BOTSWANA UNIVERSITY OF AGRICULTURE AND NATURAL RESOURCES

INFLUENCE OF HARVEST TIME AND GENOTYPE ON SEED YIELD, PETAL YIELD AND CARTHAMIDIN AND CARTHAMIN CONTENTS, AND MINERAL NUTRITIONAL CONTENT IN SAFFLOWER (*Carthamus tinctorius* L.)

A dissertation submitted in partial fulfilment of the requirements of the award of MSc Crop Science (Horticulture Stream).

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APPROVAL

This research has been examined and is deemed to have met the necessary criteria of scholarship for partial fulfilment for the degree of MSc Crop Science (Horticulture Stream).

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STATEMENT OF ORIGINALITY

This attests that the author finished the work for this dissertation between the academic years of 2022 and 2023 at the Botswana University of Agriculture and Natural Resources. Except where appropriate acknowledgment and reference have been made in the text, it does not to the greatest extent of my comprehension, consist of any previous publications or materials by other writers or materials that have been recognised as equivalent to degrees, certificates, or diplomas issued by other universities.

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DEDICATION

This dissertation is dedicated to my mother Bonang Rillah Setshogela and grandmother Tlhalefang Setshogela, I appreciate their strenuous support and prayers. To all the people who have supported me throughout my educational endeavours, thanks for making me see through to the end.

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ABSTRACT

Safflower (*Carthamus tinctorius* L.) is a versatile oil crop with drought, salinity, and severe temperature tolerance. In 2023, the Botswana government incorporated safflower into the cropping system, being among the 13 crops promoted for food security. The objective of this study was to determine the best genotype and time to harvest safflower petals for optimum petal and seed yield, and carthamidin and carthamin contents. The results indicated that phenological traits [days to emergence (8-12), days to flowering (74-117), days to end of flowering (84-126), and days to physiological maturity (99-157)], vegetative growth [height to first branching (13-34 cm), plant height (67-118cm), and number of primary branches/plant (6-12)], yield components [capitula diameter (10-19cm), number of capitula/plant (11-25), number of seeds/capitulum (13-25), and 1000-seed weight (28-39g), seed yield (1063-2697kg/ha), petal yield (91-117kg/ha)]. The carthamidin content (1-7.5%), carthamin content (0.02-0.05%). The mineral nutritional content [Ca (424-517mg/100g), Mg (273-279mg/100g), K (2214-2328mg/100g), Na (224-228mg/100g), Fe (12-17mg/100g), Zn (2-3mg/100g)]. Proximate variables [moisture content (75-80%), crude fibre (3-5%), crude protein (1-3%), fat content (3-3.4%), ash (5-9%) and carbohydrates (4-10%)]. The results showed that phenological traits (days to emergence, days to flowering, days to end of flowering, and days to physiological maturity), vegetative growth (height to first branching, plant height, and number of primary branches/plant), yield components (capitula diameter, number of capitula/plant, number of seeds/capitulum, and 1000-seed weight), and seed yield had significant genotypic variation. In both summer and winter, safflower genotypes and petal harvest time interacted significantly to influence petal yield, carthamidin and carthamin contents, and dye index. Genotypes and time of petal harvest independently had significant variation concerning safflower petal mineral nutritional content (Ca, Mg, K, Na, Fe, and Zn) and proximate variables

(moisture content, crude fibre, crude protein, fat, ash, and carbohydrate). It was concluded that the best genotype to maximise safflower seed and petal yield with high carthamidin and carthamin contents, dye index, mineral nutritional content and proximate variables was Turkey (spineless). It was also concluded that the best time to harvest safflower petals to maximise seed and petal yield with high carthamidin content which has many health benefits was either at the onset of flowering or full bloom. However, the best time to harvest safflower petals to maximise mineral nutritional content and proximate variables was variable and inconclusive.

CHAPTER 1

INTRODUCTION

1.1 Background information

The current agricultural production levels are insufficient to sustain the estimated population of over 9 billion by 2050 and thus pose challenges to food security (Alexandratos & Bruinsma, 2012). Other concerning problems such as climate change, water scarcity, and salinity have become a hindrance affecting the growth and production of crop commodities (Mayes et al., 2012; Nanduri & Shahid, 2016). Adapting agricultural production techniques and managing agricultural productivity under unfavorable growing conditions are necessary for maintaining food security in the 21st century (Jahan & Qale Nawi, 2022; Mayes et al., 2012). Moreover, consumers are conscious of healthy diets and demand natural products with therapeutic benefits (Turgumbayeva et al., 2018; Mohammadi & Tavakoli, 2015). Producers are obliged to employ cheaper substitutes for natural substances due to economic reasons (Adamska & Biernacka, 2021). For this reason, a crop such as safflower which is multipurpose, with broad genetic diversity and can grow in varied climates meets the demand of natural phyto-pharmaceuticals (Emongor, 2010; Zanetti et al., 2022; Emongor & Emongor, 2022).

Safflower (*Carthamus tinctorius* L.) is in Asteraceae or Compositae family, it is an annual multipurpose oilseed crop. It originated in Asia (Northern India, China) and the Mediterranean region, where it is commonly cultivated in arid and semi-arid climates (La Bella et al., 2019; Costantini et al., 2019). Its extensive taproot, which can develop as long as three meters deep

(Weiss, 2000; McPherson et al., 2004; Bhattarai et al., 2020; Dordas& Sioulas,2009; Khalili et al., 2014; Divya et al .,2022). Moreover, it is capable of withstanding abiotic stressors such as water stress and salinity (Bassil & Kaffka, 2002; Hosseini et al.,2010; Khalili et al., 2014; Hojati et al., 2011; Emongor et a*l*., 2015; Sirel & Aytac, 2016; Emongor & Emongor, 2022).

The crop safflower has an array of uses including leafy vegetable (Singh & Nimbkar, 2006; Moatshe et al., 2020a), medicinal and herbal (Emongor, 2010; Deetae et al.,2012 Emongor & Oagile, 2017; Delshad et al., 2018; Emongor & Emongor, 2022), edible oil (Kostik et al.,2013: Mailer et al., 2008; Kizil et al., 2008; Emongor & Emongor, 2022), animal feed (Singh & Nimbkar, 2006; Emongor & Oagile, 2017), biofuel production (Yesilyurt et al., 2020; Ilkılıç et al., 2011) cosmetics and dyes (Azami et al.,2019) and in floriculture as cut flowers (Uher, 2008; Emongor et al., 2015). The key advantage of safflower is its usage as vegetable oil, which has been deemed a healthier substitution for sunflower seed oil on account of the fatty acids in it. The oil mainly comprises of linoleic acid (70–87%) and oleic acid (11–80%) which include polyunsaturated and monounsaturated, respectively (Murthy & Anjani, 2008; Aghamohammadreza et al., 2013; Kumar et al., 2015; Sirel and Aytac, 2016; Piccinin et al., 2019; Khalid et al., 2017; Katkade et al., 2018; Moatshe et al., 2020b; Emongor & Emongor, 2022). Safflower is considered a minor, neglected, and underutilised crop comparable to most oilseed crops, even though it has many economic advantages (Ekin, 2005; Mayes et al., 2012; Emongor & Emongor, 2022; Farooq & Siddique, 2022).

Safflower is currently cultivated commercially in over sixty diverse countries, encompassing an area of over a million hectares for agriculture and generating approximately 850,000 tonnes of seeds (FAO, 2019). Kazakhstan, United States of America, Russia, Mexico, China, India,

Argentina, Turkey, Tanzania, and Australia are the top ten producers of safflower seeds (FAO, 2022; Tridge, 2021; Emongor & Emongor, 2022). In Africa, Tanzania is the leading (9th in the world) producer of safflower seed (13,721 tonnes) followed by Ethiopia (9,349 tonnes) (FAO, 2022; Tridge, 2021). The majority of cut safflowers are produced in Europe, Latin America, and Japan (Ekin, 2005; Uher, 2008; Gomashe et al., 2021). Safflower farming has become more prevalent in arid and semi-arid areas because of the plant's ability to withstand drought, extreme temperatures, and salinity (Emongor & Oagile, 2017; Khalili et al., 2014; Gengmao et al., 2015; Emongor et al., 2015; Bassil & Kaffka, 2002).

Safflower plants have globular flower heads, known as capitula, and typically have long, protruding, angular spines on the leaves and inflorescence (Dajue & Mündel, 1996; Weiss, 2000; Emongor, 2010; Emongor & Oagile, 2017). Safflower occurs in spiny and spineless are the two distinct kinds. Spiny safflower genotypes produce more oil than spineless ones (Mani et al., 2020). Safflower petals have diverse colours with varying intensities, ranging from red to yellow and white (Dajue & Mündel, 1996; Flemmer et al., 2015; Weiss, 2000; Emongor, 2010; Emongor & Oagile, 2017). The petals contain red (carthamin) and yellow (carthamidin) pigments, which are typically utilised for therapeutic purposes, as well as colouring foods, beverages, pharmaceuticals, and textiles (Ekin, 2005; Emongor, 2010; Machewad et al., 2012; Adamska & Biernacka, 2021), and cosmetics (Yue et al., 2013).

Since synthetic dye (aniline) was developed, there has been a drastic decline in the requirement for natural colourants, which is partly due to its low cost (Dajue & Mündel, 1996). The consequence of artificial colourants/food dye usage has been linked to psychological disorders such as attention deficit hyperactivity disorder (ADHD) (Arnold et al., 2012) and carcinogenic or allergic effects. This, in turn, resulted in, restrictions imposed on the use of artificial colourants in

food, pharmaceuticals, and cosmetics in most developed countries including the European Union (Singh & Nimbkar, 2006; Hughes et al., 2010; Galaffu et al.,2015; Jadhav & Joshi, 2015; Bagley, 2017; Scotter, 2011; Katz & Williams, 2011; Vogel, 2018; Gebhardt et al., 2020). The growing need for foods that are healthier, and interest in natural dyes and food colourants has increased, and the European Union (EU) has directed the food industry to rate safflower as an appropriate yellow- and orange-colouring substitute due to its minimal enrichment factor unlike to the extracts of paprika or curcuma that are frequently utilised (Bagley, 2017; Vogel, 2018).

The laborious and time-consuming nature of safflower petal gathering by hand makes it difficult and costly (Omidi & Sharifmoghaddasi, 2010; Azimi et al., 2012). Rajvanshi (2005), manufactured an innovative safflower petal harvester able to extract 3-4 kg of petals daily, permitting for one acre of petals to be collected per month. In comparison to manual harvesting, results reveal a 657% upsurge in the number of petals gathered utilising the automated harvesters (Azimi et al., 2012). Furthermore, there is a significant petal yield and quality loss when the collection is done at the last stage of flowering (Omidi & Sharifmoghaddasi, 2010).

Unlike safflower seed production research, statistics on floret/petal production are scanty. Recent research has found that when petals are harvested at different stages of flower development, the seed yield components and dye content changed (Mohammadi & Tavakoli 2015; Steberl et al., 2020a). In Turkey Kizil et al. (2008) investigated three petal harvest times (50, 75, and 100%) where carthamin content was found to be lower at the start of blooming but increased following pollination. An investigation by Mohammadi & Tavakoli, (2015) in Iran, found that safflower genotypes had a profound influence on petal yield and dye content and recommended the cultivar Zendehrood for petal production. Moreover, Steberl et al. (2020a) in Germany, reported that to maximise carthamidin and petal output, harvest the Chinese cultivar (C2) using a combine

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harvester between 111 and 118 days after sowing. Although there has been keen interest in safflower petals by several countries, to be used as food and dye colourants, and manufacture of pharmaceuticals for treatment of several human ailments. Little research to improve petal yield and dye content has been done (Mohammadi & Tavakoli, 2015; Steberl et al., 2020a; Gomashe et al., 2021). Research to improve safflower petal yield and dye content would undoubtedly aid in improving the overall crop remuneration of the farmers (Gomashe et al., 2021).

1.2 Problem Statement

Safflower research carried out in Botswana has been inclined to adaptability, population, plant nutrition, and use of safflower as animal feed rather than petal production. Thus, this study was carried out to bridge the knowledge gap on the optimal time to harvest safflower petals at their peak for maximum petal yield, seed yield, and dye content of safflower. Information regarding the ideal time to collect safflower petals will facilitate production of high-quality petals to be used in various industrial purposes such as pharmaceutical, food, cosmetic, and textile.

