-537636, followed by Gila, Kenya-9819, Sina and lastly Turkey (Figure 10). The species was first observed a week after plant emergence (rosette stage) (Figure 11). There was fluctuation in the population of Formicidae insect species from week 1 up to week 7 (elongation stage) (Figure 11). From week 8 to week 11, the population was zero. At week 12 (branching stage), the species reappeared with fluctuating population until week 14 (Figure 11). The species disappeared again, recording zero population from week 15 up to week 17 (Figure 11). At week 18 (bolling stage), the highest population of Formicidae insect species was observed (Figure 11). There was a population decreasing trend from week 18 up to week 23 (Maturity stage (Figure 11).





4.6 Major pests of safflower

During the study, only two insect species, *Helicoverpa armigera* and Aphididae species, were observed as major pests of safflower.

4.6.1 *Helicoverpa armigera*

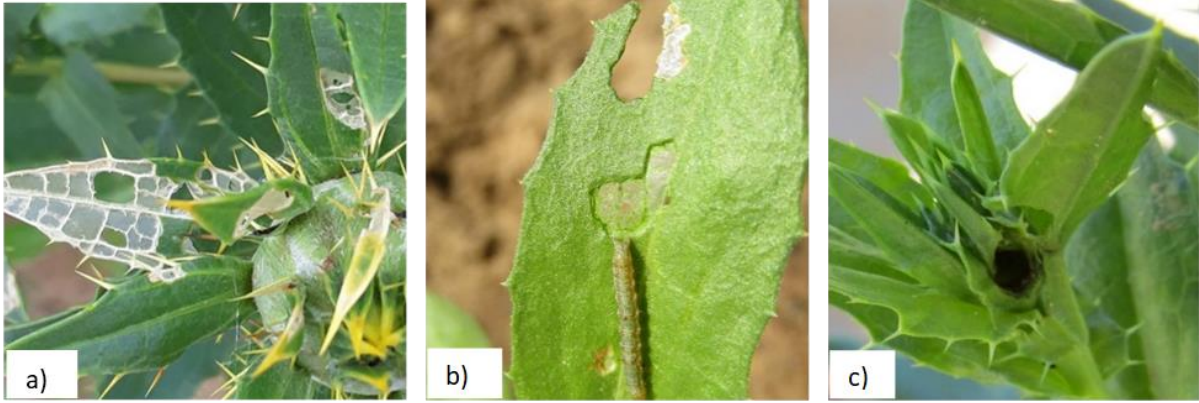


Figure 12: Damage caused by larvae of *Helicoverpa armigera* on a) bracts b) leaf c) developing capitula

Helicoverpa armigera attacked safflower as larvae during summer and winter seasons (Table 2). The population of the *Helicoverpa armigera* larvae differed significantly ($F_{2,1}=73.44$, $P=0.0001$) between seasons. The highest mean population was recorded in summer (1.7778 ± 0.21) while winter recorded lower population mean (0.14 ± 0.04). Figure 12 shows the damage caused by larvae of *Helicoverpa armigera* on safflower plants. The larvae of *Helicoverpa*

armigera caused prominent damage on bracts, leaves and capitula (Figure 12a, b, c). At rosette and elongation stages, the larvae fed on the leaves resulting in perforations (Figure 12a, b). In addition, at bolling stage, the larvae of *H. armigera* fed on bracts and bored into the developing capitula leaving perforated bracts and partially eaten capitula (Figure 12c). From observations, it has been noted that seed set was poor in flower heads that were affected during bolling and flowering stages.

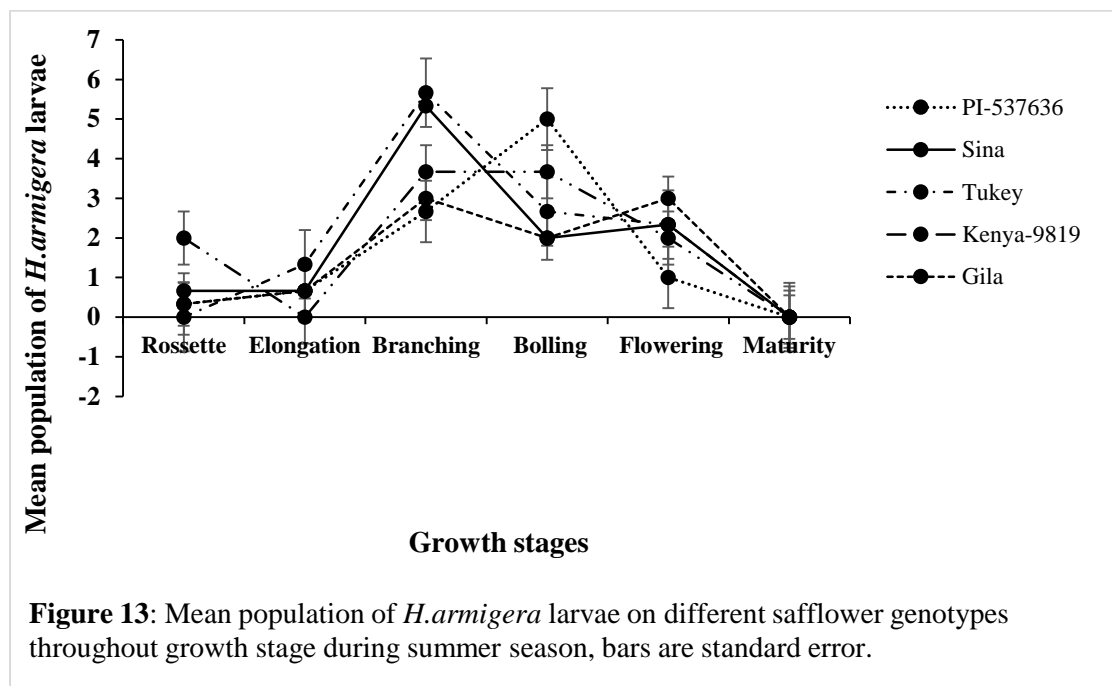
During summer, the mean population of *Helicoverpa armigera* larvae did not significantly differ ($F_{2,4}= 0.26$, $P=0.9016$) among safflower genotypes but significantly differed ($F_{2,5}=17.26$, $P=0.0001$) between safflower growth stages (Table 6). Turkey recorded the highest mean population of *Helicoverpa armigera* larvae, followed by Kenya-9819, Sina, PI537636 and Gila (Table 6). The larvae were more abundant at branching stage and less abundant at maturity stage (Figure 13).

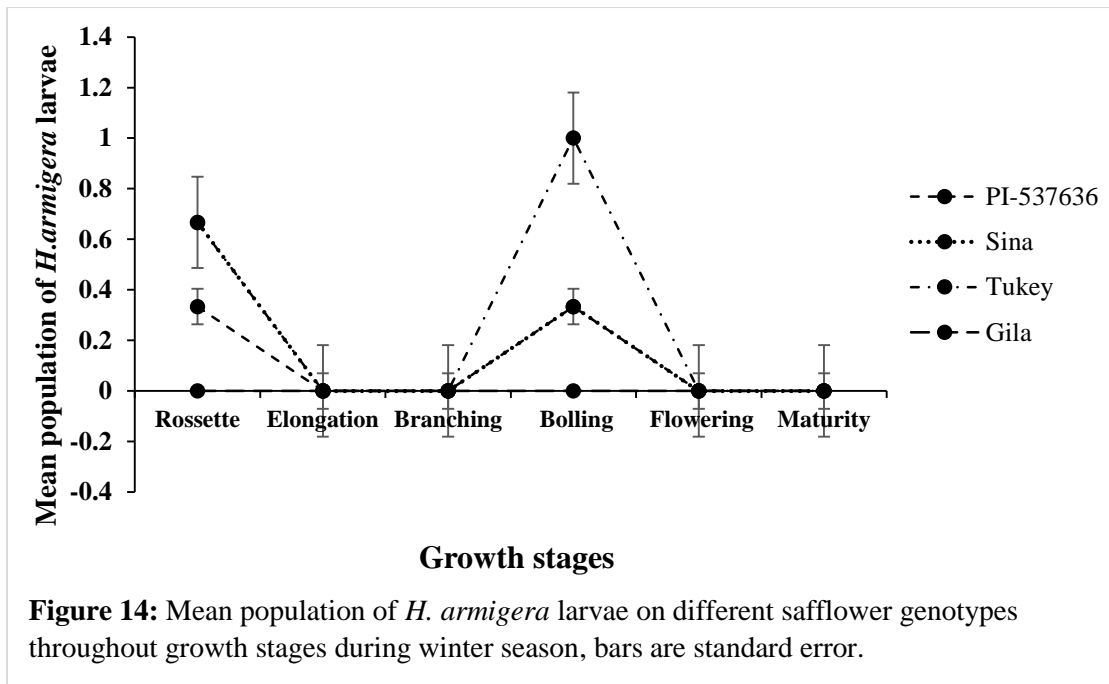
In winter, the mean population of *Helicoverpa armigera* larvae did not significantly differ ($F_{2,4}= 1.42$, $P=0.2340$) among safflower genotypes but significantly differed ($F_{2,5}=5.88$, $P=0.0001$) between safflower growth stages (Table 6). The genotype Kenya-9819 recorded the highest mean population of *Helicoverpa armigera* larvae, followed by Turkey, Sina, PI-537636 and Gila (Table 6). The larvae were more abundant at rosette stage and less abundant at elongation, branching, flowering, and maturity stage (Figure 14).

Table 6: Average population of larvae of *Helicoverpa armigera* among safflower genotypes

| Genotypes | Mean population of larvae of <i>Helicoverpa armigera</i> | |
|-------------|--|-------------------------|
| | Summer | Winter |
| Gila | 1.50±0.35 ^{aA} | 0.00±0.00 ^{aB} |
| Kenya- 9819 | 1.89±0.46 ^{aA} | 0.28±0.16 ^{aB} |
| PI-537636 | 1.72±0.52 ^{aA} | 0.11±0.08 ^{aB} |
| Sina | 1.83±0.54 ^{aA} | 0.17±0.09 ^{aB} |
| Turkey | 1.94±0.53 ^{aA} | 0.17±0.09 ^{aB} |

Means followed by the same small letter within a column are not significantly different ($P \leq 0.05$), Tukey's Honest Significant Different test. Means followed by the same capital letter within a row are not significantly different (Student's T-test, $P > 0.05$).