1.3 Justification

Agriculture is a substantial economic sector that generates employment, trading prospects, food, and revenue, all of which are associated with economic development, food security, and sustainability. However, Agriculture accounts for a measly 2.5% of the gross domestic product (GDP) in Botswana (AFDB, 2021; Prinsloo & Matema, 2021). Despite this, agriculture remains a feasible industry for alleviating poverty and job creation in Botswana (Prinsloo & Matema, 2021). The annual agricultural survey report indicated a decline in crop production; the planting area

declined by 13.2% from 135,315 hectares in 2017 to 117,416 ha in 2019 (Statistics Botswana, 2020). Yield, area planted, and harvest all decreased as production metrics due to the lack of rainfall during the 2018/19 planting season (Statistics Botswana, 2020). Drought, variable precipitation, and a reduction in irrigation water availability are the main factors impacting agricultural output in arid and semi-arid lands (ASALs) (Temoso et al., 2015). Global warming is expected to exacerbate the severity of droughts, floods, and heat waves, worsening the ASALs' food security position (IPCC, 2007; Mittler & Blumwald, 2010). Safflower can adapt to environmental factors that are common in ASALs, such as drought, extremely high or low temperatures, salinity, and low nutrient levels (Farooq et al., 2009; Salem et al., 2014; Koutroubas & Papakosta, 2010; Hojati et al., 2011; Zareie et al., 2013; Safavi et al., 2012; Harrathi et al., 2012; Yau et al., 2004; Hussain et al., 2016; Koutroubas et al., 2004; Emongor & Oagile, 2017). Safflower can additionally be grown as an edible oil crop in ASALs due to the adverse environmental conditions therein (Beyyava et al., 2011; OECD, 2020; Yeilaghi et al., 2012; Yadzi-Samadi & Bagheri, 2005; Esmaeilzadeh et al.,2022; Arslan,2007; Camas et al., 2005). For these reasons, safflower should be one of the crops that could be explored to diversify the economy of Botswana. Its adaptation to ASALs (McPherson et al., 2004; Bassil & Kaffka, 2002; Khalili et al., 2014), outstanding quality cooking oil due to the abundance of unsaturated fatty acids, especially oleic and linoleic acids (Mišurcová et al., 2011; Carvalho et al., 2006; Orsavova et al., 2015; Katkade et al., 2018; Brenna et al., 2009; Zemour et al.,2021 Piccinin et al., 2019), and its use for animal feed (Weiss, 2000; Berglund et al., 2007; Bar-Tal et al., 2008; Emongor, 2010; Kereilwe et al., 2020) makes safflower an excellent crop for the diversification of the Botswana economy. Incorporating safflower into the cropping system of Botswana may help in the local production of cooking vegetable oil, give a crop rotation alternative to break the disease and pest pattern, and

provide livestock feed (Bhattarai et al., 2020). The current petal output and yields are insufficient because of the rising demand for safflower petals in the therapeutic and culinary industries on a global scale (Singh et al., 2008; Fatahi et al., 2009; Bernard et al., 2011; Emongor & Emongor, 2022). Various behavioural and physiological issues are associated with artificial food dyes (Katz & Williams, 2011; Stevens et al., 2013; Eagle, 2014; Jadhav & Joshi, 2015; Hughes et al., 2010). There is an immense need for over 6000 tons of dried safflower petals/year in India, China, and Europe (Rajvanshi, 2005; Vogel, 2018; Singh & Nimbkar, 2006; Bagley, 2017). According to Emongor & Oagile (2017), the selling of safflower petals to domestic (BWP 1000/kg of petals) and foreign markets will boost the disposable income of Batswana farmers and reduce poverty. With a price of US$13 per kg of dried safflower petals on the international market, Botswana has the capability of meeting that demand from all around the world.

1.4 Objectives

The primary objective of the study was to determine the best genotype and time to harvest safflower petals for optimum petal and seed yield, carthamidin and carthamin contents, and mineral nutritional contents of the petals.

The specific objective of this study was to evaluate the effects of petal harvest time and genotype on petal yield, seed yield, carthamidin and carthamin contents, and mineral nutritional contents of safflower petals.

1.5 Hypotheses

1.5.1 a H$_0$: Petal harvest time has no significant effect on the petal yield, seed yield, carthamidin and carthamin contents, and mineral nutritional contents of petals.

1.5.1 b H$_a$: Petal harvest time has a significant effect on the petal yield, seed yield, carthamidin and carthamin contents, and mineral nutritional contents of safflower petals.

1.5.2 a H$_0$: Genotype has no significant effect on the petal yield, seed yield, carthamidin and carthamin contents, and mineral nutritional contents of safflower petals.

1.5.2 b H$_a$: Genotype has a significant effect on the petal yield, seed yield, carthamidin and carthamin contents, and mineral nutritional contents of safflower petals.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The following section offers a broad perspective of safflower developmental stages and then highlights the constituents and use of safflower petals. The emphasis of the review is on the effects of petal harvest time and genotype on petal yield, quality, yield components, yield, dye content, and mineral nutritional contents of safflower. The review concludes by identifying the knowledge gap that is present.

2.2 Safflower developmental stages

Dajue & Mündel (1996) delineated six stages of safflower growth: emergence, rosette, stem elongation, branching, blooming, and physiological maturity. Safflower seeds germinate and emerge from the ground in three to 14 days after sowing dependent upon the temperature (Emongor, 2010; Emongor & Oagile, 2017; OECD, 2020; Mündel, 1969; GRDC, 2017; Torabi et al., 2013; OGTR, 2019; Emongor & Emongor, 2022). While germination is possible at temperatures between 2 and 5°C, 15.6°C has been identified as the ideal temperature for germination in the literature (Emongor & Oagile, 2017; Bidgoly et al., 2018; Afzal et al., 2022; Kaffka & Kearney, 1998; Emongor & Emongor, 2022). In contrast, Torabi et al. (2013) stated that safflower seed germination occurs at 5-30°C with day lengths of 8-12 hours. Following emergence, a growing slowly rosette stage takes place, in which numerous leaves sprout close to the ground level and a strong taproot develops (Emongor, 2010; Emongor & Oagile, 2017). Safflower taproot can reach 2-3 m into the soil, permitting it to survive during droughts or sporadic rains (Weiss,

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2000; Emongor, 2010; Khalid et al., 2017). Genotype, cultural management approaches, photoperiod, and post-germination temperature influence the rosette phase which typically lasts for 20 to 39 days. Emongor et al. (2013) stated that temperature has been observed to play a big role in the duration of the rosette stage. Lower temperatures during winter prolonged this stage, which resulted in higher grain yield. In addition, cooler temperatures caused plants to grow a thick clump of leaves when planted in the spring, eventually delaying the stem elongation phase. Safflower is relatively frost (-7 to -15°C) resilient when in the rosette stage depending on genotype (Mündel et al., 1992; Emongor, 2010; El-Bassam, 2010, Li, 1989; Li et al., 1997; Johnson et al., 2006; OECD, 2020; Kolanyane, 2022; Emongor & Emongor, 2022). However, from the elongation stage to the onset of flowering, the plants may suffer from chilling injury depending on temperature and duration of exposure to temperatures below -4.2°C (Kolanyane, 2022; Kolanyane et al., 2022; Kereilwe et al., 2022).

Weed management is crucial primarily during emergence through to the rosette stage because, at this initial developmental phase, safflower poorly compete with weeds (Rezaie & Yarnia, 2009; Tanaka et al., 1997; Carapetian, 2001; GRDC, 2017; Gomashe et al.,2021; OECD, 2020; Emongor, 2010; Blackshaw et al., 1990;). Rapid stem extension and widespread branching follow the rosette stage (Singh & Nimbkar, 2006; Kaffka & Kearney, 1998; Li & Mündel, 1996). Emongor and Oagile (2017) classify established lateral branches as main, secondary, and tertiary. Branching orientations (branch to stem) on the main stem can vary between 30 to 75 degrees (Singh & Nimbkar, 2006; Li & Mündel, 1996).

 Each plant produces 3-50 globular flower heads called capitula, which are surrounded by clasping bracts that are often spiny. This form of inflorescence is distinctive of the family Asteraceae (GRDC, 2017; Dajue & Mündel, 1996; Emongor, 2010; OECD, 2020). Primary capitula are the

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first to begin flowering, followed by secondary, tertiary, and quaternary. Flowering begins on the outermost portion of the flowers and then progresses into the centre of the capitulum for approximately seven days. (Dajue & Mündel, 1996). About 20 to 180 individual florets are typically present in each capitulum (Dajue & Mündel, 1996; GRDC, 2017). Flowering time is determined by genetics, although it can be hastened by abiotic factors such as temperature and photoperiod (Zimmerman, 1972; Gilbert, 2008; Singh & Nimbkar, 2006; Emongor et al., 2017; OECD, 2020; Emongor, 2010; Moatshe, 2019). Flowering is further influenced by genotype and environment interactions (Gilbert, 2008; Zimmerman, 1972; Emongor & Emongor, 2022). The flowering phase takes 4-6 weeks depending on temperature and genotype (Weiss, 2000; Emongor & Oagile, 2017; Moatshe et al., 2020c). Orange, yellow, and red flowers are abundant during the early flowering period however, the colour after flowering is darker shade while white flowers are uncommon (Dajue & Mündel, 1996; Emongor, 2010). Mohammadi & Tavakoli (2015) reported that different safflower petal harvest periods resulted in different colours. Early harvested petals are dominated by a yellow colour, while late harvested petals are orange or reddish. Safflower petals come in a variety of colours is typically determined by the content of the bioactive constituents, which are an important aspect in defining safflower quality (Pu et al., 2019). The bioactive constituents of safflower petals with different colour shades of orange, yellow, and white are an indication of their concentration, red and white being high and low respectively. The orange to red and yellow colour indicates the pigment carthamin and carthamidin are high, respectively (Shin et al., 2008; Mohammadi & Tavakoli, 2015; Garcia, 2009; Asgary et al., 2012). According to many studies (Pandey & Kumari, 2008; Dajue & Mündel, 1996; Rudolphi et al., 2008; Knowles, 1969; Kumari & Pandey, 2005; Emongor & Emongor, 2022), safflower is roughly 90.0-94.5% self-pollinating and 4.5-10% cross-pollinating. Given the stigma as well as style extending via the

anther columns encircling them, self-pollination is quite prevalent (Claassen, 1950; GRDC, 2017; Pandey & Kumari, 2008). After the stigma has elongated, it is coated in pollen from the same flower. The likelihood of outcrossing is increased by the fact that unpollinated extended stigma can stay responsive for numerous days (Li & Mündel, 1996; GRDC, 2017; Kumari & Pandey, 2005; Emongor & Emongor, 2022). Safflower that has undergone cross-pollination has a higher capitula set (Claassen, 1950; Mündel & Bergman, 2010; OECD, 2020). Insect pollinators, genetic makeup, pollen source terms of size, and habitat all affect the rate of cross-pollination that occurs (Nabloussi et al., 2013; Li & Mündel, 1996; Rudolphi et al., 2008; Mündel & Bergman, 2010; Pandey & Kumari, 2008; OGTR, 2019; OECD, 2020). Honeybees are the primary insects that pollinate safflower (Emongor & Oagile, 2017; Chaney, 1985; Van Deynze et al., 2005; Saeidi & Adam, 2011; Khalil et al., 1986; Pandey & Kumari, 2008; Sajjad et al., 2008; Bukero et al., 2015). The pollination of safflowers is carried out by insects other than honeybees (Chand et al., 2000; Esfahani et al., 2012; FAO, 2014; Emongor & Oagile, 2017; Khalil et al., 1986; Basiame, 2022). Reviewing the literature (Langridge & Goodman, 1980; Esfahani et al., 2012; Khalil et al., 1986; Emongor & Oagile, 2017; Basiame, 2022) indicated that nineteen insect species from five orders (Coleoptera, Lepidoptera, Hymenoptera, Hemiptera, and Diptera) frequent safflower florets. Safflower seed set is not affected by wind pollination (Claassen, 1950; Emongor & Oagile, 2017; Li & Mündel, 1996; OGTR, 2019). Achenes are generated following pollination, each capitulum produces between 13 and 70 achenes, which mature thirty-five days post flowering (OGTR, 2019; Emongor & Oagile, 2017; Moatshe, 2019). Safflower seeds have a 33-60% hull to 40-67% kernel ratio at maturity, and their oil content varies from 20-45 percent subject to genotype and growth conditions (Dajue & Mündel, 1996; Emongor & Oagile, 2017; Moatshe, 2019).

2.3 Safflower petal constituents and uses.

2.3.1 Constituents of safflower petals

Safflower petals are the precursor of carthamin, a naturally produced red pigment (Figure 1). Carthamin ($C_{43}H_{42}O_{22}$) has molecular weight of 910.8 g/mol (Adamska & Biernacka, 2021). The red pigment is mostly used to colour chocolates, cosmetics, and clothing (Shin et al., 2008; Garcia, 2009) because of its poor solubility in water. Carthamin has been explored as a potential replacement for nitrate and nitrite, whose established carcinogenic effects are well documented, and thus carthamin can be used as colouring for the dietary fibre meat processed for human consumption (Kim et al., 2015).
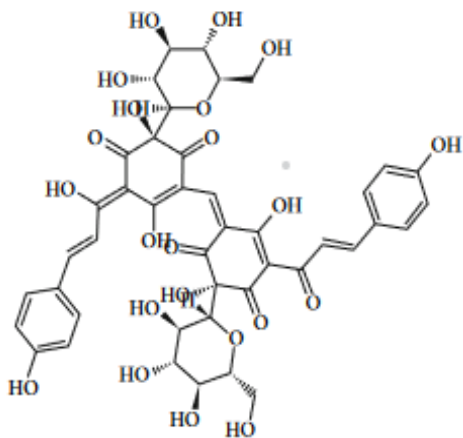
Figure 1: Carthamin structure
(Source: Adamska & Biernacka, 2021).

Carthamidin (Figure 2) is the water-soluble yellow pigment in safflower petals and is approximately 26–36% in content (Adamska & Biernacka, 2021). The molecular formula of carthamidin is $C_{15}H_{12}O_6$ and its molecular weight of 288.25 g/mol (Adamska & Biernacka, 2021;

NCBI, 2023). The yellow pigment has several components; safflor yellow B and saffiomin A along with additional minor substances, like cartormin, coumarins, and steroids (Li et al., 2016; Zhang et al., 2017). Yellow safflower pigment is used primarily as an all-natural colorant for foods in dyed juices and jelly, ice cream, sweets, and yogurt in part to the water's solubility (Bernard et al., 2011; Machewad et al. 2012).
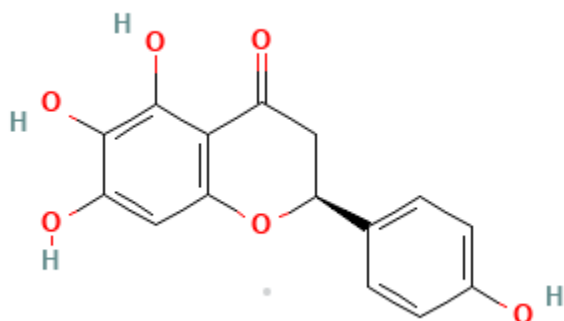
Figure 2: Carthamidin structure
 (Source: NCBI, 2023).

Various active compounds, which include phenylethanoid glycosides, flavonoids, coumarins, hydroxysafflor yellow A, fatty-acids, coumarins, and steroids, have been acknowledged in safflower petals (Zhou et al., 2009; Li et al., 2016; Zhang et al., 2017; Zhao et al., 2020a).

The petal colour of safflower is an essential quality component that serves as an indicator of quality after harvest, processing, and storage (Kim et al., 2020). The colour of safflower florets is affected by the stage of flowering (Figure 3). Safflower red and yellow pigments content is not constant (Figure 3). The colouring shade of the flower looks yellow then reddens at the start of flowering depending on the genotype but becomes red when senescence sets in (Erbas & Mutlucan, 2023).
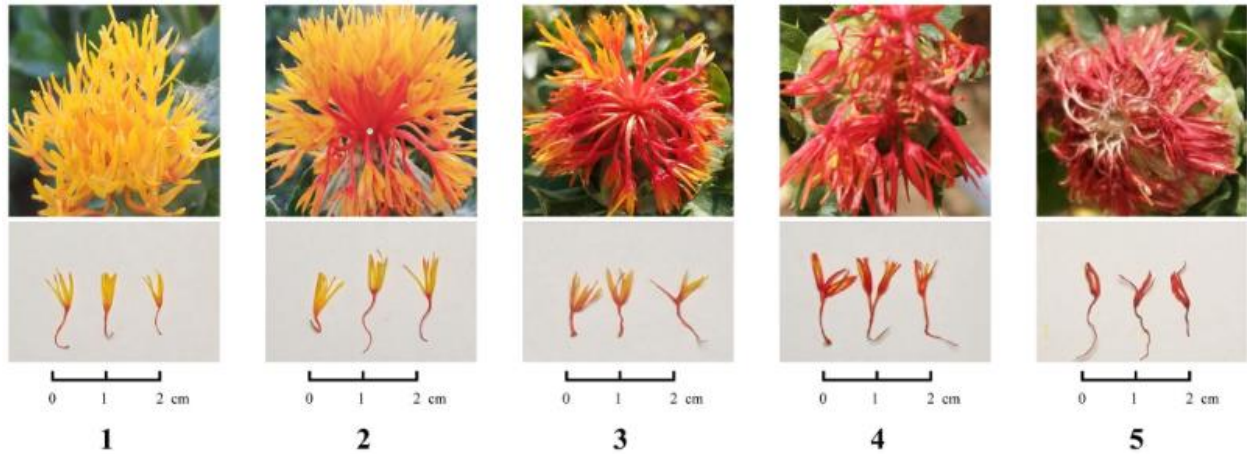
Figure 3: Safflower colour evolution at different stages

(Source: Pu et al., 2021).

The specifics of the blooming phase are depicted in Figure 3, which was separated into five stages.

Stage 1: The florets were all yellow.

Stage 2: A few red spots formed on the bottom of the flower.

Stage 3: Additional red segments on a single tubular bloom.

Stage 4: Most petals were red, with just a few yellow spots, and the flowers started to lose moisture.

Stage 5: There were no yellow sections visible, only red regions, with dried petals and no moisture (Pu et al., 2021).

The colour of carthamin in flowers progressively evolves from yellow to red during the biosynthesis process (Cho et al., 2000). The biosynthesis of carthamin (Figure 4) from chalcone (2,4,6,4'-tetrahydroxychalcone) with a pair of molecules of glucose have been elucidated (Cho et al., 2000; Cho & Hahn 2000; Kazuma et al., 2000; Zhang et al., 2017; Li et al., 2016).
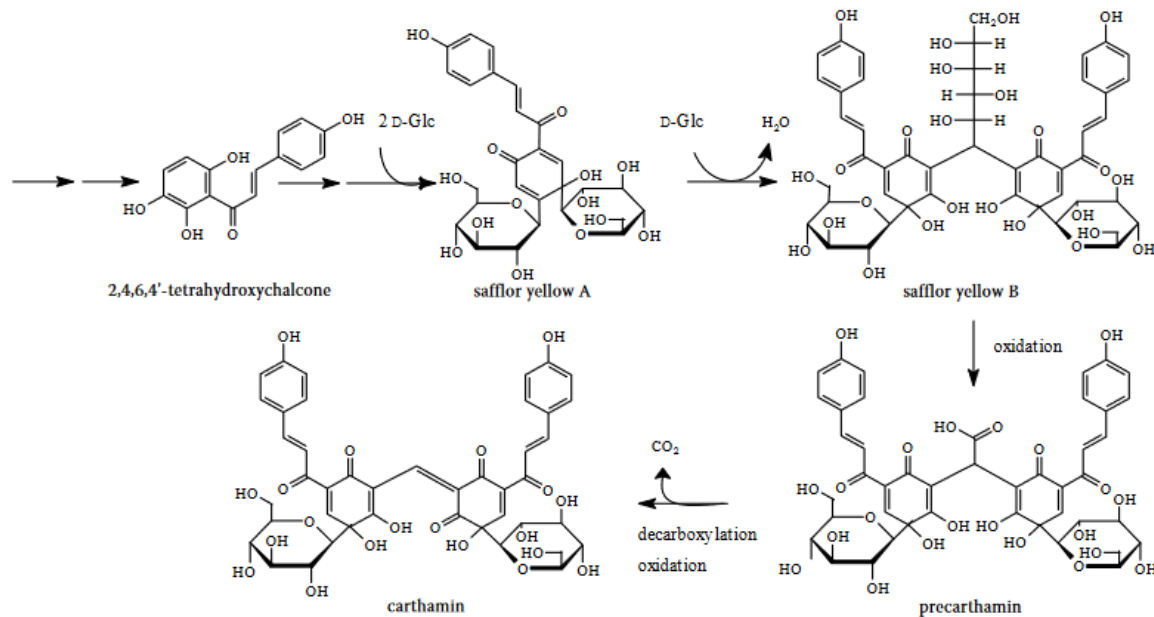
Figure 4: Carthamin biosynthesis pathway

(Source: Cho & Hahn 2000; Kazuma et al., 2000; Li et al., 2016; Cho et al., 2000; Zhang et al., 2017).

2.2.2 Uses of safflower petals

The most recent prohibitions on the usage of artificial colouring agents in foods and pharmaceuticals in the European Union, the United States of America, and Japan, combined with consumer demand for naturally derived colorants driven by health concerns, have boosted the popularity of safflower petal extracts derived as an alternative source of organic food and pharmaceutical colorants (Katz & Williams, 2011; Dajue & Mündel, 1996; Emongor, 2010; Bagley, 2017; Singh & Nimbkar, 2006; Hughes et al., 2010; Vogel, 2018). As a result, several prohibitions and restrictions have been imposed on the usage of artificial colourants and they should be substituted with natural antioxidants. With growing knowledge of toxicity and overuse

of artificial food additives, the synthetic dye industry has been on the decrease. There have been investigations linking synthetic colourants sensitivity to many forms of pollution and negative toxicological side effects, including mental illnesses such as deficit/hyperactivity disorder in human beings (ADHD) (Hughes et al., 2010; Bagley, 2017; Arnold et al., 2012; Jadhav & Joshi, 2015; Katz & Williams, 2011; Vogel, 2018). Different studies have reported that these inorganic food colourants adversely impact the behaviour of children, and cause carcinogenic or allergic effects (Bateman et al., 2004; Arnold et al., 2012; Kumar & Sinha, 2004; Křížová, 2015). According to Coultate & Blackburn (2018), 0.01% to 0.1% of children developed symptoms like eczema or asthma because of tartrazine used in colouring baby foods. Consumption of tartrazine and five other food colourants, Quinoline Yellow WS, Sunset Yellow, Carmoisine, Ponceau 4R, and Allura Red AC, were concomitant to an increased prevalence rate of hyperactivity in children (McCann et al., 2007). Furthermore, due to the developing cognizance of environmental consciousness, safe and healthy intake, the demand for natural colourants has risen (Kumar & Sinha, 2004; Křížová, 2015; Yusuf et al., 2017). Other natural colourants from plants such as turmeric (*Curcuma longa*) which is in ginger family, Zingiberaceae, which comprises dried rhizomes, and saffron (*Crocus sativus)* belonging to the family Iridaceae, which includes the dried crimson stigma (Brudzyńska et al., 2021). The cost of saffron and safflower as an unprocessed product for manufacturing is influenced by crop yield. A mere 8% of the overall flower output is usable saffron (spice). According to Kumar et al. (2008), a plant may generate no more than nine pistil stigmas (about three blooms, each having three stigmas). Considering that pistil typically weighs two milligrams, a plant can only provide up to 18 milligrams of raw material. Approximately 150,000 flowers are used to produce a kilogram of saffron powder (Brudzyska et al., 2021; Fernandez, 2004; Adamska & Biernacka, 2021). Safflower's complete blooms (petals

and stamens) generated in massive inflorescences with plants holding 20–250 blossoms serve as the raw material (Singh & Nimbkar, 2006; Emongor et al., 2015; Moatshe, 2019).

According to Zhaomu and Lijie (2001), China generates 1,800-2,600 tons of safflower flowers yearly, these are processed to source dyes and production medications. Safflower petals also serve a purpose in traditional medicine (Wang et al., 2020; Wang & Li, 1985; Zhou, 1992; Li et al., 2016; Adamaska & Biernacka, 2021; Mani et al., 2020). Safflower petal herbal tea and extracts are used to treat cramps during menstruation, after childbirth hemorrhage, pneumonia, chronic lung disease, rheumatism, and back pain (Wang et al., 2020; Adamska & Biernacka, 2021; Luo et al., 2019) and cardiology and obstetrical difficulties (Ao et al., 2018; More et al., 2005; Mani et al., 2020; Han et al., 2016; Ma et al., 2019; Zhang et al., 2019). Anticoagulant, neuroprotective, antihypertensive, vasodilating, anti-oxidative, and immune-protective properties are found in safflower petal extracts (Sun et al., 2018; Xu et al., 2017; Liu et al., 2018; Tan et al., 2020; Lee et al., 2020; Zhang et al., 2019; Bie et al., 2010), and anti-cancer medications (Chen et al ., 2022; Jin et al., 2019; Ma et al., 2019; Alahmadi et al., 2023; Qu et al., 2019), having favorable effects on melanin biosynthesis (Yin et al., 2015; Chen et al.,2013; Adamska & Biernacka, 2021). Cardiovascular and cerebrovascular disease (CCD) constitutes one of the worldwide primary causes of death and it has augmented with a high death rate (Donahue & Hendrikse, 2018; Collins et al., 2017; Qu et al., 2016; Zhao et al., 2020a). Natural products as an example hydroxysafflor yellow A, digoxin, and aspirin, have significant benefits for prevention from CCD (Dai & Ge, 2012; Qu et al., 2016; Desborough & Keeling, 2017; Zhao et al., 2020a; Eichhorn & Gheorghiade, 2002). Hydroxysafflor yellow A extracted from safflower petals has been shown to offer a multitude of pharmaceutical functions of improved blood circulation, eliminated blood stasis,

antioxidants, anti-inflammatory, and anticoagulation function that are crucial in preventing CCD (Sun et al., 2018; Wu et al., 2012; Sun et al., 2010; Ma et al., 2019; Zhou et al., 2019; Bacchetti et al., 2020).

2.4 Safflower petal production

Petal production from safflower is predominantly carried out by hand, making it a tremendously labour and time-intensive process (Azimi et al., 2012; Yun et al., 2016). The absence of industrially manufactured harvesting devices has hampered large-scale safflower petal production (Yun et al., 2016). To boost efficiency, there are two currently available designs for producing safflower harvesting machines. The first method is to use the cutting harvest technique, which involves using a rotary cutter to chop off the petals. When cutting safflower petals, however, the exact alignment of the slicing side of the blade is difficult. As an outcome, these petals readily shatter, reducing both effectiveness and quality (Yun et al., 2016). When harvesting, the air-blast gatherer uses a flower- gulping tube that is lined up with safflower petals (Azim et al., 2012; Yun et al., 2016). This equipment's fan creates a negative force in the flow of the airfield. As a result of the stream of air effect, the petals divide from the head. On the other hand, when the petals pile up, harvest efficiency declines, and fan power consumption rises (Azimi et al., 2012; Yun et al., 2016). Thus, establishing an automated harvesting system that can enhance both harvesting efficiency and safflower petal quality is critical. Typically, petal picking is done by hand at the final stages of flowering once the colour and value are poor (Omidi & Sharifmoghaddasi, 2010).