4.6.2 Aphididae species

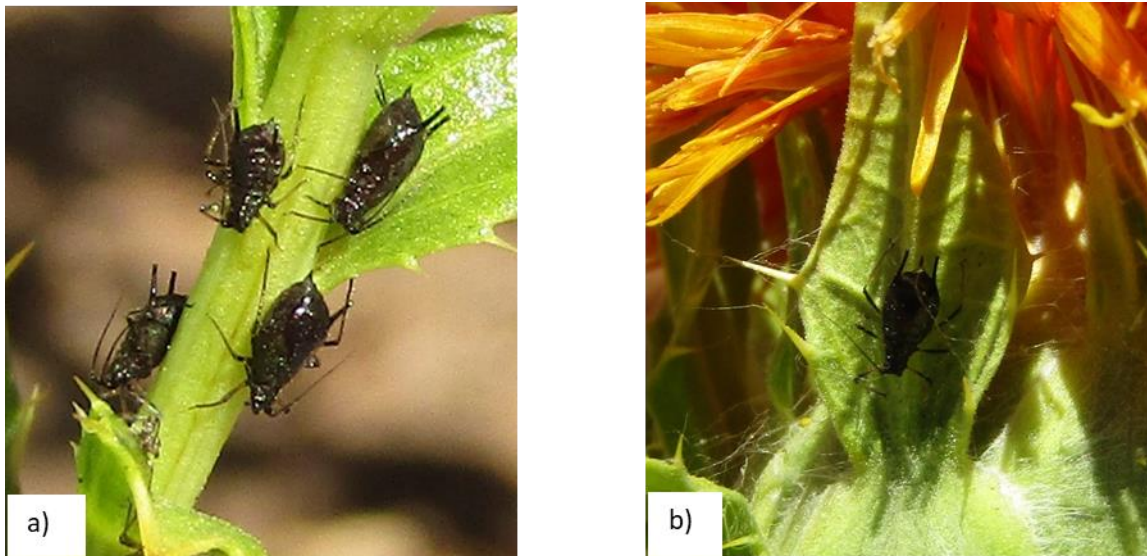


Figure 15: Aphididae species feeding on safflower a) stem b) leaf

The Aphididae insect species was considered a major pest of safflower, causing prominent damage with nymphs and adults sucking sap from the tender parts of safflower plant. Figure 15 shows Aphididae insect species feeding on the stem and leaves of safflower plants. The Aphididae insect species were absent in summer but present in winter (Table 2). The pest started to attack safflower at rosette stage and persisted up to flowering stage (Figure 16). The population of Aphididae insect species did not differ significantly ($F_{2,4}=2.25$, $P=0.0716$)

among safflower genotypes (Table 7) but differed significantly ($F_{2,5}=5.23$, $P=0.0003$) between safflower growth stages (Figure 16). The highest population mean was recorded on the genotype Kenya-9819, followed by PI- 537636, Gila, Turkey and lastly Sina (Table 7). Aphididae insect species were more abundant at the rosette stage and less abundant at maturity stage (Figure 16). From observations, at the rosette stage, plants infested with Aphididae insect species were weaker than those which were not infested. The pest produced secretions on the surface of the plant parts affected.

Table 7: Mean population of Aphididae species among safflower genotypes during winter season

| Genotype | Mean population of Aphididae species |
|--------------------|---|
| Gila | 1.33±0.35 ^a |
| Kenya- 9819 | 3.33±1.49 ^a |
| PI-537636 | 2.33±0.92 ^a |
| Sina | 0.61±0.44 ^a |
| Turkey | 0.89±0.29 ^a |

Means followed by the same small letter within a column are not significantly different ($P \leq 0.05$), Tukey's Honesty Significant Different test. Means followed by the same capital letter within a row are not significantly different (Student's T-test, $P > 0.05$).

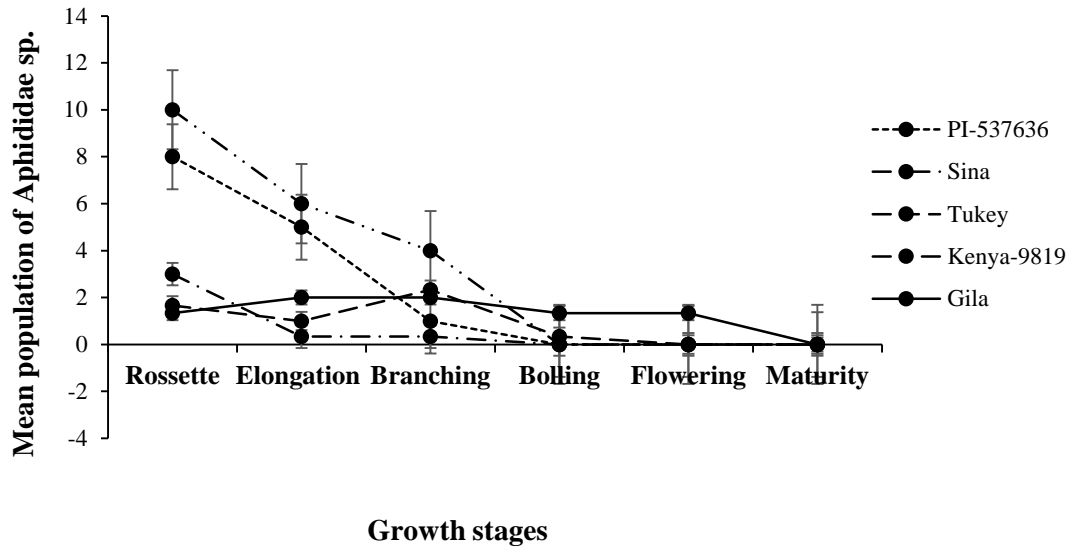


Figure 16: Mean population of Aphididae species on different safflower genotypes throughout growth stage during winter season; error bars are standard error

4.7 Plant parts affected

Table 8: Pests feeding on safflower, their status and feeding behaviour

| Insect species | Status | Feeding behaviour |
|---|---------------|---------------------------------|
| 1. Pests feeding on the leaves | | |
| <i>Amarasca biguttula biguttula</i> | Minor | Piercing and Sucking |
| <i>Helicoverpa armigera</i> | Major | Borer and chewing |
| <i>Thrips tabaci</i> | Minor | Piercing, scraping and Scraping |
| Curculionidae sp. | Minor | Piercing and Sucking |
| Tephritidae sp. | Minor | Piercing and sucking |
| Aphididae species | Major | Piercing and sucking |
| 2. Pests feeding inside the flower head | | |
| <i>Spilostethus pandurus</i> | Minor | Piercing and sucking |
| <i>Helicoverpa armigera</i> | Major | Borer and chewing |
| <i>Zonocerus elegans</i> | Minor | Biting and chewing |
| <i>Thrips tabaci</i> | Minor | Piercing, scraping and sucking |
| Curculionidae sp. | Minor | Piercing and sucking |
| Tephritidae sp. | Minor | Piercing and sucking |
| <i>Calidea panaethiopica</i> | Minor | Piercing and sucking |
| Aphididae species | Major | Piercing and sucking |
| 3. Pests feeding on the outside of flower head | | |
| <i>Amarasca biguttula biguttula</i> | Minor | Piercing and sucking |
| <i>Helicoverpa armigera</i> | Major | Borer and chewing |
| <i>Spilostethus pandurus</i> | Minor | Piercing and sucking |
| <i>Elasmucha grisea</i> | Minor | Piercing and sucking |
| <i>Calidea panaethiopica</i> | Minor | Piercing and sucking |
| <i>Zonocerus elegans</i> | Minor | Biting and chewing |
| Tephritidae sp. | Minor | Piercing and sucking |
| <i>Thrips tabaci</i> | Minor | Piercing and sucking |
| 4. Pests feeding on the stem | | |
| Aphididae species | Major | Piercing and sucking |

Based on feeding behaviour, seven (7) species were grouped as piercing and sucking, one (1) borer and chewing and one (1) biting and chewing (Table 8). The pests were also grouped

according to the plant part they were feeding on; those that were feeding on leaves, inside the flower head, outside the flower head and those that fed on the stem (Table 8). *Amrasca biguttula biguttula*, *Helicoverpa armigera*, *Thrips tabaci*, Curculionidae sp., Tephritidae sp. and Aphididae species were feeding on the leaves of safflower (Table 8). *Spilostethus pandurus*, *Helicoverpa armigera*, *Zonocerus elegans*, *Thrips tabaci*, Curculionidae species, Tephritidae sp., *Calidea panaethiopica* and Aphididae species were feeding on the inside part of the flower head (Table 8). While *Amrasca biguttula biguttula*, *Helicoverpa armigera*, *Spilostethus pandurus*, *Elasmucha grisea*, *Calidea panaethiopica*, *Zonocerus elegans*, *Thrips tabaci* and Tephritidae species were feeding on the outside part of the flower head (Table 8). Aphididae species was the only pest found to be feeding on the stem of safflower plant. *Apis mellifera* was the only pollinator recorded during the study; while *Cheilomenes lunata*, spider, Formicidae species and *Exochomus flavipes* were recorded as predatory insects.

4.8 Insects species diversity

Diversity indices of insect orders collected from safflower are shown in Table 9. The diversity indices indicate that, out of 8 orders, Hemiptera had the highest number of species (4). During summer, Hemiptera was the most diverse order (0.91), while in winter it was the second most diverse after Coleoptera (0.81). Order Hymenoptera had the highest species evenness (0.89) in summer while Coleopteran had the highest species evenness (0.74) in winter. Thysanoptera recorded the highest number of individuals during summer and winter, amazingly the order recorded the lowest Margalef's species richness in both seasons as 0.13 and 0.12, respectively. The highest Margalef's species richness was recorded in Coleoptera as 0.59 and 0.69, for summer and winter, respectively.

Table 10 shows diversity indices of safflower insects collected on different weeks in summer and winter. The diversity indices indicates that Safflower attained maturity earlier in summer than in winter. During summer season, weeks 6,8 and 9 had the highest number of species (11).

The lowest species richness (4) was recorded at week 1,12 and 13. These weeks also recorded low Margalef's richness index at 0.88,0.76 and 0.83, respectively. Week 10 recorded the highest number of individuals (1392) while the lowest number of individuals was recorded at week 11. The highest dominance (0.68) was at week 8 and 6. However, the highest Shannon diversity index (1.49), species evenness (0.62) and Margalef's richness index (2.04) were recorded at week 8. Even though week 10 had the highest number of individuals, the week recorded the lowest dominance (0.05), Shannon diversity index (0.18) and species evenness (0.08).During winter season, the highest species richness was observed at week 20 while the lowest species richness (0) was at week 8,9,10,11,14,15,16 and 17. These weeks also recorded the lowest number of individuals, dominance, Shannon diversity index, evenness and Margalef's richness index. The greatest number of individuals was recorded at week 19. Unexpectedly, week 23 recorded the highest dominance (0.71), evenness (0.83) and Margalef's richness index (2.06) even though it recorded only 7 individuals. The highest diversity (1.31) was recorded at week 3 which had only 6 species and 37 individuals.