2.5 Time of petal harvest on yield components

The yielding components of safflower include capitula number/plant, capitulum size, the number of achenes (seed) for each capitulum, and achene quantity (1000-seed weight) (Gonzalez et al., 1994): Emongor et al., 2013 & 2017; Moatshe, 2019). Even though yield components are genetically controlled, they react to agricultural management practices with different levels of adaptability (Emongor et al., 2013; Gonzalez et al., 1994; Emongor et al., 2017; Moatshe, 2019). Kizil et al. (2008) reported that safflower genotypes achieved maximum plant height when petals were harvested at 50% flowering. No matter when the petals were harvested, plants that were taller were observed in all varieties when grown under cooler climates (Kizil et al., 2008). Petal harvesting time also significantly affected the first branching height (Kizil et al., 2008). Petal harvesting at 50% flowering significantly increased first branching height compared to petal harvesting at 75 or 100% flowering (Kizil et al., 2008). The number of capitula/plant displayed a positive and significant correlation with petal yield (Malekshan et al., 2015). Malekshan et al. (2015) established that among the genotypes under study, the genotype 'Zendehrod' was appropriate for multifaceted production and 'Goldasht' was right for petal production as it produced crimson petals.

2.6 Time of petal harvest on safflower petal and seed yield, and carthamin and carthamidin contents

2.6.1 Petal yield

According to Mohammadi and Tavakoli (2015), the time of harvest influenced petal yield; the highest yield was observed when petals were harvested at the onset of flowering but dwindled when harvested later. Spiny cultivars were observed to have lower petal output than spineless ones

(Patil et al., 2005; Kizil et al., 2008). Another factor that influences petal yield is temperature. Maximum yield was reported to be obtained at high temperatures during flowering period (Steberl et al., 2020a). The results reported by Omidi and Sharifmoghaddasi (2010) indicated that the height of branching was correlated positively with petal yield per plant. The imperative direct impact on safflower petal yield were plant height, branching height, and number of seed per capitulum (Omidi & Sharifmoghaddasi, 2010). High-yielding safflower cultivars are taller with numerous branches, superior capitula, less ineffectual heads, and a more prolonged duration of flowering (Omidi & Sharifmoghhaddasi, 2010; Emongor & Oagile, 2017; Emongor et al., 2017). The colour composition and petals production from safflower differ based on cultivar and date of harvest (Mohammadi & Tavakoli, 2015; Kizil et al., 2008).

Steberl et al. (2020a) reported significant interactions of cultivar and specifications for threshing, cultivar, and harvest scheduling on safflower petal yield when machine harvested. The yield of processed safflower florets was highest during 2018 (622 kg/ha) than in 2017 (512 kg/ha) (Steberl et al., 2020a). Threshed safflower petal yields increased when harvested after full bloom (Steberl et al., 2020a). The German safflower cultivar (CV1), with thresh variable pre-set at P2 (1142 kg/ha), yielded the highest possible threshed petal yield, while the Chinese cultivar (CV2) yields were greatest when the threshing factor settings P1 was applied (1049 kg/ha) when harvested after petals had withered. When harvested at the beginning of blooming and full bloom, both cultivars had the least safflower petal yields on parameter option P2. (Steberl et al., 2020a). The highest safflower petal yields with cultivar CV1 occurred when gathered at the beginning of flowering (294 kg/ha) and full bloom (393 kg/ha) (Steberl et al., 2020a). Studies in which hand harvesting (manual) of safflower petals was done reported petal yields ranging from 230 to 648 kg/ha, based upon the variety, timing of harvest, and the year (Weiss, 2000; Azari et al., 2005; Kizil et al., 2008;

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Nagaraj, 2009; Mohammad & Tavakoli, 2015; Steberl et al., 2020b). Steberl et al. (2020b) reported that lower safflower density (40 plants/m^2) lead to significantly higher number of branches, capitula number per plant, and petal yield than from plants of high density (75 plants/m^2). Harvesting safflower petals approximately 14-21 days after flowering produced a significantly greater petal yield in contrast to the onset of flowering (Steberl et al., 2020b). The Chinese safflower cultivar (182-459 kg/ha) had significantly higher petal yield than the German safflower cultivar (91-168 kg/ha) (Steberl et al., 2020b). The petals are harvested mostly from non-spiny safflower crops. There are several non-spiny cultivars and hybrids available, for petal production ranging from 120 to 150 kg/ha under rain-fed systems and 180 to 250 kg/ha with irrigation. Once the crop matures, the petals may be harvested, allowing dye and oilseed to be extracted from the same crop (Omidi & Sharifmoghaddasi (2010).

2.6.2 Seed yield

It has been shown that the timing of harvesting safflower petals has no effect on seed output, but that delayed harvesting of petals diminishes petal yield (Kizil et al., 2008; Malekshan et al., 2015). When it comes to seed weight, Omidi and Sharifmoghaddasi (2010) found that gathering petals every three days after flowering started led to a higher seed weight per capitulum, whilst picking the petals 9 days shortly after flowering began resulted in the lowest seed weight per capitulum.

2.6.3 Carthamin and carthamidin content

The percentage of carthamidin was reported to be highest when petals were harvested at the onset of flowering. However, when petal harvesting time was done after pollination (onset of petal wilting) carthamidin content was low (Mohammadi & Tavakoli, 2015). A lower percentage of carthamin was reported once petals were picked at the commencement of flowering however

increased post pollination (Kizil et al., 2008). Kizil et al. (2008) further reported that carthamin and carthamidin contents depended on the safflower cultivar, with the cultivar 'Dicer' significantly out yielding 'Yenice' and '5-154'. A maximal of 6.13% overall dyestuff content (carthamin and carthamin) was documented with the genotype 'Dincer' when harvested at 100% flowering and the least dyestuff content of 4.86% was recorded with the genotype '5-154' at 50% flowering petal collection time (Kizil et al., 2008). The results of Tavakoli (2014) agreed with those of Kizil et al. (2008), who reported that there was a significant cultivar effect on carthamidin and carthamin contents. The highest level of carthamidin was obtained from the cultivar 'Zendehrood' but must be picked at the start of flowering (Tavakoli, 2014). However, if carthamin was needed, the cultivar 'Zendehrood' following pollination, the florets ought to be harvested and at the commencement of wilted petals (Tavakoli, 2014). While Steberl et al. (2020b) reported that lower safflower density (40 plants/m^2) resulted in significantly higher carthamidin content than from plants of high density (75 plants/m^2). Harvesting safflower petals approximately 2-3 weeks post flowering, there was a substantial higher carthamidin yield than at the onset of flowering (Steberl et al., 2020b). The Chinese safflower cultivar had significantly higher carthamidin content (5.97-8.12%) and carthamidin yield (13.28-34.13 kg/ha) than the German safflower cultivar (2.68-5.72% of carthamidin) and (2.94-5.93 kg/ha carthamidin yield), respectively (Steberl et al., 2020b). Typically, the carthamidin content and carthamidin yield ranged between 2.53–8.29% and yielded 0.04–37.86 kg/ha (Steberl et al., 2020b). In addition to the time of petal harvesting, the amount of light intensity, temperature, soil fertility, sowing dates, and plant density are examples of such parameters that have been reported to influence carthamidin and carthamin contents (Steberl et al., 2020b). However, it has been documented that drier climatic conditions could possibly have a

favourable impact, leading to a rise of secondary phytochemicals such colorants (Salem et al., 2014).

2.7 Safflower petals mineral composition and proximate content

Table 1 below shows the proximate content and mineral composition of safflower petals reported in literature (Omidi & Sharifmoghaddasi, 2010; Machewad et al., 2012; Barashovets & Popova, 2016, Kim et al., 2000; Al-Snafi et al., 2015 and Waghmode et al., 2016).

Table 1: Mineral composition and proximate content of safflower petals.

Constituents	Value
Calcium	530-708 mg/100g
Iron	5.5-55.1 mg/100g
Magnesium	142-207 mg/100g
Potassium	2040-3992 mg/100g
Copper	1.10-4.73 mg/100g
Sodium	17.0-1043 mg/100g
Zinc	2.6-2.88mg/100g
Moisture	4.7-7.5 %
Crude protein	1.8-26.3%
Crude fat	4.8-11.5%
Crude fibre	11.6%
Ash	5.7-10.8%
Total sugars	7.36-11.81%

From the literature review above, for optimisation of petal, seed yield, and carthamin and carthamidin contents factors such as cultivar, plant density, harvest times and environment factors must be considered in safflower cultivation (Kizil et al., 2008; Mohammadi and Tavakoli, 2015). According to Steberl et al. (2020b), the optimal petal harvest time to maximise petal output, seed yield, carthamin, and carthamidin contents was two to three weeks after flowering, whereas Mohammadi and Tavakoli (2015) emphasised that harvesting petals after pollination optimised carthamin content. From the literature, there is scanty information on how safflower harvest time influence's petal and seed yield, yield components, carthamin and carthamidin contents, and proximate and mineral contents of petals. Therefore, the information generated from the proposed study will add to this knowledge gap.

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental site

Two field experiments were carried out to study the effect of petal harvest timing and genotype on safflower petal yield, quality, yield components, yield, and dye content. The experiments were conducted at the Botswana University of Agriculture and Natural Resources Content Farm in Sebele, Botswana, in 2021 and 2022. The Farm is situated in Southern Botswana's semi-arid area in latitude 59º24'S, longitude 95º25'E, and elevation 993 m above the sea level. The soils are medium to coarse-grained, shallow, ferruginous sandy loams (De Wilt & Nachtengale, 1996; Emongor & Mabe, 2012). Precipitation and evapotranspiration per annum vary between 250 to 600 mm and 1800 - 3000 mm, respectively (De Wilt & Nachtengale, 1996).

3.2 Experimental Design

The experimental design was a split-plot in randomized complete blocks with three replications. The treatments were three safflower genotypes and four petal harvest times assessed evaluated in both growth seasons, summer, and winter. The genotypes were Sina (spiny, yellow flowers), Kenya-9819 (spiny, yellow flowers), and Turkey (spineless, red/orange flowers) randomly assigned to the main plots. The four petal harvest times included the onset of flowering, full bloom, post-pollination, and end of flowering (petals have wilted control) randomly assigned to sub-plots. The sub-plot size was 9 m^2, while the main plots were 70 m^2. The total experimental units (plots) were 36.

3.3 Cultural and Management practices

The surface of the plot area was ploughed, disc harrowed, and cleared to achieve fine soil tilth. Application rates for the base fertilizer were 80 and 30 kg ha-1 of nitrogen and phosphorus, respectively. Direct sowing of safflower seed was placed at 4.5 cm deep, at a rating of two seeds each hill. After 15 days of emergence, the crop was thinned, retaining a single seedling each hill. Safflower plants underwent management procedures including weeding, disease, and insect control to encourage healthy growth. Each of the experimental units received supplemental watering each a week for a total of two hours (11 mm), utilising overhead sprinkler systems.

3.4 Data collection

Days to emergence, days to end of flowering, days to physiological maturation, height of the plant, numbers of main branches, capitulum diameter, the number of capitula per plant, total number of seeds per capitulum, thousand seed weight, the yield of seeds, petal yield carthamin, and carthamidin content were the dependent variables determined.

3.4.1 Days to emergence

The number of days to emergence was derived between the days of seed planting to the date of emergence.

3.4.2 Days to flowering.

The total number of days to flowering plants were kept track of for each treatment and replication from the day of seed sowing until 50% of the capitulum had produced flowers.

3.4.3 Days to end flowering.

Days to the end of flowering were chronicled from the date of seed planting until 95% of the capitulum had opened flowers per treatment per replication.

3.4.4 Days to maturity

The days of maturity were calculated from the day of seed sowing until the plants attained physiological maturity (when nearly all of the plant's leaves had become brown and only a hint of lush green remained within the capitulum bracts).

3.4.5 Height of first branch

Was obtained by randomly choosing 15 plants for every treatment and replication. The measurement of height to the primary branch at ground level was taken with a tape measure.

3.4.6 Plant height

Plant height was measured by selecting at random fifteen plants for each treatment and replication. At physiological maturity, the principal stem's length was measured above the surface of the soil to the apex of primary stem of the plant utilising a tape measure.

3.4.7 Number of primary branches

Fifteen randomly selected plants from each treatment and replication were tagged, and the total amount of primary branches per plant was tallied.

3.4.8 Capitulum diameter

At the end of the flowering duration, the capitulum dimensions of the 15 randomly selected plants for every subsequent replication of each treatment was assessed by employing a digital vernier calliper.

3.4.9 Number of capitula per plant

The aggregate amount of capitula produced from an individual plant at the stage of maturity of the 15 selected at random plants per treatment each replication was calculated.

3.4.10 Number of seeds per capitulum

Using a seed counter (Contador 1; Pfeuffer, Germany), the quantity of seeds per capitulum was calculated by counting the numeral of seeds acquired on a group of capitula gathered from random sample of 15 plants.

3.4.11 One-thousand seed weight

One-thousand seed weight was determined from a sample of 1000 seeds from each treatment for every replication using the Mettler PM 400 electronic scale.

3.4.12 Seed yield

The seed yield was calculated using a 4 m^2 zone from the center of each plot. For the purpose of separating the seeds from the chaff, the harvested capitula were threshed and winnowed. The seeds were then weighed using a Mettler PM 400 digital balance.

3.4.13 Petal yield

Petal yield was obtained within an area of 4 m^2 from the centre of each plot. Harvesting was done manually every three days. The harvested petals were placed in paper bags and then placed within an oven at 60°C for 24 hours. Thereafter, petals were weighed using a Mettler PM 400 digital balance, and cumulative weight constituted the yield which was calculated and expressed as kg/ha.

3.4.14 Petal dye index determination

Petal colour was determined visually and was recorded in 0 to 5 scores representing the colour (0 = white; one = yellow, two= amber, three= orange, four = vermilion, and five = red). The dye index was calculated using the formula below:

$$\text{Dye index} = \frac{\sum(\text{dye score x number of flowers at a particular colour})}{\text{Total of flowers assessed}}$$

3.4.15 Determination of carthamidin content

Carthamidin was extracted according to the method of Mohammadi & Tavakoli (2015), with minor modifications. Samples of safflower petals were dried for 12 hours in an oven at 40°C. A size two sieve was used to grind and filter the powdered petals. Then a sample of 0.1 g dried petals was weighed using an analytic balance (ME403 Mettle Toledo; Switzerland). The sample was transferred to a 250 ml volumetric flask, and then 200 ml of citric acid/di-sodium hydrogen phosphate buffer solution (pH level of 5) was added and left overnight. Then the mixture was filtered using filter paper (Filtron, qualitative 90 mm filter paper, India). Then a spectrophotometer

(Genesys 10s uv-vis, Thermo Fisher Scientific, USA) was used to quantify carthamidin at 400-408 nm. The following formula was applied to compute the proportion of colouring matter (P):

$$P = \frac{A}{487} \times \frac{200}{W}$$

Where W was the sample weight (grams), A is the highest absorbance of the sample in the 400-408 nm range, and 487 which was carthamidin specific absorbance value. Carthamidin yield was estimated by multiplying the carthamidin percent by the petal yield.

3.4.16 Determination of carthamin content

Carthamin was extraction following the method of Mohammadi and Tavakoli (2015), and a sample of 0.1 g dried petals was weighed using an analytical balance. The sample was transferred to a 250 ml volumetric flask, and then 100 ml of citric acid/disodium hydrogen phosphate buffer solution (pH level of 5) was added and left overnight. Following filtration using filter papers, the remaining petals (residue) were immersed for 1 hour in 100 ml of distilled water (repetitive for three times). The remaining petals were air-dried before being immersed in 15 ml dimethylformamide in a 125 ml conical flask for about three hours and filtered. A spectrophotometer (Genesys 10s uv-vis (Thermo Fisher Scientific, USA) was then used to measure carthamin at 525-535 nm. The following equation will be applied to determine the percent of colouring matter (P):

$$P = \frac{A}{992} \times \frac{200}{W}$$

Where W was the sample weight in grams, A was the highest absorbance of the sample in the 525-535 nm wavelength range, and 992 was the specific absorbance of carthamin. By multiplying the proportion of carthamin in the yield of petals, the carthamin yield was obtained.

3.5. Proximate composition analysis

3.5.1 Moisture content determination

 Crucibles were put in an oven pre-heated at 105°C for 24 hours, dried, cooled using a desiccator, and weighed (W0). A 5.0 g sample was placed in a crucible and measured crucible + sample (W1). Before reweighing, the crucible bearing the sample was then oven-dried at 105°C for 48 hours and chilled in a desiccator (W2).

The moisture content was determined as follows:

$$\% \ Moisture \ content = (W1 - W2)/ \ (W0) \times 100$$

3.5.2 Crude Fibre Determination

In accordance with (AOAC, 1996), this was established after the dry sample was pulverized and around 1.0 g (W0) was measured into a fritted glass crucible. Next, the sample was hydrolysed in a hot extractor using a 0.128 M sulphuric acid solution before being boiled in a 0.223 M potassium hydroxide solution. Before being moved to a cold extractor and being cleaned with acetone, the residue was first rinsed with preheated distilled water. Prior to being fired in a muffle furnace at 550°C for two hours, the residue and crucibles were oven dried at 105°C for an entire night and weighed (W1). The leftover ash was weighed (W2) after cooling in an oven at 105°C overnight, and subsequently brought to ambient temperature in a desiccator. The formula used to get the crude fibre % is

$$\% \ Crude \ Fibre = (W1 - W2)/ \ (W0) \times 100$$

3.5.3 Crude Protein Determination

The pulverized safflower petals were broken down in a digestion block at 330°C for seven hours. Nitrogen (N) was measured after digestion using distillation and titration using the micro-Kjeldahl technique (AOAC, 1996). By increasing the N content% by 6.25, the quantity of crude protein was calculated (AOAC, 1996). Protein content was calculated using AOAC-approved method 979.09. Using an analytical scale, the petal sample was measured and put to the digestion flask in 1 g mass. After that, a 6 mL acid mixture (5:1 concentrated H_3PO_4: H_2SO_4) and 3.5 mL of 30% H_2O_2 were progressively added to the digestion flask. When the tubes were shaken, there was a dramatic reaction. After the violent reaction was stopped, 3 g of the catalyst mixture (0.5:100 Se: K_2SO_4) was added to the digesting flask. The resultant solution was digested for one hour at 370°C. Following digestion, the contents of the flask were diluted by using water and 40% concentrated sodium hydroxide was added to neutralize the acid and slightly alkalinize the resultant solution. The ammonia was then distilled into a receiving flask comprising 4% boric acid solution. The borate ion was created via the interaction of boric acid and ammonia, which was titrated afterwards alongside a standard acid (0.1 N sulphuric acid solution) until the green hue changed pink. The following formulae was used to compute the nitrogen content:

$$\text{Nitrogen}(\%) = \frac{(V2 - V1) * N * 14.007}{W} \times 100$$

Where: V2 was the volume in milliliters of the prescribed sulphuric acid solution used for the sample titration.

V1 = the volume in milliliters of the recommended amount of sulfuric acid utilised in the blank determination titration N = Normality of the standard sulphuric acid

W = weight in grams of sample

$Crude\ protein\ content(\%)$
$= Nitrogen(\%) * 6.25\ (nitrogen\ to\ protein\ conversion\ factor)$

3.5.4 Determination of the fat content

Safflower petals that had been dried and crushed were used as a sample of 0.5 g. The sample was weighed, put in extraction bags that had already been pre-weighed, sealed, and submerged in petroleum ether for 60 minutes (XT10 Ankom Extraction System Ankom Technology, USA). Once the extracting process was done, the sealed bagged samples were cooled for 15 minutes before being dried in a hot oven for 15 minutes. The bag along with samples were subsequently weighed. The fat content was determined by comparing the beginning and finishing weights, and it was then reported as a percentage of the original weight of the ground desiccated safflower petal.

3.5.5 Determination of ash content

The crucibles used in the experiment were sterilized with small amounts of hydrochloric acid before being rinsed using distilled water. It was subsequently dried in the oven at 120°C until being heated in a furnace at 550°C for three hours. After that, the crucibles were removed from the furnace and placed in desiccators to cool. Then weighed on a digital scale and the tare (zero) the scale, the crucibles' mass was calculated as M1. In the porcelain crucible, a 2 g quantity of sample safflower petals powdered was weighed and recorded as M2. The samples were burned on a hotplate at 120°C for four hours, before the entire substance become carbonized. The samples were then heated in a 550°C furnace for eight hours before they were free of carbon and the residue became grey/white. After burning, by using tongs, the crucibles were transferred to a desiccator to cool before recording its weight M3. To compute the ash percentage in a sample formula below was used:

$$\text{Ash } (\%) = (M3 - M1)/(M2 - M3) \times 100$$

Where, M1 = mass of the dried crucible; M2 = mass of the crucible with the sample; M3 = mass of the crucible and the ash.

3.5.6 Determination of Carbohydrates

To determine carbohydrates, the following formulae were used.

Percentage of carbohydrate was given by $100 - \sum \% \,(\text{Ash} + \text{Moisture} + \text{fat} + \text{protein})$.

3.6 Mineral analysis procedure

Safflower petals were oven-dried at 66°C. Followed by a 1.25 g composite sample digested in a BD block at 330°C for seven hours in 20 ml of sulphuric acid (98%) and 4 ml of hydrogen peroxide (@ 30%). After digestion, the samples were then diluted with an appropriate volume of deionized water. For calcium and magnesium, the final sample contained also 1% of lanthanum to avoid anionic interferences. Calcium (Ca), magnesium (Mg), potassium (K), Sodium (Na), Iron (Fe), and Zinc (Zn) absorbance were measured using Agilent 240FS Atomic Absorption Spectroscopy (Agilent Technologies, USA) connected to the SpectrA A software that provided sample results/report. Total mineral content was reported as mg/100 g on a dry weight basis.

3.7 Statistical Analysis

Data collected was subjected to analysis of variance (ANOVA) using the General Linear Model (PROC GLM) procedure of the Statistical Analysis System (SAS) program package. Multiple comparisons among means were done using Protected Least Significant Difference (LSD) at P = 0.05.

CHAPTER 4

RESULTS

4.1 Effect of genotype and harvest time on phenological stages

4.1.1 Emergence

Genotype had a significant ($P<0.05$) effect on seed emergence of safflower grown both in summer and winter, but there was no interaction of genotype and harvest time on their influence on days to seedling emergence (Table 2). Safflower cultivated in both the summer and the winter, the genotype Turkey took 9.1 and 11.5 days to emerge, respectively, which were significantly ($P<0.05$) longer than the days the genotypes Sina and Kenya-9819 took to emerge (Table 2). The genotypes Sina and Kenya-9819 did not significantly ($P>0.05$) contrast in their days to emergence in both summer and winter grown safflower (Table 2). In general, safflower planted in summer emerged earlier by 1.78 days than winter (Table 2). Harvest time had no significant ($P>0.05$) influence on days to seed emergence of safflower in both summer and winter plantings (Table 2).

4.1.2 Days to flowering.

Genotype had a significant ($P<0.001$) effect on days to flowering of safflower grown in both summer and winter, but there was no significant ($P>0.05$) interaction of genotype and harvest time on days to flowering, thus only the key effects were explained (Table 2). The genotype Turkey took 80.11 and 117.17 days to flowering in summer and winter, respectively, which were significantly ($P<0.05$) longer than the days genotypes Sina and Kenya-9819 to get to flowering stage (Table 2). During winter the genotypes Sina and Kenya-9819 did not significantly ($P>0.05$) differ in their days to flowering. Summer planted safflower took 37.14 days to arrive at flowering which was significantly ($P<0.05$) earlier than winter planted safflower (Table 2). In both summer

and winter planted safflower harvest time had no significant (P>0.05) influence on days to flowering (Table 2).

4.1.3 Days to end of flowering.

Safflower genotype had a significant (P<0.001) influence on days of end of flowering in both summer and winter (Table 2). There was no significant (P>0.05) interaction of genotype and harvest times on days to end of flowering, hence only main effects were outlined. Safflower grown in summer took between 83.67-90.36 days to end of flowering based upon the genotype. Whereas during winter safflower required between 118.95- 126.14 days to end of flowering subject to the genotype (Table 2). Genotypes Sina, Kenya-9819 and Turkey significantly (P<0.05) diverged in their days to reach to end of flowering in both summer and winter season. The genotype Turkey took 90.36 days to the end of flowering which was significantly (P<0.05) the longest period followed by Kenya (84.90 days) then Sina (83.67 days) in summer (Table 2). During winter, the genotype Turkey took 126.14 days to reach the end of flowering which was significantly (P<0.05) the longest period followed by Sina (120.13 days) and Kenya-9819 (118.95 days). Conventionally, summer planted safflower reached to the end of flowering by 35.43 days less than winter planted safflower (Table 2).

Harvest time had a significant (P<0.05) effect on days to the end of flowering period in winter (Table 2). However, it did not have a significant (P>0.05) effect on days to the end of flowering in summer. In winter, the harvest times, onset flowering, full bloom and end of flowering did not significantly (P>0.05) differ in their days to reach the end of flowering, but significantly (P<0.05) took longer days to reach to the end of flowering than post-pollination harvest time (Table 2).

4.1.4 Days to physiological maturity

Safflower genotypes had a significant (P<0.001) effect on days to physiological maturity of the plants grown both in summer and winter (Table 2). There was no significant (P>0.05) interaction of genotype and harvest times on safflower physiological maturity. There was a significant (P<0.05) difference in days to reach physiological maturity among all the genotypes (Table 2).The genotype Turkey required 107.3 and 156.91 days to reach physiological maturity in summer and winter, respectively which were significantly (P<0.05) longer than days genotypes Kenya-9819 and Sina took to reach physiological maturity (Table 2) The genotype Sina took 99.25 and 139.55 days to reach physiological maturity in summer and winter, correspondingly, which were significantly (P<0.05) shorter than days other genotypes took (Table 2).

Petal harvest time had a significant (P<0.01) effect on days to reach physiological maturity in both summer and winter grown safflower (Table 2). Summer planted safflower reached physiological maturity 44.01 days earlier than winter planted ones. In both summer and winter, harvesting safflower petals at onset of flowering, full bloom and end of flowering caused the plants to take significantly (P<0.05) longer days to reach physiological maturity by 0.73 and 1.54 days, respectively than plants where petal harvesting was done at post-pollination (Table 2). However, petal harvesting at onset of flowering, full bloom and end of flowering caused the plants to reach physiological maturity at statistically the same time of 103 and 147 days in summer and summer, respectively.

Table 2: Effect of genotype and time of petal harvest on days to emergence, flowering, end of flowering, and physiological maturity of safflower.