Table 11 shows computed diversity indices of insects collected from different safflower growth stages during summer and winter season, respectively. In general, flowering stage had the highest number of individuals and species in both seasons. During summer, the highest number of species (11) was recorded at flowering and bolling stage. The highest number of individuals (1909) was at flowering stage. Even though flowering and bolling recorded the same number of species, bolling recorded the highest dominance (0.64), Shannon diversity index (1.36), species evenness (0.57) and Margalef's richness index (1.75). The lowest number of species (6) was observed at maturity stage. Maturity and flowering stage recorded same species evenness (0.29) even though flowering stage had the highest number of species and maturity stage had the lowest number of species. The lowest number of individuals (102) and Margalef's richness index (0.39) were recorded at elongation stage. Similarly, during winter season,

flowering stage recorded the highest number of species (12) and individuals (2329). However, the flowering stage had the lowest dominance (0.17), Shannon diversity index (0.49) and species evenness (0.20) with second highest Margalef's richness index (1.55). In contrary, during winter season elongation stage had the highest dominance (0.63) while maturity stage recorded the highest Margalef's richness index (2.48). The lowest number of species and individuals in winter were observed at maturity as 4 and 5, respectively. The lowest Margalef's richness index (0.94) was recorded at branching stage which had the second highest number of individuals (1650).

Table 12 shows a calculation of a suite of diversity indices and richness estimators for insects collected from different safflower genotypes during summer and winter season, respectively. In summer, Sina and Turkey recorded the highest number of species richness (13) and highest Margalef's richness index at 2.00 and 1.99, respectively. Sina had the highest dominance (0.68) and species evenness (0.57). Kenya recorded the lowest number of individuals (610) as well as Shannon diversity index (1.31) and species evenness (0.52). The lowest number of species (12) was recorded on PI-537636, Kenya-9819 and Gila. Furthermore, genotype PI-537636 and Kenya-9819 recorded the lowest dominance (0.58). During winter, P1-53636 recorded the highest number of individuals (1230), dominance (0.43), Shannon diversity index (0.94) and species evenness (0.38). Turkey had the highest species richness (13) and lowest number of individuals (755) compared to other genotypes. Table 17 reveals that, even though Turkey had the lowest number of individuals, dominance, Shannon diversity index and species evenness are almost similar to those of PI-537636 which recorded the highest number of individuals. Moreover, Turkey recorded the highest Margalef's richness index (1.96).

Table 16 present diversity indices of insects collected from safflower during summer and winter season. The two seasons recorded the same number of species (13) with winter recording the highest number of individuals (5459). Summer revealed the highest Shannon diversity

index (0.62) and Margalef's richness index (1.60). Additionally, summer had the highest species evenness (0.54) and dominance (0.62)

Table 9: Diversity indices of insects orders collected from safflower

| Indices | Season | Insect Orders and other Arthropods | | | | | | | |
|------------------------------------|--------|------------------------------------|------------|---------|-----------|-------------|-------------|--------------|---------|
| | | Coleoptera | Orthoptera | Diptera | Hemiptera | Hymenoptera | Lepidoptera | Thysanoptera | Araneae |
| Species Richness(S) | Summer | 2 | 1 | 1 | 4 | 2 | 1 | 1 | 1 |
| | Winter | 3 | - | 1 | 4 | 2 | 1 | 1 | 1 |
| Individuals | Summer | 30 | 19 | 65 | 1106 | 68 | 160 | 1914 | 12 |
| | winter | 77 | - | 13 | 823 | 271 | 13 | 4240 | 22 |
| Simpson`s Dominance Index, D | Summer | 0.24 | 0 | 0 | 0.53 | 0.43 | 0 | 0 | 0 |
| | winter | 0.47 | - | 0 | 0.37 | 0.23 | 0 | 0 | 0 |
| Shannon Diversity Index, H | Summer | 0.39 | 0 | 0 | 0.91 | 0.62 | 0 | 0 | 0 |
| | winter | 0.81 | - | 0 | 0.68 | 0.38 | 0 | 0 | 0 |
| Pielou`s Species Evenness, J | Summer | 0.57 | - | - | 0.65 | 0.89 | - | - | - |
| | winter | 0.74 | - | - | 0.49 | 0.56 | - | - | - |
| Margalef`s Richness Index | Summer | 0.59 | 0.34 | 0.24 | 0.57 | 0.47 | 0.20 | 0.13 | 0.40 |
| | winter | 0.69 | - | 0.39 | 0.37 | 0.36 | 0.39 | 0.12 | 0.32 |

Table 10: Diversity indices of insect species collected on different weeks

| Weeks | Species Richness, S | | Individuals | | Simpson`s Dominance Index, D | | Shannon Diversity Index, (H') | | Pielou`s Species Evenness, J | | Margalef`s Richness Index | |
|-----------|---------------------|---------------|---------------|---------------|------------------------------|---------------|-------------------------------|---------------|------------------------------|---------------|---------------------------|---------------|
| | <i>Summer</i> | <i>Winter</i> | <i>Summer</i> | <i>Winter</i> | <i>Summer</i> | <i>Winter</i> | <i>Summer</i> | <i>Winter</i> | <i>Summer</i> | <i>Winter</i> | <i>Summer</i> | <i>Winter</i> |
| 1 | 4 | 3 | 95 | 31 | 0.43 | 0.29 | 0.8 | 0.52 | 0.57 | 0.48 | 0.88 | 0.87 |
| 2 | 6 | 3 | 82 | 58 | 0.41 | 0.07 | 0.85 | 0.17 | 0.47 | 0.16 | 1.36 | 0.74 |
| 3 | 8 | 6 | 96 | 37 | 0.41 | 0.62 | 0.89 | 1.31 | 0.43 | 0.3 | 1.75 | 1.66 |
| 4 | 7 | 4 | 158 | 84 | 0.53 | 0.14 | 1.05 | 0.32 | 0.54 | 0.23 | 1.38 | 0.9 |
| 5 | 7 | 6 | 119 | 65 | 0.59 | 0.6 | 1.17 | 1.16 | 0.6 | 0.65 | 1.46 | 1.44 |
| 6 | 11 | 6 | 279 | 188 | 0.68 | 0.69 | 1.41 | 1.27 | 0.59 | 0.71 | 1.95 | 1.15 |
| 7 | 9 | 6 | 255 | 251 | 0.59 | 0.63 | 1.22 | 1.16 | 0.55 | 0.65 | 1.62 | 1.09 |
| 8 | 11 | 0 | 219 | 0 | 0.68 | 0 | 1.49 | 0 | 0.62 | 0 | 2.04 | 0 |
| 9 | 11 | 0 | 298 | 0 | 0.67 | 0 | 1.46 | 0 | 0.61 | 0 | 1.93 | 0 |
| 10 | 9 | 0 | 1392 | 0 | 0.05 | 0 | 0.18 | 0 | 0.08 | 0 | 1.24 | 0 |
| 11 | 6 | 0 | 63 | 0 | 0.6 | 0 | 1.22 | 0 | 0.68 | 0 | 1.45 | 0 |
| 12 | 4 | 5 | 187 | 222 | 0.1 | 0.43 | 0.23 | 0.87 | 0.17 | 0.54 | 0.76 | 0.93 |
| 13 | 4 | 6 | 125 | 477 | 0.15 | 0.24 | 0.33 | 0.56 | 0.24 | 0.31 | 0.83 | 0.97 |
| 14 | - | 0 | - | 0 | - | 0 | - | 0 | - | 0 | - | 0 |
| 15 | - | 0 | - | 0 | - | 0 | - | 0 | - | 0 | - | 0 |
| 16 | - | 0 | - | 0 | - | 0 | - | 0 | - | 0 | - | 0 |
| 17 | - | 0 | - | 0 | - | 0 | - | 0 | - | 0 | - | 0 |
| 18 | - | 9 | - | 771 | - | 0.51 | - | 1.04 | - | 0.47 | - | 1.35 |
| 19 | - | 9 | - | 1059 | - | 0.18 | - | 0.48 | - | 0.22 | - | 1.29 |
| 20 | - | 11 | - | 757 | - | 0.17 | - | 0.48 | - | 0.2 | - | 1.66 |
| 21 | - | 10 | - | 394 | - | 0.2 | - | 0.55 | - | 0.24 | - | 1.67 |
| 22 | - | 6 | - | 134 | - | 0.18 | - | 0.47 | - | 0.26 | - | 1.23 |
| 23 | - | 4 | - | 7 | - | 0.71 | - | 1.15 | - | 0.83 | - | 2.06 |

Table 11: Diversity indices of safflower insects collected at different growth stages

| Indices | Season | Growth Stages | | | | | |
|---|---------------|---------------|------------|-----------|---------|-----------|----------|
| | | Rosette | Elongation | Branching | Bolling | Flowering | Maturity |
| Species Richness, S | <i>Summer</i> | 7 | 8 | 8 | 11 | 11 | 6 |
| | <i>Winter</i> | 9 | 6 | 7 | 9 | 12 | 4 |
| Individuals | <i>Summer</i> | 177 | 102 | 277 | 534 | 1909 | 375 |
| | <i>Winter</i> | 463 | 251 | 1650 | 759 | 2329 | 5 |
| Simpson`s Dominance Index, D | <i>Summer</i> | 0.43 | 0.39 | 0.56 | 0.64 | 0.28 | 0.22 |
| | <i>Winter</i> | 0.56 | 0.63 | 0.25 | 0.5 | 0.17 | 0.9 |
| Shannon Diversity Index, H | <i>Summer</i> | 0.96 | 0.89 | 1.14 | 1.36 | 0.71 | 0.52 |
| | <i>Winter</i> | 1.16 | 1.16 | 0.59 | 1.02 | 0.49 | 1.33 |
| Pielou`s Species Evenness, J | <i>Summer</i> | 0.49 | 0.43 | 0.55 | 0.57 | 0.29 | 0.29 |
| | <i>Winter</i> | 0.53 | 0.65 | 0.3 | 0.47 | 0.2 | 0.96 |
| Margalef`s Richness Index | <i>Summer</i> | 1.35 | 0.39 | 1.42 | 1.75 | 1.46 | 1.01 |
| | <i>Winter</i> | 1.47 | 1.09 | 0.94 | 1.36 | 1.55 | 2.48 |

Table 12: Diversity indices of insects collected from different safflower genotypes

| Indices | Season | Genotypes | | | | |
|---------------------------------|---------------|-----------|------|------------|------|--------|
| | | PI-537636 | Sina | Kenya-9819 | Gila | Turkey |
| Species Richness, S | <i>Summer</i> | 12 | 13 | 12 | 12 | 13 |
| | <i>Winter</i> | 12 | 11 | 12 | 9 | 13 |
| Individuals | <i>Summer</i> | 641 | 659 | 610 | 769 | 695 |
| | <i>Winter</i> | 1230 | 1036 | 1331 | 1107 | 755 |
| Simpson`s Dominance Index, D | <i>Summer</i> | 0.58 | 0.68 | 0.58 | 0.64 | 0.61 |
| | <i>Winter</i> | 0.43 | 0.3 | 0.4 | 0.34 | 0.42 |
| Shannon Diversity Index, H | <i>Summer</i> | 1.32 | 1.47 | 1.31 | 1.39 | 1.37 |
| | <i>Winter</i> | 0.94 | 0.72 | 0.9 | 0.76 | 0.94 |
| Pielou`s Species Evenness, J | <i>Summer</i> | 0.53 | 0.57 | 0.52 | 0.56 | 0.53 |
| | <i>Winter</i> | 0.38 | 0.3 | 0.36 | 0.35 | 0.37 |
| Margalef`s Richness Index | <i>Summer</i> | 1.86 | 2 | 1.87 | 1.81 | 1.99 |
| | <i>Winter</i> | 1.69 | 1.58 | 1.67 | 1.28 | 1.96 |

Table 13: Diversity indices of safflower insects collected during summer and winter season

| Season | Species Richness, S | Individuals | Simpson's Dominance Index, D | Shannon Diversity Index, H | Pielou's Species Evenness, J | Margalef's Richness |
|--------|---------------------|-------------|------------------------------|----------------------------|------------------------------|---------------------|
| Summer | 13 | 3374 | 0.62 | 1.39 | 0.54 | 1.60 |
| Winter | 13 | 5459 | 0.38 | 0.87 | 0.34 | 1.51 |

4.9 Seed yield

Table 14: Seed yield from different safflower genotypes

| Genotype | Seed yield (kg/ha) | |
|-------------------|-----------------------------|-----------------------------|
| | Summer | Winter |
| Gila | 543.47±103.28 ^{aA} | 344.83±8.14 ^{aA} |
| Kenya-9819 | 388.80±108.45 ^{aA} | 473.87±42.32 ^{aA} |
| PI-537636 | 462.67±178.04 ^{aA} | 697.73±151.34 ^{aA} |
| Sina | 426.27±56.43 ^{aA} | 301.47±117.43 ^{aA} |
| Turkey | 665.33±120.92 ^{aA} | 566.40±14.16 ^{aA} |

Means followed by the same small letter within a column are not significantly different ($P \leq 0.05$), Tukey's Honest Significant Different test. Means followed by the same capital letter within a row are not significantly different (Student's T-test, $P > 0.05$).

The seed yield of safflower genotypes is presented in Table 14. There was no significant difference in the yield ($F_{2,4} = 1.07$, $P = 0.4328$) obtained from different safflower genotypes during summer, with Turkey recording the highest yield and Kenya-9819 recorded the lowest seed yield (Table 14). Similarly, there was no significant difference in the seed yield ($F_{2,4} = 2.75$, $P = 0.1046$) among safflower genotypes in winter. PI-537636 recorded the highest yield while Sina recorded the lowest yield in winter (Table 14). The results also show that all genotypes did not significantly ($P > 0.05$) differ in their yield between summer and winter (Table 14).

Figure 17 indicates the relationships of safflower seed yield and population of potential pests of safflower during summer. There were weak and positive linear relationships between yield and the population of *Amrasca biguttula biguttula* ($r = 0.20$), *Helicoverpa armigera* ($r = 0.32$) and *Calidea panaethiopica* ($r = 0.21$) in safflower grown in summer (Figure 17 a, b, d). Also, there were weak negative linear relationship observed between safflower seed yield and the populations of *Thrips tabaci* ($r = -0.13$) (Figure 17c)

Figure 18 indicates the relationship of safflower seed yield and potential pests of safflower during winter. There was a non-significant ($P > 0.05$) linear increase of safflower seed yield with increase in *Amrasca biguttula biguttula* ($r = 0.48$) (Figure 21a) and Aphididae ($r = 0.04$) (Figure 21d) insect species in winter grown safflower. There was a non-significant ($P > 0.05$) negative correlation ($r = -0.21$) between safflower seed yield and *Thrips tabaci* insect species in winter (Figure 21c). As the population of *Thrips tabaci* insect species increased on safflower plants in winter, seed yield decreased (Figure 21c). *H. armigera* (Figure 21b) insect species had a non-significant quadratic relationship with safflower seed yield in winter. As the population of *H. armigera* species increased, safflower seed yield increased non-significantly ($P > 0.05$) ($r = 0.0.20$) up to a maximum insect species population, thereafter seed yield decreased.

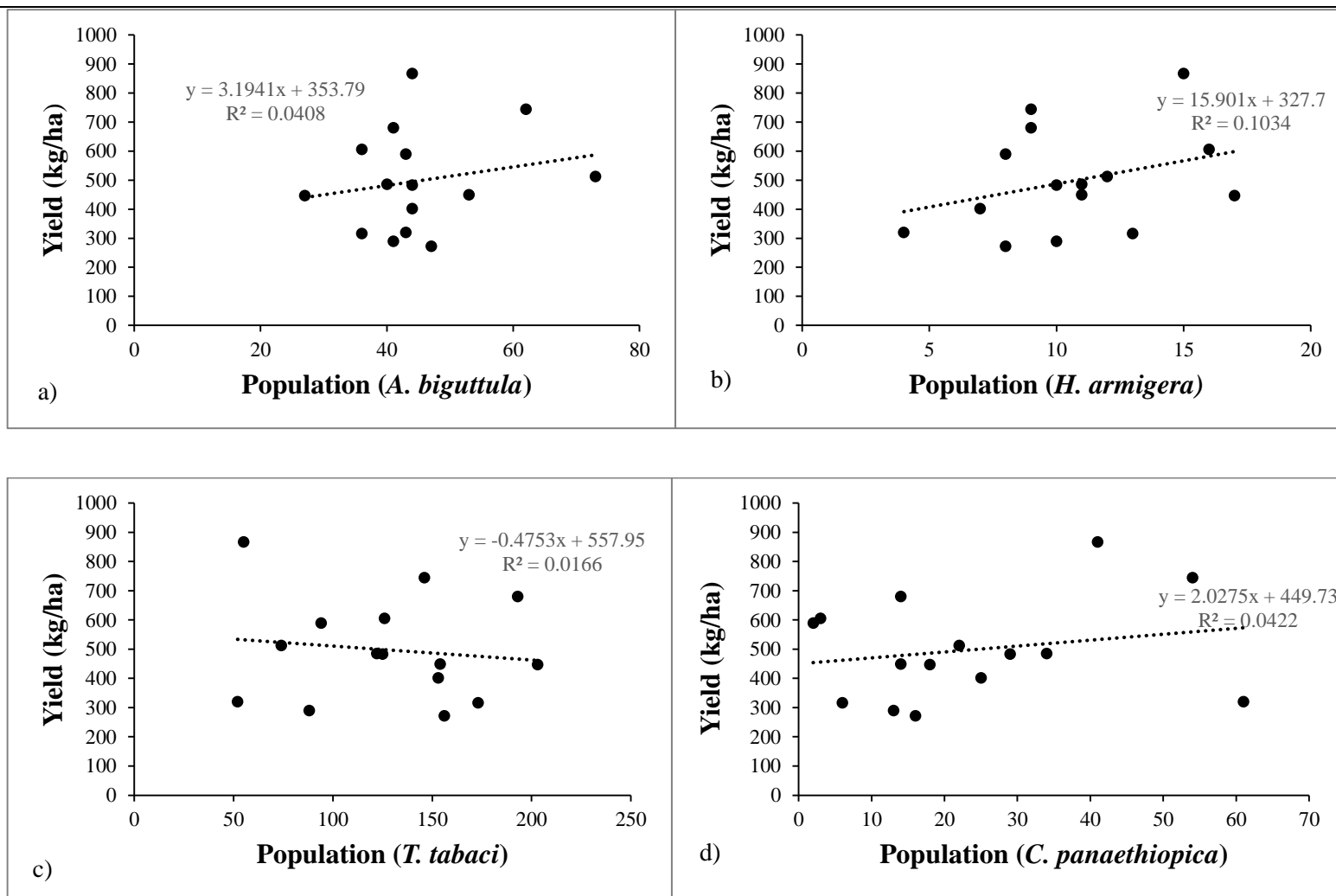


Figure 17: Relationship between seed yield and insect species population a) *Amrasca biguttula biguttula* b) *Helicoverpa armigera* c) *Thrips tabaci* d) *Calidea panaethiopica* in summer.