Main effects and interactions	Days to emergence		Days to flowering		Days to end of flowering		Days to physiological maturity	
	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter
Genotype(G)								
Sina	8.25 b	9.50b	74.09c	111.29b	83.68c	120.13b	99.25c	139.55c
Kenya-9819	8.08 b	9.78b	74.73b	111.89b	84.90b	118.95c	101.87b	143.99b
Turkey	9.12a	11.50a	80.11a	117.17a	90.36a	126.15a	107.30a	156.91a
Significance	*	**	***	***	***	***	***	***
LSD	0.814	0.921	0.583	1.226	0.131	0.837	0.701	1.391
Harvest time (H)								
Onset of flowering(H1)	8.47 a	10.30a	76.15a	113.35a	86.39a	121.87a	102.97a	147.08a
Full bloom(H2)	8.37 a	10.26a	76.46a	113.55a	86.22a	120.93b	103.20a	147.52a
Post pollination(H3)	8.70 a	10.22a	76.47a	113.70a	86.35a	121.97a	102.26b	145.66b
End of flowering(H4)	8.40 a	10.26a	76.17a	113.21a	86.30a	122.21a	102.80a	147.00a
Significance	NS	NS	NS	NS	NS	*	**	*
LSD	0.362	0.463	0.582	0.702	0.289	0.819	0.513	2.274
Interactions								
G*H	NS	NS	NS	NS	NS	NS	NS	NS

 *, **, ***, NS. Significant at 0.05, 00.1, 0.001 or not significantly, respectively. Means separated using the Least Significant Difference (LSD) at P<0.05, means within column followed by the same letter(s) are not significantly different

4.2 Effect of genotype and harvest time on vegetative growth

4.2.1 Height of first branch

Genotype had a significant (P<0.05) effect on height of the first branching for safflower plants

grown in summer and winter (Table 3). In both the seasons, genotypes Sina and Kenya-9819 did

not significantly (P>0.05) differ in their heights of first branch development (Table 3). Plants of

the genotype Turkey in both summer and winter started to branch at significantly (P<0.05) higher heights than other genotypes under study (Table 3). Overall, first branching height ranged between 12.58-14.6 cm and 29.28-34.36 cm in summer and winter grown safflower, respectively, depending on genotype (Table 3). Time of petal harvest time had no significant influence on the height to first branch development of safflower plants irrespective of season (Table 3). There was no significant (P>0.05) interaction of genotype and harvest time on safflower height of first branch development (Table 3).

4.2.2 Plant height

Safflower genotypes had significant (P<0.01) variation on plant height for plants grown in summer and winter, but there was no significant (P>0.05) interaction of genotype and time of petal harvest on plant height (Table 3). The genotype Turkey had plant heights of 84.97 and 118.06 cm when grown in summer and winter, respectively, which was significantly (P<0.05) taller than plant heights of genotypes Sina and Kenya-9819 (Table 3). There was no significant (P>0.05) genetic variation of plant height for the genotypes Sina and Kenya-9819 (Table 3). Safflower plants grown in winter had significantly (P<0.05) taller (91.21 cm) plants than summer (73.43 cm) grown plants irrespective of genotype (Table 3). The plant height span within 67.89-84.97 cm and 76.85-118.06 cm in summer and winter, singly, contingent on genotype (Table 3).

4.2.3 Number of primary branches

There was no significant (P>0.05) interaction of genotypes and petal harvest time on primary branch number of safflower. However, safflower genotypes significantly (P<0.05) influenced primary branch number (Table 3). The genotypes Sina and Turkey had no significant (P>0.05) variation in the number of primary branches/plant in both winter and summer, but their primary

branches were significantly (P<0.05) more than those of plants from the genotype Kenya-9819 (Table 3). Safflower grown in winter had significantly (P<0.05) more branches per plant (10.6) compared to those grown in summer (8.2) (Table 3).

Table 3: Effect of genotype and time of petal harvest on height of the first branch, plant height and number of primary branches

Main effects and interactions	Height of the first branch(cm)		Plant height (cm)		Number of primary branches/plant	
	Summer	Winter	Summer	Winter	Summer	Winter
Genotype(G)						
Sina	12.58b	29.28b	67.89b	76.85b	8.58a	10.95a
Kenya-9819	12.50b	29.91b	67.44b	78.73b	6.33b	8.95b
Turkey	14.63a	34.36a	84.97a	118.06a	9.67a	11.91a
Significance	**	*	**	***	*	*
LSD	0.890	3.475	5.312	9.062	2.025	1.438
Harvest time (H)						
Onset of flowering(H1)	12.83ab	31.38a	72.41a	90.05a	8.22a	10.22a
Full bloom(H2)	12.84ab	31.12a	74.38a	93.23a	8.89a	11.56a
Post-pollination(H3)	12.62b	31.29a	73.12a	90.82a	8.11a	10.11a
End of flowering(H4)	14.11a	30.94a	73.82a	90.76a	7.56a	9.89a
Significance	NS	NS	NS	NS	NS	NS
LSD	1.460	2.043	3.651	4.470	1.467	1.534
Interactions						
G*H	NS	NS	NS	NS	NS	NS

 *, **, ***, NS. Significant at 0.05, 00.1, 0.001 or not significantly, respectively. Means separated using the Least Significant Difference (LSD) at P<0.05, means within column followed by the same letter(s) are not significantly different

4.3 Effect of genotype and harvest time on yield and yield parameters of safflower

4.3.1 Capitula diameter

There was no significant (P>0.05) interaction of genotype and petal harvest time on safflower capitula diameter, but main effects had significant (P<0.05) influence on capitula diameter (Table 4). Safflower genotype had a significant (P<0.01) influence on the capitula diameter in summer

and winter (Table 4). In summer the genotype Turkey had plants with capitula diameter of 12.43 mm which was significantly ($P<0.05$) higher than capitula diameter generated by the plants of the genotypes Sina and Kenya-9819 (Table 4). Also, in summer, the genotypes Sina and Kenya-9818 did not statistically ($P>0.05$) differ in their capitula diameter (Table 4). In winter plants of the genotype Turkey produced a capitula diameter of 19.39 mm which was significantly ($P<0.05$) larger than that of the Sina but not Kenya-9819 (Table 4). Safflower plants grown in winter (17.07 mm) produced significantly ($P<0.05$) larger capitula diameter than summer (10.85 mm) plants (Table 4).

Harvest time had a significant ($P<0.05$) effect on capitula diameter both in summer and winter (Table 4). In summer planted safflower, petal harvesting at post-pollination (petals mid-withered) and end of flowering period significantly ($P<0.05$) produced plants with larger capitula diameter than plants where petal harvesting was done at the onset of flowering and full bloom (Table 4). However, plants where petal harvesting was done at post-pollination and end of flowering period did not statistically ($P>0.05$) vary in their capitula diameter in summer (Table 4). In winter, petal harvesting at full bloom stage significantly ($P<0.05$) produced safflower plants with larger capitula diameter than other petal harvest times to the exclusion of petal harvesting at the onset of flowering (Table 4). Petal harvesting at the end of flowering produced plants with significantly ($P<0.05$) smaller capitula diameter than other petal harvesting times with exception of post-pollination (Table 4).

4.3.2 Number of capitula per plant

Safflower genotypes significantly ($P<0.05$) affected capitula number/plant (Table 4). The genotype Turkey had plants with significantly ($P<0.05$) higher number of capitula per plant than other genotypes in all seasons with exception of genotype Kenya-9819 grown in summer (Table

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4). In summer, the genotypes Sina and Kenya-9819, and Turkey and Kenya-9819 did not significantly (P>0.05) vary in their capitula number/plant (Table 4). In winter grown safflower, all genotypes significantly (P<0.05) varied in their capitula number/plant, but the genotype Sina had significantly (P<0.05) lower capitula number/plant than other genotypes in winter (Table 4). Safflower plants grown in winter had capitula number/plant of 22.28 which was significantly (P<0.05) higher than summer (12.13) depending on genotype (Table 4). The capitula number/plant ranged from 10.97-24.56 depending on genotype and planting season (Table 4). Petal harvest time and the interaction of genotype and time of petal harvest had no significant (P>0.05) effect on the number of capitulum per plant (Table 4).

4.3.3 Number of seeds per capitulum

Genotypes and petal harvest time interaction had no significant (P>0.05) effect on seed number/capitulum, therefore main effects are reported (Table 4). Genotypes had a significant (P<0.05) influence on the number of seeds/capitulum in safflower plants grown in summer and winter (Table 4). In both summer and winter grown safflower, the genotype Turkey had significantly (P<0.05) higher number of seeds/capitulum than other genotypes with exemption of the genotype Kenya-9819 (Table 4). Wintertime cultivated safflower plants had number of seeds/capitulum of 22.77 which was significantly (P<0.05) higher than that of summer (14.22) grown plants (Table 4). Petal harvest times had a significant (P<0.01) influence on the number of seeds/capitulum for plants grown in summer but not winter (Table 4). In summer, harvesting petals at post-pollination and end of flowering significantly (P<0.05) increased the number of seeds/capitulum than harvesting petals at the onset of flowering and full bloom (Table 4). Petal harvesting at the onset of flowering and full bloom, and post-pollination and end of flowering, (in that order), in summer did not significantly (P<0.05) contrast in seed number/capitulum (Table 4).

Table 4: Effect of genotype and time of petal harvest on capitulum diameter, number of capitula per plant and number of seeds per capitulum of safflower

Main effects and interactions	Capitula diameter (mm)		Number of capitula per plant		Number of seeds per capitulum	
	Summer	Winter	Summer	Winter	Summer	Winter
Genotype(G)						
Sina	9.91b	14.16b	10.97b	19.80c	13.10b	20.12b
Kenya -9819	10.20b	17.66a	12.14ab	22.49b	14.09ab	22.99a
Turkey	12.43a	19.39a	13.29a	24.56a	15.48a	25.19a
Significance	**	*	*	**	*	*
LSD	0.8864	2.468	1.404	2.061	1.4651	2.381
Harvest time (H)						
onset of flowering (H1)	10.15c	17.53ab	11.79a	21.93a	13.76b	22.21a
Full bloom (H2)	10.59b	17.63a	11.74a	22.13a	13.80b	22.89a
Post pollination (H3)	11.37a	16.26bc	12.77a	22.58a	14.79a	23.41a
End of flowering (H4)	11.28a	16.05c	12.23a	22.50a	14.58a	22.54a
Significance	***	*	NS	NS	**	NS
LSD	0.4213	1.360	1.240	1.093	0.3578	1.230
Interactions						
G*H	NS	NS	NS	NS	NS	NS

*, **, ***, NS. Significant at 0.05, 0.01, 0.001 or not significantly, respectively. Means separated using Least Significant Difference (LSD) at P<0.05, means within column followed by the same letter(s) are not significantly different

4.3.4 Thousand seed weight

Safflower genotypes had significant (P<0.05) influence on 1000-seed weight both in summer and winter (Table 5). However, there was no significant (P>0.05) interaction of genotype and harvest times on safflower thousand seed weight (Table 5). The genotype Turkey had significantly (P<0.05) higher 1000-seed weight of 32.74 and 39.04 g in summertime and winter, respectively,

than other genotypes (Table 5). The genotypes Kenya-9819 and Sina did not significantly (P>0.05) vary in their 1000-seed weights in both summer and winter (Table 5). Winter grown safflower produced plants with heavier 1000-seed of 37.96 g than summer (29.31 g). Petal harvest time had no significant (P>0.05) effect on 1000-seed weight of safflower when planted in summer (Table 5). In winter planted safflower, petal harvest time significantly (P>0.05) influenced 1000-seed weight (Table 5). Petal harvesting at the onset of flowering caused safflower plants to produce significantly (P<0.05) heavier 1000-seed weight than other petal harvest times in winter (Table 5). Petal harvesting at full bloom, post-pollination, and end of flowering in winter did not cause significant (P>0.05) variation in 1000-seed weight of safflower (Table 5).

4.3.5 Seed Yield

Safflower genotypes had significant (P<0.05) influence on seed yield in both summer and winter, but there was no significant (P>0.05) interaction of genotype and petal harvest time or petal harvest time on seed yield (Table 5). In both summer and winter grown safflower there was significant (P<0.05) genotypic variation on seed yield (Table 5). The genotype Turkey had significantly (P<0.05) higher seed yield of 1479 and 2697 kg/ha in summer and winter, respectively, than other genotypes (Table 5). However, the genotypes Kenya-9819 and Sina did not significantly (P>0.05) vary in their seed yield in summer and winter (Table 5). Similarly, the seed yield of the genotypes Turkey and Sina did not significantly (P>0.05) differ both in summer and winter (Table 4). Winter grown safflower had an average seed yield of 2511 kg/ha which was significantly (P<0.05) higher than the seed yield of 1241 kg/ha generated by summer plants reliant on genotype (Table 5).

Table 5: Effect of genotype and time of petal harvest on 1000 seed weight, and seed yield of safflower

Main effects and interactions	1000-seed weight (g)		Seed yield (kg/ha)	
	Summer	Winter	Summer	Winter
Genotype(G)				
Sina	27.56b	37.74b	1181ab	2475ab
Kenya -9819	27.64b	37.00b	1063b	2362b
Turkey	32.74a	39.14a	1479a	2697a
Significance	*	*	*	*
LSD	3.446	1.364	311.3	227.1
Harvest time (H)				
onset of flowering (H1)	29.54a	39.39a	1276a	2554a
Full bloom (H2)	29.18a	37.33b	1215a	2488a
Post-pollination (H3)	29.52a	37.64b	1258a	2512a
End of flowering (H4)	29.01a	37.49b	1215a	2488a
Significance	NS	**	NS	NS
LSD	1.986	1.193	148.4	146.8
Interactions				
G*H	NS	NS	NS	NS

*, **, ***, NS. Significant at 0.05, 0.01, 0.001 or not significantly, respectively. Means separated using the Least Significant Difference (LSD) at P<0.05, means within column followed by the same letter(s) are not significantly different.