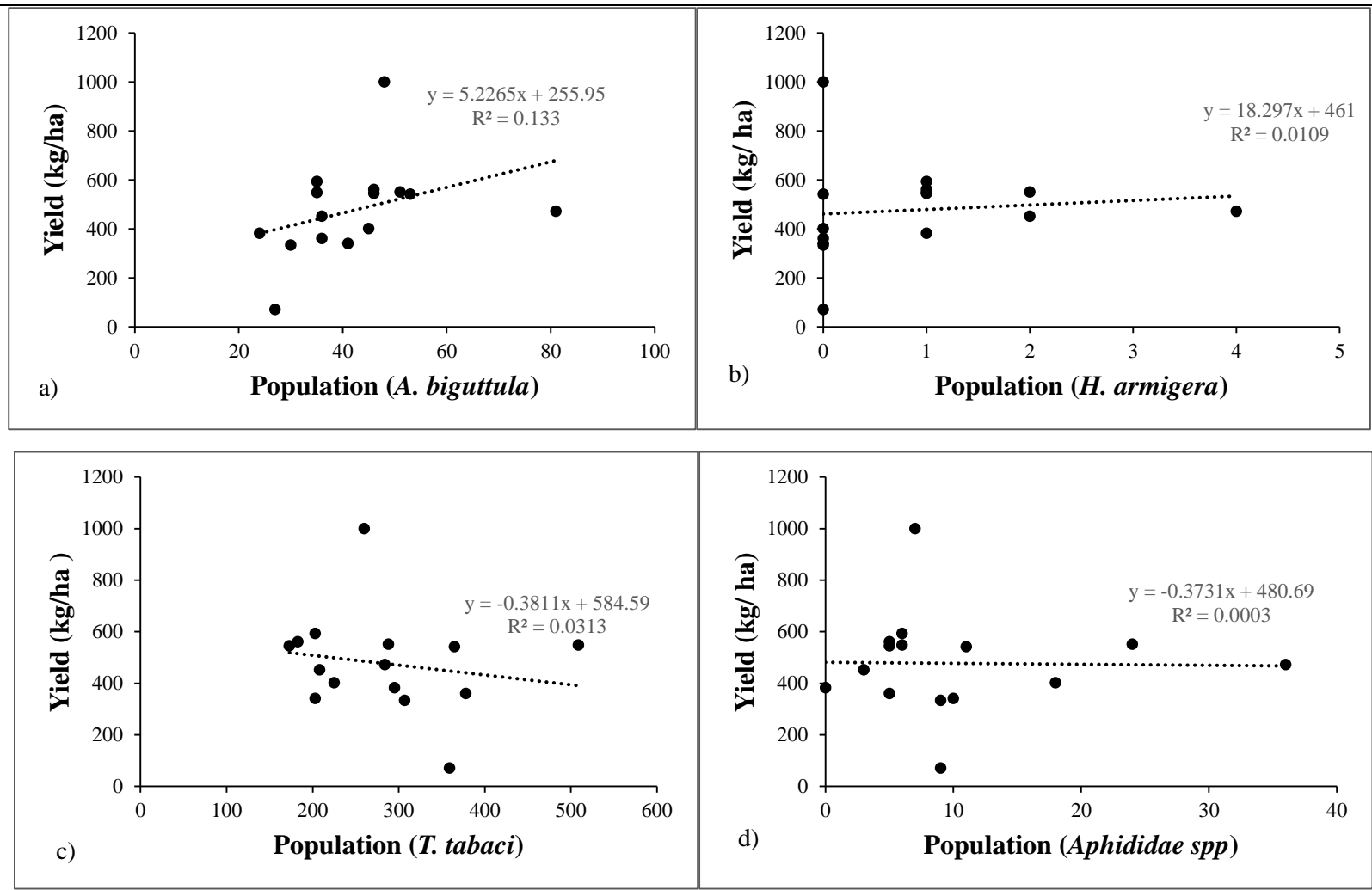


Figure 18: Relationships between seed yield and species population in winter a) *Amrasca biguttula biguttula* b) *Helicoverpa armigera* c) *Thrips tabaci* d) Aphididae species

4.10 Effects of weather parameters on species population

Figure 19 present relationships between temperature, rainfall, relative humidity and total insects population during summer season. Temperature, rainfall and relative humidity ranged from 15.9°C and 22.4°C, 0mm to 9.5mm and 25% to 85%, respectively. Total insects population increased linearly with temperature (Figure 19a). However, rainfall had a non-linear relationship with total insect population as represented in Figure 19(b). Furthermore, the relationship between relative humidity and total insects population was negative and quadratic function, therefore, total insects population decreased as relative humidity increased (Figure 19c). The corresponding correlation coefficients were 0.32, -0.16 and -0.20, for temperature, rainfall and relative humidity respectively.

Figure 20 indicates the relationship between temperature, rainfall and relative humidity and total insects population during winter season. Temperature ranged from 0.8°C and 17.5°C, rainfall ranged from 0mm to 13.9mm while relative humidity ranged from 20% to 91%. Total insects population had a curvilinear relationship with temperature and linear relationship with rainfall and relative humidity (Figure 20a, b, c). This depicts that as temperature, rainfall and relative humidity increases, total number of insects also increases. The corresponding correlation coefficients were 0.32, 0.34 and 0.11 for temperature, rainfall and relative humidity, respectively.

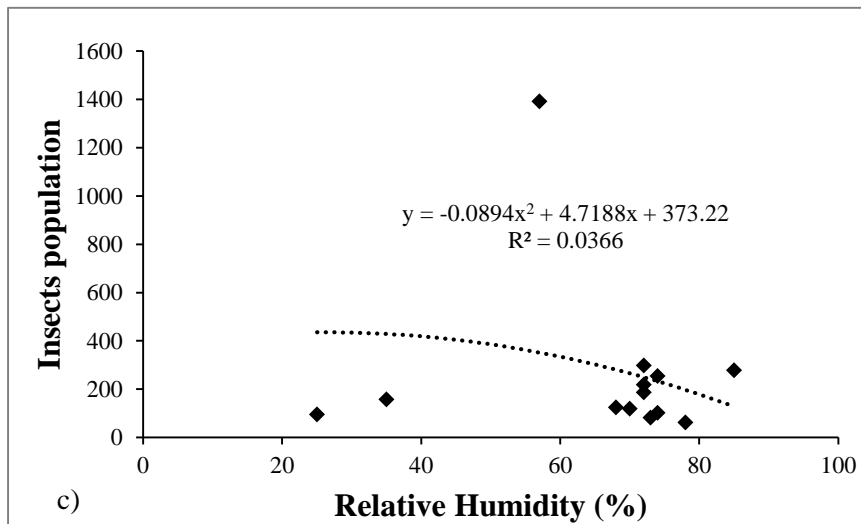
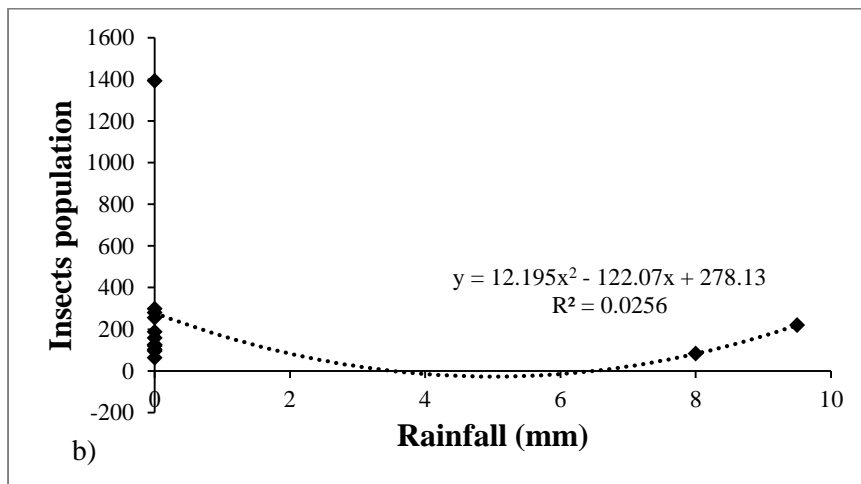
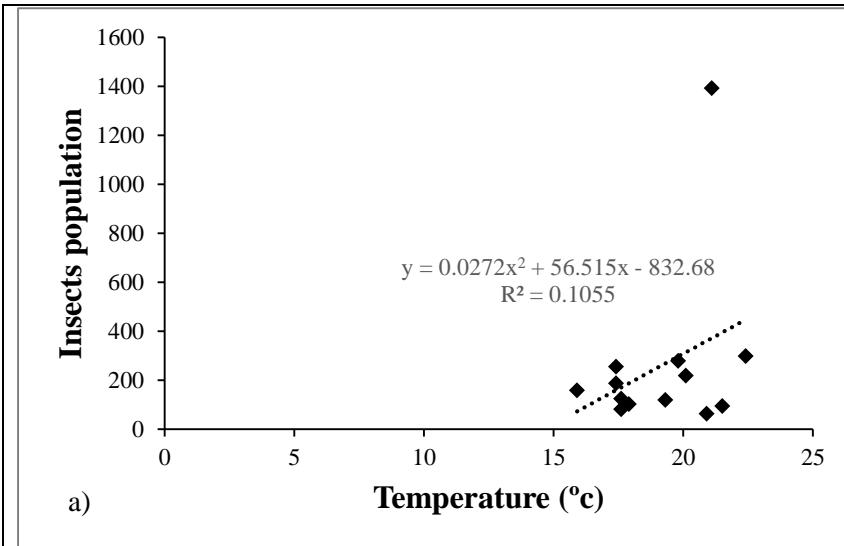


Figure 19: Relationship between a) temperature, b) rainfall and c) relative humidity and total insects' population during winter season.

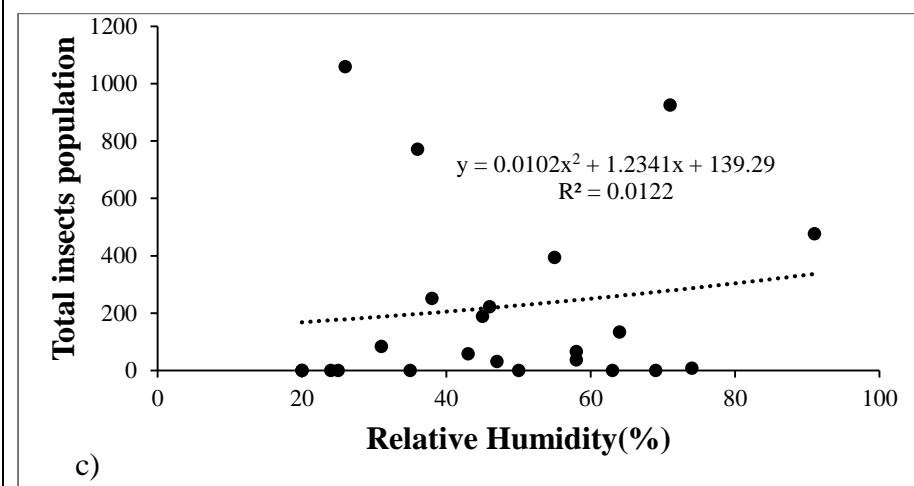
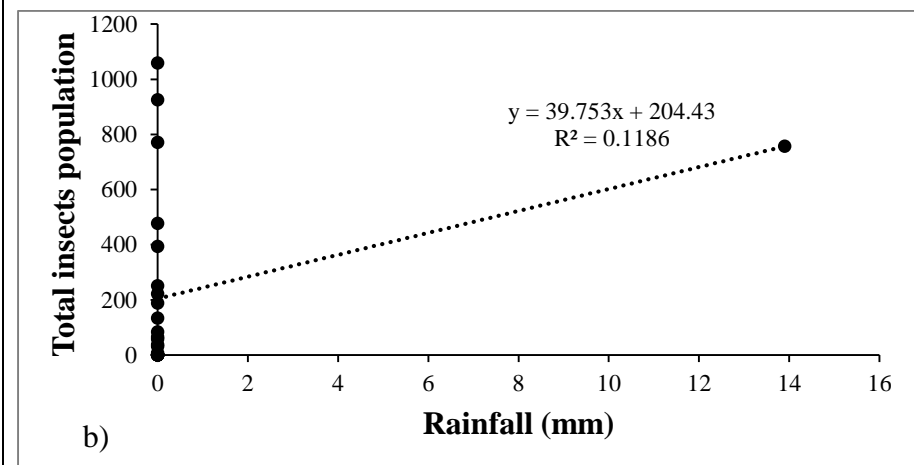
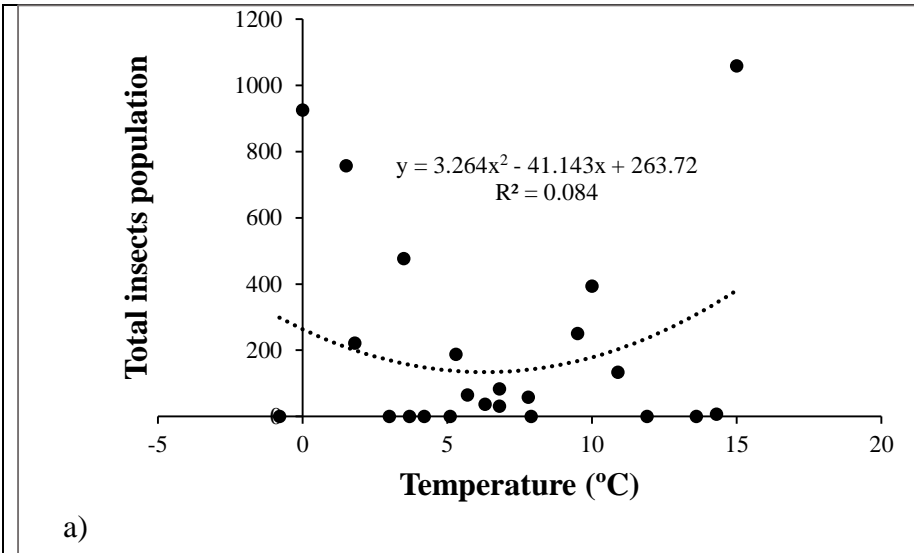


Figure 20: Relationship between a) temperature, b) rainfall and c) relative humidity and total insects' population during winter season.

Table 15: Correlation coefficient (r) of potential pests of safflower with relative humidity, temperature and rainfall

| <i>Insect species</i> | <i>Season</i> | <i>Correlation coefficient (r)</i> | | |
|----------------------------------|---------------|------------------------------------|--------------------------|-------------------------------|
| | | Temperature (°C) | Rainfall (mm) | Relative Humidity (RH) |
| <i>T.tabaci</i> | Summer | 0.34 | -0.11 | -0.09 |
| | Winter | 0.19 | 0.37 | 0.13 |
| <i>A.biguttula biguttula</i> | Summer | 0.08 | 0.06 | -0.21 |
| | Winter | -0.10 | 0.00 | -0.04 |
| <i>C.panaethiopica</i> | Summer | 0.31 | -0.16 | 0.17 |
| | Winter | - | - | - |
| <i>H.armigera</i> | Summer | -0.10 | -0.10 | -0.13 |
| | Winter | -0.12 | -0.08 | -0.06 |
| <i>Aphididae sp.</i> | Summer | - | - | - |
| | Winter | -0.19 | -0.07 | 0.07 |

Table 15 presented correlation coefficients between temperature, rainfall, relative humidity and population of different potential pests of safflower recorded. During summer, there was a non-significant ($P > 0.05$) positive correlation between temperature and population of *Thrips tabaci* ($r=0.34$). Temperature contributed an insignificant positive impact on abundance of *Thrips tabaci* during summer. However, non-significant ($P > 0.05$) negative correlations were recorded between temperature and species population of *Helicoverpa armigera* ($r= -0.10$), *Calidea panaethiopica* ($r=-0.31$) and *Amarasca biguttula biguttula* ($r=-0.08$), indicating a decrease in populations of these pests with an increase in temperature. Table 15 revealed that abundance of *Thrips tabaci* ($r=-0.11$), *Calidea panaethiopica* ($r=-0.16$) and *Helicoverpa armigera* ($r=-0.10$) had non-significant negative correlation with rainfall, whereas population of *Amrasca*

biguttula biguttula ($r=0.06$) had a non- significant positive correlation with rainfall. Between relative humidity and species population, *Thrips tabaci* ($r=-0.09$), *Amrasca biguttula biguttula* (-0.21) and *Helicoverpa armigera* ($r=-0.13$) decreased as relative humidity increased, except for population of *Calidea panethiopica* which increased non- significantly ($P > 0.05$) with an increase in relative humidity. During winter, correlation of *Thrips tabaci* population was non- significant and positive with temperature ($r= 0.19$), rainfall (0.37) and relative humidity ($r=0.13$). It is evident that the temperature had a non- significant and negative effect on population of *Amrasca biguttula biguttula* ($r=-0.10$), *Helicoverpa armigera* ($r=-0.12$) and Aphididae species ($r=-0.19$). Non- significant and negative effect with relative rainfall was noticed on population of *Helicoverpa armigera* ($r= -0.08$) and Aphididae species ($r= -0.07$). Table 15 showed that there was no relationship between insect species of *Amrasca biguttula biguttula* ($r=0.00$) and rainfall during winter. *Helicoverpa armigera* ($r=-0.06$) and *Amrasca biguttula biguttula* ($r=-0.04$) population showed a non- significant and negative correlation with relative humidity, whereas a positive and non- significant relationship was noticed between Aphididae species ($r=0.07$) and relative humidity.

CHAPTER 5

5.0 DISCUSSION

Variations in the abundance and diversity of a species is an adaptive phenomenon evolved to take advantage from the environmental conditions. The temporal fluctuations in the abundance are an important manifestation of populations' response to the environmental conditions. These fluctuations are more showing in populations of lower organisms such as insects (Dar et al., 2021). Insects are generally more sensitive to environmental changes and its life stages are adaptively timed according to the environmental conditions (Robinet and Roques, 2010). Biodiversity has high significance in various ecosystem services, which show the status of the area and can determine various factors (Xin *et al.*, 2012; Moghimian and Kooch, 2013). However, alterations of land use, environment disintegration, supplement change, and stress can affect the movement of energy and cycling of supplements (Wilsey and Potvin 2000). Nahmani *et al.* (2005) reported that determination of diversity, richness, Evenness, and abundance of fauna are required for ecological studies, habitat management, and conservation programs in any ecosystem.

5.1 Species abundance and diversity

The diversity of insects associated with safflower was found to belong to 14 families and eight orders comprising of Hemiptera (five species), Coleoptera (three species), Hymenoptera (two species), Orthoptera (one species), Diptera (one species), Lepidoptera (one species), Thysanoptera (one species) and Araneae (one species). The highest number of Hemiptera species was attributed to the morphological characteristics of safflower plant. Safflower is a seed forming plant with a lot of leaves and colourful flowers. These characteristics made the plant to be more susceptible to sap sucking pests and those that feed on seeds. Most of the hemipterans recorded in this study were found on the vegetative and floral parts of safflower plants and this could possibly imply feeding. This is supported by findings of Panizzi (2000) who reported that among different part of plants, leaves, seeds and developing fruits are the

main feeding sites of Hemiptera insect species. Hemipterans are mostly described by their stylet, which they insert into plant tissues, injecting a watery saliva that contains digestive enzymes and sucking out the sap. Esfahani et al. (2012) and Kaffka & Kearney (1998) reported similar Hemipteran insect species in Iran and California, respectively, feeding on safflower. These insects fed on the aerial organs of safflower plants, specifically damaging the surface of the flower head. Some pests such as Lygaeidae bugs, aphids and thrips cause latex to leave the flower head that result in seeds turning dark and spoiled (Esfahani et al., 2012). The major insect pests of safflower causing economic losses in Iran are in the order Hemiptera include *Uroleucon carthami* (safflower aphid), *Empoasca decipiens* (green leafhopper), *Oxycarenus palens* (cotton seed bug), *Oxycarenus hyalipennis* (cotton seed bug), and *Lygus* sp (Esfahani et al., 2012; Sabzalian et al., 2010). Saeidi et al. (2015) reported a total number of 4261 insect specimens, under 31 families and 92 species, on safflower in Iran. Out of the 31 families collected, 10 families were pests, 2 families belonged to predators and beneficial insects, and 7 families belonged to parasitoids and beneficial insects and finally, 12 families of these insects were saprophage and polyphagous (Saeidi et al., 2015). Javed et al. (2013) reported population of different insect pests including safflower aphid, jassid, bugs and pod borer on five different varieties of safflower at different stages of growth of the plants.

5.2 Species abundance and diversity among Safflower genotypes and plant growth stages
Plant genotype play an important role in shaping associated insects populations and communities. It is known to affect community composition, ecosystem function and herbivore performance through differences in nutrient concentration and defensive compounds (Moran et al., 2013). Plant characters such as architecture, trichome density, leaf toughness, and nutrient and secondary chemical content, have been shown to affect insect feeding preference, performance characters such as growth, survival, and fecundity (Zangerl 1990). These findings reveal that plant traits can strongly affect the success of individual insects and also characters

that can influence population growth rates. However, from the results of the current study, there is experimental evidence that differences among host-plant (safflower) genotypes do not have the capacity to contribute to differences in insect species incidence and composition. It is thus clear that the species had no preference of safflower genotype. However, none of the genotypes was found free from the attack by arthropods.

The study pertaining to incidence of insect species on safflower genotypes indicated that none of the genotypes was found free from the attack by arthropods. The insect species had no preference of safflower genotype(s). In terms of the seasonal comparisons of these insect species, it was revealed that there was high significant differences among the population levels of insect species between seasons. Highest population of insects was recorded in winter than in summer. This may be attributed to the extended period the plants took to reach physiological maturity. During winter, safflower took 23 weeks to reach physiological maturity while in summer it took 13 weeks to mature. In addition, the safflower plants in the winter were vigorously growing and taller than in the summer. Prolonged growth period to maturity exposed safflower plants to insects for a longer period, hence more individual species were recorded in winter. Moatshe et al. (2020) reported that safflower takes 99-116 and 135-147 days after emergence to reach physiological maturity in summer and winter, respectively. Similar results of longer growth period of safflower in winter than summer, or under cooler growing conditions depending on genotype is reported in literature (Emongor et al., 2015; Moatshe et al., 2020). Dhopte (2017) showed that short photoperiod prolonged safflower growth stages and tolerance to low temperature depended on variety and stage of development. Javed et al. (2013) reported that the insurgence of insect population on safflower is significantly influenced by genotype and phenological stages of safflower.

In all the studied genotypes, insect population significantly differed over the phenological stages of safflower regardless of the season. The insect population increased from rosette to

flowering stage, reaching peak at flowering, thereafter the population decreased until harvest. The present findings show that the peak population of insects occurred at the same phenological stage regardless of genotype implying that safflower genotypes can not be employed as a biological measure to control pests on safflower. In general, as plant ages, its physiological characteristics changes, passing through different phenological and developmental stages of growth (Groover, 2017). The increase in insect population on safflower from rosette to flowering stage may be due to an increase in number of leaves and leaf area. According to Moatshe et al. (2020), leaf area index (LAI) of safflower increase with increase in days after sowing, reaching a maximum at 50% flowering. Thereafter, LAI decrease irrespective of genotype, plant density or season (Moatshe et al., 2020). Similar observations were earlier reported by Ul et al. (2014) who found that LAI increase from the earlier stages of safflower growth due to continuous increase in leaf number and expansion of leaf area reaching a peak at 126 days after planting. Thereafter, LAI decrease attributed to progressive leaves senescence of lower due to shading of older leaves (Ul et al., 2014).