4.3.6 Petal yield

In both summer and winter grown safflower, safflower genotypes and harvest time interacted significantly (P<0.05) to impact on petal yield (Figure 5, 6). The genotype Turkey and petals harvested at the onset of flowering had significantly (P<0.05) the highest petal yield of 98.3 kg/ha compared to other genotypes and harvest times besides the genotypes Turkey harvested at full bloom and post-pollination, and Sina harvested at onset of flowering and full bloom in summer grown safflower (Figure 5). The genotype Sina harvested at end of flowering produced significantly (P<0.05) the lowest petal yield of 90.3 kg/ha paralleled to other genotypes and harvest

times with omission of the genotype Kenya-9819 harvested at post-pollination and end of flowering in summer (Figure 5). The genotype Kenya-9819 harvested at onset of flowering, full bloom, and post-pollination did not significantly (P>0.05) differ in their petal yields in summer (Figure 5). In summer grown safflower, the petal yield ranged between 90.3-98.3 kg/ha depending on genotype and petal harvest time (Figure 5).

Effect of genotype and harvest time interaction on safflower petal yield in summer. Means separated using the Least Significant difference (LSD) at P = 0.05; means followed by the same letter(s) are not significantly different.

Figure 5 : Effect of genotype and harvest time interaction on safflower petal yield in summer.

In winter the genotype Turkey and petals harvested at full bloom had significantly (P<0.05) the highest petal yield of 116.5 kg/ha in contrast to other genotypes and harvest times with exception of the genotypes Turkey collected at onset of flowering and post-pollination, and Sina harvested at onset of flowering and full bloom (Figure 6). The genotype Sina harvested at end of flowering

produced significantly (P<0.05) the lowest petal yield of 107.6 kg/ha compared to other genotypes and harvest times with exception of the genotype Kenya-9819 harvested at end of flowering in winter (Figure 6). During winter the genotype Kenya-9819 harvested at onset of flowering, full bloom, and post-pollination did not significantly (P>0.05) vary in their petal yields neither did it differ with the petal yield of the genotype Sina harvested at post-pollination (Figure 6). Petal yields of the genotypes Sina and Turkey harvested at onset of flowering and full bloom, and post-pollination and end of flowering did not significantly (P>0.05) vary in winter (Figure 6). Safflower planted in winter, produced petal yield ranging between 107.6 and 116.2 kg/ha depending on genotype and petal harvest time (Figure 6).
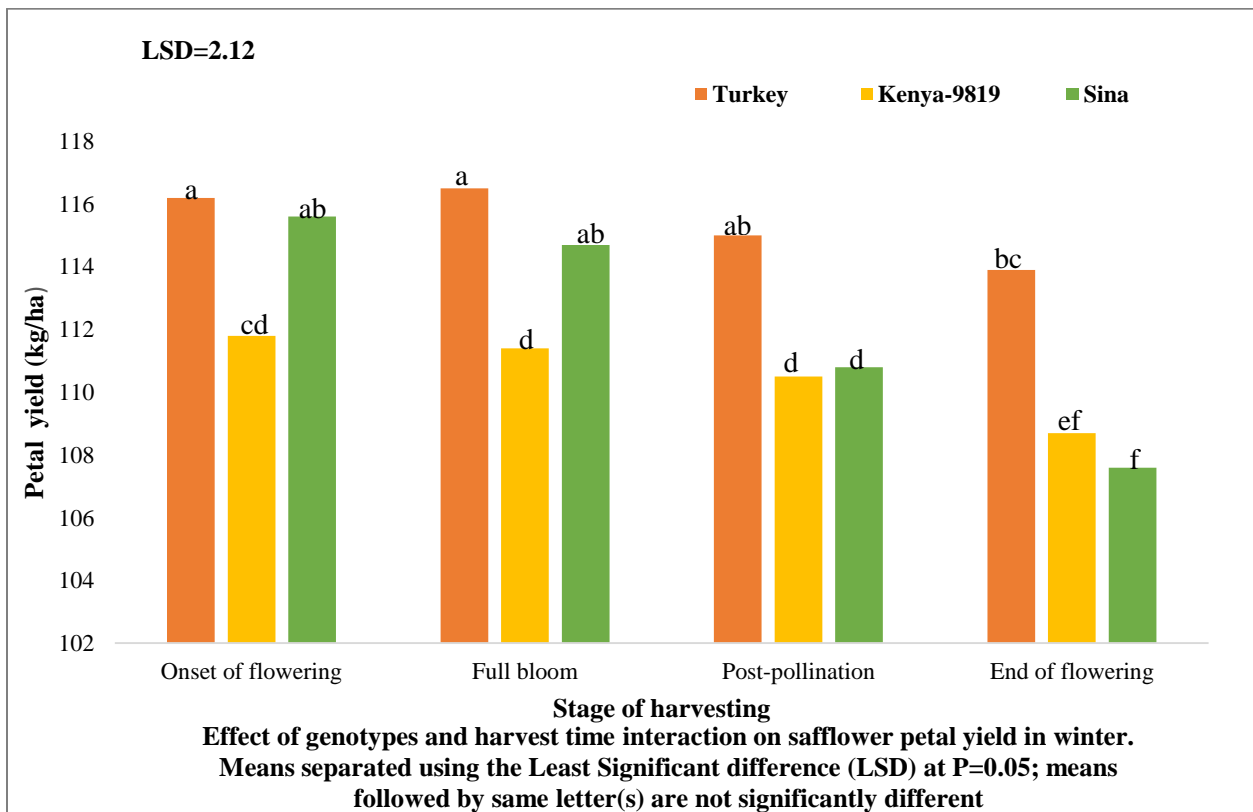
Figure 6: Effect of genotypes and harvest time interaction on safflower petal yield in winter.

4.4 Effect of genotype and harvest time on dye content

4.4.1 Carthamidin content

Safflower genotypes and harvest time interacted significantly (P<0.05) to influence the carthamidin petal content in both summer and winter (Figure 7, 8). The genotype Sina and petals harvested at the onset of flowering had significantly (P<0.05) the highest carthamidin content of 7.5% in comparison to other genotypes and harvest times besides the genotype Sina harvested at full bloom for safflower grown in summer (Figure 7). The genotype Kenya-9819 harvested at end of flowering produced significantly (P<0.05) the lowest petal carthamidin content of 1.6% in contrast to other genotypes and harvest times during summer (Figure 7). The genotype Sina petals harvested at post-pollination had petal carthamidin content of 5.8% which did not significantly (P>0.05) differ with petal carthamidin contents of the genotypes Kenya-9819 and Turkey harvested at onset of flowering in summer (Figure 7). Moreover, the genotypes Turkey and Kenya-9819 petals harvested at full bloom and post-pollination did not statistically (P>0.05) dissimilar in their carthamidin contents in summer (Figure 7). Based on genotype and petal harvest time, the range of petal carthamidin content was 1.6-7.5% for summer grown safflower (Figure 7).
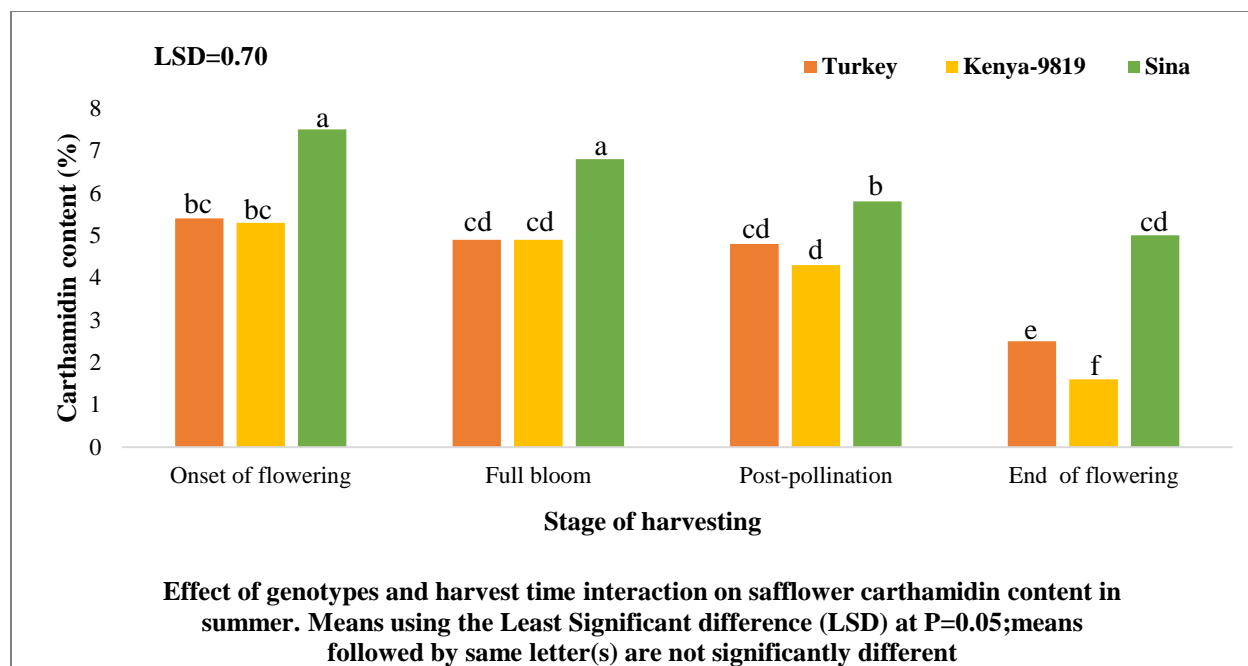
Effect of genotypes and harvest time interaction on safflower carthamidin content in summer. Means using the Least Significant difference (LSD) at P=0.05;means followed by same letter(s) are not significantly different

Figure 7: Effect of genotypes and harvest time interaction on safflower carthamidin content in summer.

At the onset of flowering the genotype Sina significantly (P<0.05) produced petals with the highest carthamidin content of 7.0% in comparison to other genotypes and harvest times for safflower grown in winter (Figure 8). During winter, the genotype Kenya-9819 harvested at end of flowering produced significantly (P<0.05) the lowest petal carthamidin content of 1.0% in contrast to other genotypes and harvest times (Figure 8). The genotype Sina petals harvested at full bloom had significantly (P<0.05) the second highest carthamidin content of 6% compared to other genotypes harvested at different times in winter (Figure 8). The genotype Sina harvested post-pollination did not significantly (P>0.05) differ in their carthamidin contents with genotypes Turkey and Kenya-9819 harvested at the onset of flowering in winter (Figure 8). The range of carthamidin content was between 1.0-7.0% depending on the safflower genotype and petal harvest time during winter (Figure 8).

50
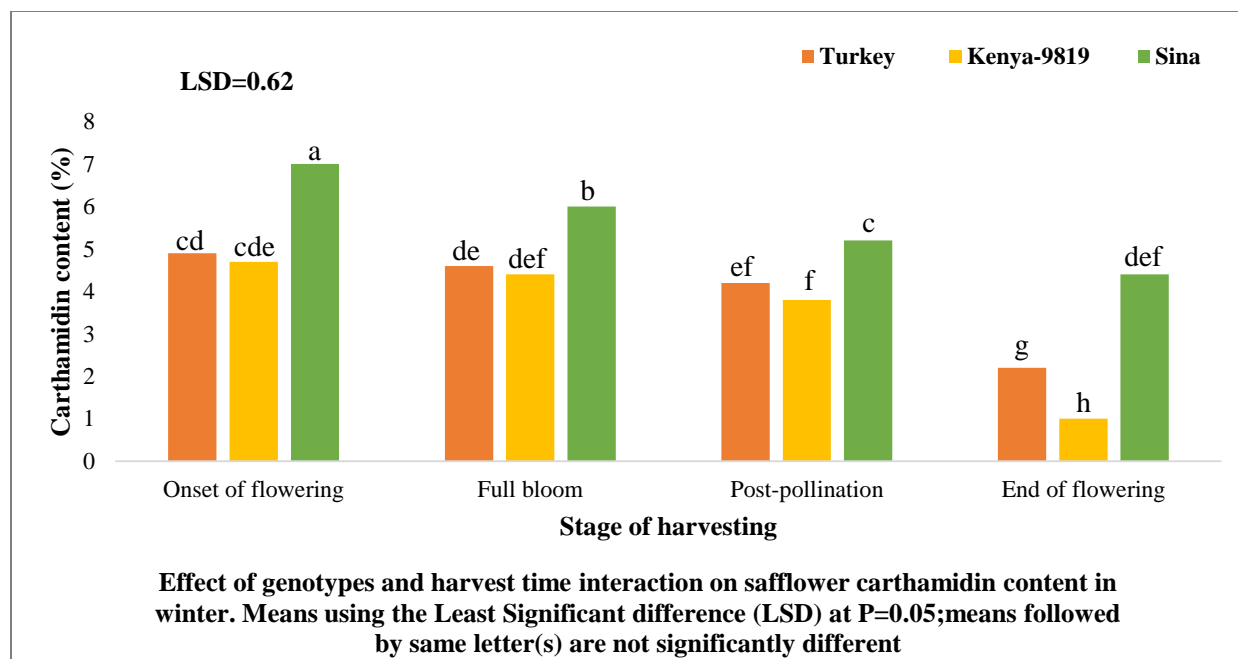
Figure 8: Effect of genotypes and harvest time interaction on safflower carthamidin content in winter.