5.4 Plant parts affected

Several injurious insect pests were reported to cause severe and economical losses on various parts of safflower plants (Esfahani et al., 2012). In the current study, it was observed that all aerial parts of safflower plant: including leaves, flower head and stem, were attacked by insect pests. Among the ten (10) pest species that attacked safflower, eight (8) of the species had piercing and sucking mouth parts, one (1) had borer and chewing mouth parts and one (1) had biting and chewing mouth parts. Species that fed on the inside of the flower head (capitula) included those that fed on flowers and seeds. While those that fed on the outside of flower head specifically damaged the surface of the flower head. Esfahani et al., (2012) reported that insect pests that fed on the outside of the safflower flower head produced latex which made the flower head and internal seeds dark, with damaged flower heads leaning sideways. Earlier, Saeidi et

al. (2011) reported similar results when they found that insect pests consumed all aerial parts of safflower including leaves, shoot tips and capitula in Iran.

5.3 Potential insect species of safflower and their effect on safflower seed yield

Safflower production was reported to be under threat from a variety of insects pests which cause low seed yield (Saeidi et al., 2011; Javed et al., 2013). In India, safflower has been reported to be damage by 36 species of pests and out of these the safflower aphid, *Uroleucon compositae* (Theobald), capsule borer (*Helicoverpa armigera*) and leaf eating caterpillar (*Perigea capensis* Walker) were regarded as major pests of safflower (Bharaj et al., 2003). The most serious crop damage by insects usually occurs because of infestations either at the time of germination or flowering, when young seedlings or developing capitula are the targets of attack (Esfahani et al., 2012; Vaani et al., 2016). In the current study, classification of insects into 'major' and 'minor' pests was based on observations on the incidence, abundance and the degree of importance of the damage caused by these pests in the field. Analysis on abundance and damage of insect species on safflower revealed five potential insect species of safflower in Botswana: *Thrips tabaci*, *Amrasca biguttula biguttula*, *Calidea panaethiopica*, *Helicoverpa armigera* and Aphididae species. Numerically, *Thrips tabaci*, *Amrasca biguttula biguttula* and *Calidea panaethiopica* were considered the most abundant insects of safflower. *Helicoverpa armigera* and Aphididae species were considered major pests of safflower because of their overwhelming effect on plant damage when compared to other insect species. Even though *Thrips tabaci*, *Amrasca biguttula biguttula* and *Calidea panaethiopica* were relatively abundant, the degree of damage caused by these pests was not prominent hence they were considered minor pests. Similar results have been reported by Saeidi and Adam (2011) that the damage inflicted by *Amrasca biguttula biguttula* in safflower is minor and observed as chlorotic spots on the leaves.

To reach an economic crop yield it is often necessary to tolerate some level of damage caused by the insect pest. Assessment of impact of potential insect pests on safflower seed yield was based on the relationship between yields and population of a given pest. Correlation analysis between seed yield and population of potential pests identified in the current study revealed weak and very weak associations of these pests and safflower seed yield. This suggest that high population and damage inflicted by potential safflower pests did not lead to any significant safflower seed yield losses. Previous studies by Carison (1964) and Elfadl et al.(2009) reported that safflower plants have compensative ability. According to Carison (1964), safflower tolerated head damage. In his study, it was found that safflower seed yield of plants with some of the heads damaged by pests during the onset of flowering were not affected and this was attributed to significant increase in seeds produced by the remaining seed heads (capitula). Elfadl et al. (2009) revealed that safflower uses residual soil nitrogen efficiently to compensate for low plant density.

5.3.1 *Thrips tabaci*

Thrips tabaci was found to be abundant in summer and winter season. The abundance of *T. tabaci* was associated to its polyphagus nature and its high reproductive rate. Sathe and Mithari (2015) noted a similar observation that *T. tabaci* is a polyphagus pest. The greater abundance of *T. tabaci* at flowering stage can also be attributed to the structure of the flower head, which may have offered a suitable place for the pest to hide from predators and harsh conditions leading to reduction in its mortality. As a result, colonization rates and population size of *Thrips tabaci* was expected to be larger during flowering than in other growth stages. According to Diaz- Montano *et al.* (2011) *T. tabaci* has frequency of producing more generations with short generation time, high survival of cryptic (nonfeeding prepupa and pupa) instars and ability to reproduce without mating (parthenogenesis).

T.tabaci was feeding on almost every part of safflower plant; under the bracts of the buds, leaves and in the flower head. The population of this pest had negative and insignificant association with safflower seed yield in both seasons. This suggested that as population of *T. tabaci* increased there was an insignificant decrease in the yield of safflower seeds. The results also revealed that seasons did not have an effect in the impact *T.tabaci* has on safflower seed yield. Obopile *et al.*(2008) reported *T. tabaci* to be one of the major pests of onion in Botswana.

5.3.2 *Amrasca biguttula biguttula*

Amrasca biguttula biguttula was present in summer and winter as one of the most abundant insect species of safflower. There was a similar trend of population fluctuations between different safflower genotypes indicating that the pest did not have preference of safflower genotype. *A. biguttula biguttula* fed on safflower by piercing and sucking sap from the leaves and flower head. The pest has been reported to be amongst the most important sucking insects of okra, cotton, cowpeas, pigeon pea and sunflower plants (Rajpup *et al.*, 2015; Srivastava *et al.*, 2019). The nymphs and adults of *A. biguttula biguttula* introduce salivary toxins during feeding and the affected leaves curl downwards; turn yellowish then brownish before drying and shedding (Chandani and Tukaram, 2015). Kamble *et al.*(2014) found that *A. biguttula biguttula* caused vertical destructive pattern on sunflower, in which the upper leaves of the sunflower plants were more susceptible to *A. biguttula biguttula* nymphs attack than lower leaves.

Correlation test has revealed a non-significant positive association between the population of *A. biguttula biguttula* and safflower seed yield. This explains that an increase in population of *A.biguttulla biguttula* does not imposed any effect in the yield of safflower seed yield, yield still increased even with an increase in the population of the mentioned pest. According to Ingale *et al.* (2019), *Amrasca biguttula biguttula* caused crop loss of up to 46 percent on sunflower. In cotton, yield losses due to *A.biguttula biguttula* could be as high as 100-114 kg

of fiber per hectare and in sunflower attack combined with whiteflies may result in losses of 9.2% (Dhawan & Peshin, 2009).

5.3.3 *Calidea panaethiopica*

Abundance of *Calidea panaethiopica* was statistically similar among safflower genotypes, implying that the pest does not have preference of genotype. Maximum population of *C.panaethiopica* was recorded at maturity stage when safflower heads were completely filled and dry. This indicated that the pest preferred seeds of safflower more than other parts of the plant. This observation complemented the findings of Djimmy and Nacro (2015) who discovered that larvae and adults of *C.panaethiopica* fed on flowers, fruits and seeds of *Jatropha curcas L.* According to Djimmy et al. (2016) *C.panaethiopica* is a polyphagous insect of many host plants containing toxic compounds such as *Ricinus communis*, *J.podagrica* and *Gossypium* species. While *C.panaethiopica* has not been reported on safflower before, the present study provides a first record of *C.panaethiopica* on safflower in Botswana. This may be due to climatic conditions, genotype, or unavailability of alternative crops. Niesel (2010) confirmed that cotton in Tanzania, sorghum and sunflower in South Africa are host plants for *Calidea dregii*, which is from the same family as *Calidea panaethiopica*.

A weak and positive association was noted between the population of *C.panaethiopica* and safflower seed yield. This exposes that an increase in the population of *C.panaethiopica* does not affect safflower seed yield hence an increase in seed yield even when population of *C.panaethiopica* increased. Yield loss of up to 59% on *J.curcas L.* seeds due *C.panaethiopica* was recorded in South Sudanian Zone of Burkinafaso (Djimmy & Nacro, 2015).

5.3.4 *Helicoverpa armigera*

Population of *Helicoverpa armigera* was noted in summer and winter grown safflower. The population of *Helicoverpa armigera* on different safflower genotypes was statistically similar but significant variation was observed on growth stages. The results of the current study are in

partial agreement with findings of Javed *et al.* (2013) who reported significant difference in the population of *H. armigera* on different varieties of safflower and dates of observations. The larvae of *H. armigera* were biting and chewing every part of safflower plant including leaves, bracts, flowers and developing head except stem. In both seasons, the highest population of *H. armigera* was observed in genotype Turkey. This was attributed to the physical structure of the genotype. Compared to other genotypes, Turkey is spineless. According to Kariyat *et al.* (2017), spines can act as an effective defence against caterpillar feeding by restricting its movement and increasing the time taken to access feeding sites, with possible consequences including longer developmental periods and increased exposure to predators. In summer, *H. armigera* was abundant at branching stage while in winter the pest was abundant at rosette stage. This was attributed to the prolonged rosette stage of safflower plants in winter (40 days) than summer (21 days) (Emongor *et al.*, 2015).

Correlation between the population of larvae of *H. armigera* and safflower seed yield was non-significantly but positive in summer and winter seasons. This showed that *H. armigera* does not have any noticeable effect on safflower seed yield despite variations in seasons. This resulted in an increase in seed yield with an increase in *H. armigera* population. Obopile & Mosinkie (2007) and Munthali *et al.* (2004), confirmed the presence of *Helicoverpa armigera* in cultivated habitats of Botswana. According to Obopile & Mosinkie (2007), *H. armigera* was estimated to cause yield losses of 15 to 30% on sorghum in Botswana.

5.3.5 Aphididae species

Aphididae species were available in winter season but absent during summer. The population of Aphididae species did not differ significantly among genotypes but differed significantly with growth stages. This finding expresses that genotype cannot be used as a biological control of Aphididae species on safflower. The highest infestation was recorded at the rosette stage, and this was attributed to the tender parts of safflower plant parts at this stage, which included

leaves, stems, and flower heads. The nymphs and adults of Aphididae species were sucking sap from the tender parts of safflower plant. Contrary to what was expected, Aphididae species had a very weak and positive association with safflower seed yield. This implied that as population of Aphididae species increased it did not affect safflower seed yield, but seed yield increased.

Barton & Ives (2014) reported that insect species live within communities of other interacting species, as a result an external pressure that directly affects one species can indirectly affect all other members of the community. In the present study, it was found that population of *Exochomus flavipes* and *Formicidae* were more associated with Aphididae species. According to Raymond et al.(2000), the presence of aphids and their wastes was necessary to attract coccinellids. Aphids also attracted a greater population of ants hence increase in its population in winter season. Recently. A study by Duque et al. (2021), indicated a mutualist relationship between ants and aphids. Aphids produced honeydew as they concentrate nutrients in the sap which they ingest during feeding (Munthali & Tshogofatso, 2014). As ants fed on the honeydew produced by aphids, the ants provided protection against predators. The positive effect of ants resulted in the reproductive effectiveness of aphid colonies.

5.5 Diversity of safflower insects

Diversity indices indicates that all insect orders collected during this study had low level of species richness. From the results, the highest margalef's richness index score was recorded on Coleoptera as 0.59 and 0.69, for summer and winter, respectively. This agreed with Margalef (1958) who indicated that species richness index can be computed using which categories richness level as $R < 2.5 = \text{low}$, $2.5 > R > 4 = \text{medium}$ and $R > 4 = \text{high}$. Diversity for all the studied safflower genotypes is the same, in both summer and winter season. Shannon diversity index values for genotypes ranged from 1.32 to 1.47 in summer and 0.72 to 0.95 in winter. This implied that insects did not have preference over safflower genotypes and all genotypes were

attacked the same. Sorensen similarity coefficient was also used to compare the similarity between the genotypes. The results also confirmed that, there was no significant difference in distribution of species composition between safflower genotypes in both seasons as the similarity index between genotypes was from 96% to 100% in summer and from 81.71% to 100% in winter.

The number of individual insects caught in summer and winter were more at flowering stage. On observations, at flowering stage safflower has entered a reproductive phase and is a source of food for insects which appear to act as pests and pollinators. The highest number of individuals at flowering stage may also be due to the insects having multiplied and grown as time has run and increased population. According to Moore (2013), diversity depends on two factors, species richness and evenness. Even though flowering stage occupied a greater share of population in summer and winter seasons, species evenness for this stage was low in both seasons, with summer recording 0.29 and winter recording 0.20. This was consistent with the findings of Cho et al (1988) who explained evenness index criteria as: $E < 0.4 = \text{low}$, $0.4 < E < 0.6 = \text{medium}$, $E > 0.6 = \text{high}$. This indicated that distribution pattern of insects species at flowering stage was not balanced, one or a few species were dominant. Low levels of species richness at maturity stage occurs because safflower as a food source of these insects has reached a stage of hardening seeds and physiologically mature, so the food source of some of the insects were not suitable as before.

In the present study, all the diversity indices for insects of safflower were more in summer grown safflower than winter. Even though a greater number of insects were collected during winter, the value of diversity, Shannon-wiener index, was maximum in summer and minimum in winter which suggested that in summer the diversities of insects varied more, and the season was good for growth of insects compared to winter. In both seasons the diversity levels of insects were medium (diversity criteria). Similarly, summer showed high species richness and

species evenness compared to winter. This implied that safflower insect complex was evenly distributed amongst different insects' species in summer than in winter. Margalef's richness index confirmed the findings of the current study which showed high insect species richness in summer than winter. It was observed that vegetation of the two seasons varied in their richness as more rains were received in summer than in winter. Therefore, the difference in diversity of insect species between the two seasons could be explained by differences in availability of food resources and shelter, hence summer having a greater diversity compared to winter. Haddad et al.(2009) demonstrated that strong arthropod richness was positively related to plant species richness. However, Saeidi et al. (2015) reported no significant difference in the number of insects collected between warm and cool months in safflower, and this does not corroborate with the findings of the present study.

5.6 Weather parameters and species population

Weather parameters are known to have profound influence on the occurrence, growth, development, and population build-up of insect pests in crop ecosystem, and ultimately on the extent of damage to the crop and yield loss thereof (Kalita et al., 2015). The overall correlation analysis of the present study on weather parameters and insect species population showed non-significant association between insect species population with temperature, relative humidity, and rainfall. Explicitly, temperature, relative humidity and rainfall did not play any precise function in the multiplication or reduction of safflower insect species. The findings of the current study are not in agreement with those reported by Namni et al. (2017). Namni et al. (2017) observed increased significant abundance of the insect population with increase in temperature, relative humidity, and rainfall. Low relative humidity and temperature have been reported to delay development of eggs and larvae, reduced egg hatching and larval survival as compared to high relative humidity and temperature (Guarneri et al., 2003; Han et al., 2008; Norhisham et al., 2013). Rahman & Khan (2012) reported significant positive correlations of

temperature ($r = 0.58$), relative humidity ($r = 0.61$), and rainfall ($r = 0.79$) with incidence of pests in jute mallow (*Corchorus olitorius* L). While Singh et al. (2013) reported weak negative correlation of aphid population in okra with minimum and mean temperature, rainfall, and maximum and minimum relative humidity. Whereas positive correlations of aphid population with maximum temperature and coccinellids (Singh et al., 2013).

The analysis disclosed that temperature played an insignificant but positive role in population of *Thrips tabaci* in both summer and winter season in the present study. The finding of the present study was in agreement with the findings of Panwar (2015) who reported positive correlation of temperature with *Thrips tabaci* population in Bt- and non-Bt cotton. Further, it was depicted that in summer, the correlations between population of *Thrips tabaci* with rainfall and relative humidity were non-significantly negative in safflower, but in winter the correlations between the population of *Thrips tabaci* and humidity and rainfall were non-significantly but positive. Janu et al. (2017) found that all the weather parameters were non-significantly correlated with the *Thrips tabaci* population in *Gossypium hirsutum*. In a study performed by Khan et al. (2011), temperature displayed a negative and significant correlation with thrips and positive significant correlation with relative humidity in *Vigna radiata*. An analysis of correlation of *Helicoverpa armigera* population with temperature, relative humidity and rainfall exhibited no-significant negative association in both summer and winter season in safflower. These findings of the present study are in partial agreement with those of Galav et al (2018) who reported a significant positive association with temperature, but negative association with relative humidity and non-significant negative association with rainfall in chick pea. While Singh et al. (2015) reported a significant positive role of temperature on the larval population of the *Helicoverpa armigera* in Chickpea.

The results of the current study revealed that *Amrasca biguttula biguttula* population had a non-significant but negative correlations with temperature and relative humidity, but a non-

significant positive correlation with rainfall. The findings of the current study disagree with the findings of Jayasimha et al.(2012), who reported that *Amrasca biguttula biguttula* population had a significant positive and negative correlations with temperature and rainfall, respectively, in okra. In another study, Rajpup et al. (2015) reported a positive correlation between *Amrasca* population with temperature and relative humidity on sunflower. Sandhi & Sadhu (2018) also reported that *Amrasca biguttula biguttula* population had a significant negative correlation with the maximum temperature, but a non-significant positive correlation with the minimum temperature in okra. However, they further reported a non-significant and negative correlation between *Amrasca biguttula biguttula* population and rainfall, but a significant positive correlation with relative humidity (Sandhi & Sadhu, 2018). Also a significant negative correlation between population of *Calidea panaethiopica* with temperature and rainfall were recorded in the current study, however a non-significant positive relationship was found with relative humidity.

CHAPTER 6

6.1 CONCLUSION

The present study has shown that safflower has low insect biodiversity by providing the checklist and the diversity of insects' species. It is documented for the very first time the insect fauna of safflower in Botswana. Insect species of safflower belonged to 15 species and 14 families under 8 taxonomic orders. The study showed that, 87.5% of all the orders observed comprised of insects with the remaining part comprising of Arachnida (Spiders). The pest, predator and pollinator insect revealed the highest occurrence in order Hemiptera, Coleoptera and Hymenoptera, respectively. Based on population density of different insects, *Thrips tabaci*, *Amrasca biguttula biguttula* and *Calidea panaethiopica* were the most abundant pests of safflower. It was shown that *Helicoverpa armigera* and Aphididae species were the predominant serious pests of safflower crop, having detrimental effect on vegetative growth stages of the plant.

Safflower genotypes did not differ significantly in abundance and diversity of insect species, but the severity differed with phenological stages of safflower. The flowering and maturity stages had the highest and lowest number of insect species, respectively. While winter supported a relatively high abundance of insects compared to summer. The values of diversity indices revealed that summer and winter values did not significantly differ in diversity and species richness.

Piercing and sucking insect species were the most abundant and all the upper parts of safflower plants were susceptible to infestation by safflower pests. The pests had no significant effect on seed yield because of the compensation ability of safflower plant. Correlation studies showed that the population build-up of insect species was not influenced by weather parameters.

6.2 RECOMMENDATIONS

Based on the results of the current study, the author recommend farmers to plant Kenya-9819 during summer and Turkey during winter as they recorded the lowest population of insects per plant in the respective seasons. Summer has been found to be the best season for safflower plantation since plants take a shorter period to reach maturity, which is a best pest management strategy by reducing the exposure of the plants to pests. Farmers should also intense monitoring and pests control measures at flowering stage as it was found to be the most insect populated growth stage in both summer and winter season.

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APPENDIX

Appendix 1: Weather parameter readings for the first trial (summer)

| Weeks after crop emergence | Weather parameters | | |
|----------------------------|--------------------|---------------|-------------------------|
| | Temp. (°C) | Rainfall (mm) | Relative Humidity (R/H) |
| 1 | 21.5 | 0 | 25 |
| 2 | 17.6 | 8 | 73 |
| 3 | 17.9 | 0 | 74 |
| 4 | 15.9 | 0 | 35 |
| 5 | 19.3 | 0 | 70 |
| 6 | 19.8 | 0 | 85 |
| 7 | 17.4 | 0 | 74 |
| 8 | 20.1 | 9.5 | 72 |
| 9 | 22.4 | 0 | 72 |
| 10 | 21.1 | 0 | 57 |
| 11 | 20.9 | 0 | 78 |
| 12 | 17.4 | 0 | 72 |
| 13 | 17.6 | 0 | 68 |

Appendix 2: Weather parameter readings for the second trial (winter)

| Weeks after crop emergence | Weather parameters | | |
|----------------------------|--------------------|---------------|-------------------------|
| | Temp. (°C) | Rainfall (mm) | Relative Humidity (R/H) |
| 1 | 6.8 | 0 | 47 |
| 2 | 7.8 | 0 | 43 |
| 3 | 6.3 | 0 | 58 |
| 4 | 6.8 | 0 | 31 |
| 5 | 5.7 | 0 | 58 |
| 6 | 5.3 | 0 | 45 |
| 7 | 9.5 | 0 | 38 |
| 8 | 3.7 | 0 | 69 |
| 9 | 4.2 | 0 | 24 |
| 10 | 7.9 | 0 | 20 |
| 11 | 3 | 0 | 35 |
| 12 | 1.8 | 0 | 46 |
| 13 | 3.5 | 0 | 91 |
| 14 | 0 | 0 | 71 |
| 15 | -0.8 | 0 | 50 |
| 16 | 5.1 | 0 | 25 |
| 17 | 11.9 | 0 | 20 |
| 18 | 5.1 | 0 | 36 |
| 19 | 15 | 0 | 26 |
| 20 | 17.5 | 13.9 | 51 |
| 21 | 10 | 0 | 55 |
| 22 | 10.9 | 0 | 64 |
| 23 | 14.3 | 0 | 74 |
| 24 | 13.6 | 0 | 63 |