4.4.2 Carthamin content

Safflower genotypes and harvest time interacted significantly (P<0.05) to affect petal carthamin content (Figure 9, 10). The genotype Turkey and petals harvested at the post-pollination had significantly (P<0.05) the highest petal carthamin content of 0.049 % in contrast to other genotypes and harvest times in summertime planted safflower (Figure 9). The genotype Kenya-9819 gathered at onset of flowering produced significantly (P<0.05) the lowest petal carthamin of 0.024% compared to other genotypes and harvest times with exception of the genotype Sina harvested at the onset of flowering in summer (Figure 9). In summer, genotypes Sina, Kenya-9819, and Turkey petals collected post-pollination and end of flowering, post-pollination, also end of flowering, respectively did not significantly (P>0.05) change in their carthamin contents (Figure 9). In summer grown safflower, the petal carthamin content ranked between 0.024-0.049% depending on genotype and petal harvest time (Figure 9).

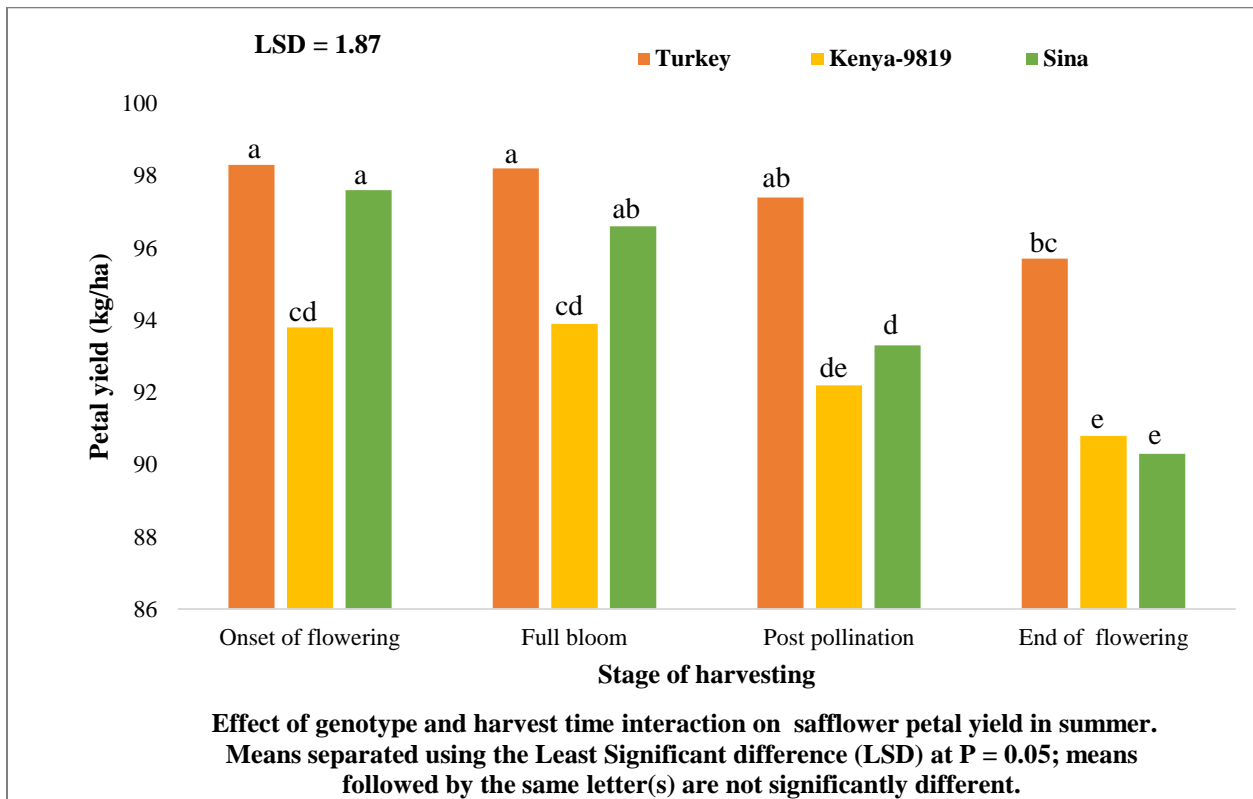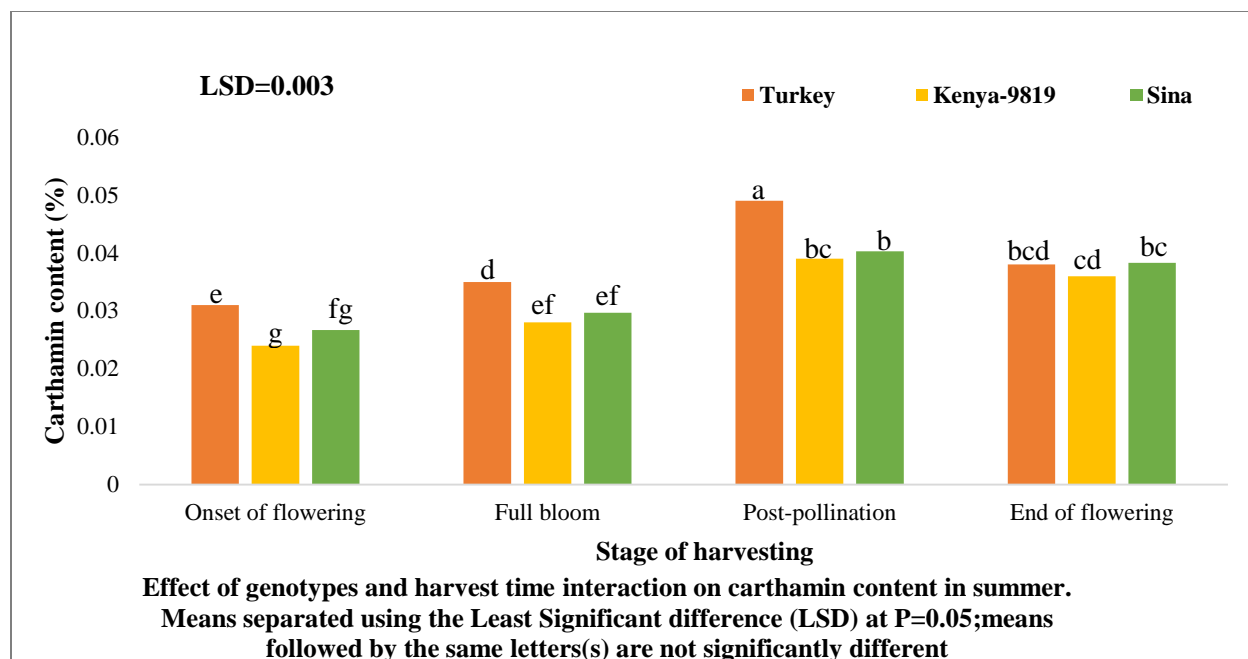**Figure 9:** Effect of genotypes and harvest time interaction on carthamin content in summer.

The genotype Turkey and petals harvested at post-pollination had significantly ($P<0.05$) higher carthamin content of 0.044% compared to other genotypes and harvest times for safflower grown in winter (Figure 10). The genotype Kenya-9819 petals harvested at onset of flowering had significantly ($P<0.05$) lower carthamin content of 0.018% than other genotypes and harvest times with the exception of the genotypes Kenya-9819 and Sina harvested at full bloom and onset of flowering, respectively in winter (Figure 10).The genotypes Kenya-9819, Sina and Turkey petals harvested at post-pollination and end of flowering, post-pollination and end of flowering, respectively, did not significantly ($P>0.05$) differ in their petal carthamin contents in winter (Figure 10). Furthermore, the genotypes Turkey and Sina petals harvested at onset of flowering and full bloom, and full bloom and end of flowering, correspondingly, did significantly ($P>0.05$) differ in their carthamin contents in winter (Figure 10). Carthamin content ranging within 0.018 and 0.044 % subject on the genotype and petal harvest time (Figure 10).

LSD=0.005

Turkey   Kenya-9819   Sina

Stage of harvesting
Effect of genotypes and harvest time interaction on carthamin content in winter.
Means separated using the Least Significant difference (LSD) at P=0.05;means
followed by the same letter(s) are not significantly different
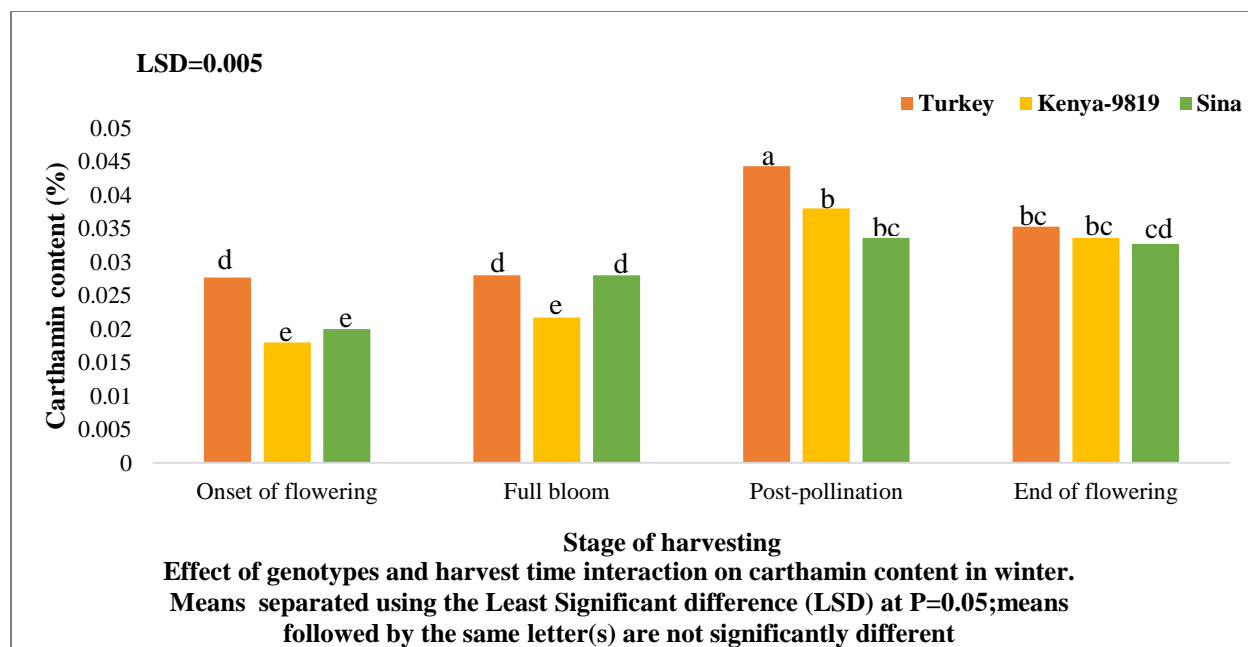
Figure 10: Effect of genotypes and harvest time interaction on carthamin content in winter.

4.4.3 Dye index

Safflower genotypes and harvest times interacted significantly (P<0.05) to influence petal dye index in summer and winter (Figure 11, 12).The genotype Turkey and petals collected at post-pollination had significantly (P<0.05) the highest dye index of 5 in comparison to other genotypes and harvest times with exception of the genotypes Turkey, Kenya-9819, and Sina petals harvested at the full bloom and end of flowering, post-pollination and end of flowering, and end of flowering, respectively, in summer cultivated safflower (Figure 11).The genotype Sina petals harvested at full bloom had significantly (P<0.05) the lowest petal dye index of 1.82 compared to other genotypes and harvest times excluding the genotypes Sina and Kenya-9819 petals harvested at onset of flowering in summer (Figure 11). The genotype Sina petals harvested at the end of flowering did not significantly (P>0.05) differ in their index with genotypes Turkey and Kenya-

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9819 petals harvested at onset of flowering and full bloom, and post-pollination, respectively in summer (Figure 11). In summer safflower petal dye index spanning between 1.82 and 5.0 dependent in the genotype and petal harvest time (Figure 11).

LSD=0.755

Legend: ■ Turkey ■ Kenya-9819 ■ Sina

X-axis (Stage of harvesting): Onset of flowering, Full bloom, Post-pollination, End of flowering
Y-axis (Petal dye index): 0 to 6

Effect of genotypes and harvest time interaction on safflower petal dye index in summer. Means separated using the Least Significant difference (LSD) at P=0.05; means followed by the same letter(s) are not significantly different

Figure 11: Effect of genotypes and harvest time interaction on safflower petal dye index in summer.

In winter grown safflower, the genotype Turkey petals harvested at end of flowering had significantly (P<0.05) the highest petal dye index of 5.0 in contrast to other genotypes and harvest times except for the genotypes Turkey, Kenya-9819, and Sina petals harvested at post pollination, and end of flowering, respectively, during wintertime (Figure 12). For winter grown safflower, the genotype Sina harvested at full bloom had significantly (P<0.05) the lowest petal dye index of 1.0 in contrasted to other genotypes and harvest times except for the genotypes Sina and Kenya-9819 petals harvested at the onset of flowering (Figure 12). In winter, safflower petal dye index ranged among 1.0 and 5.0 depending on genotype and petal harvest time (Figure 12).

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Effect of genotypes and harvest time interaction on safflower petal dye index in winter. Means separated using the Least Significant differnce (LSD) at P=0.05;means followed by the same letter(s) are not significantly different
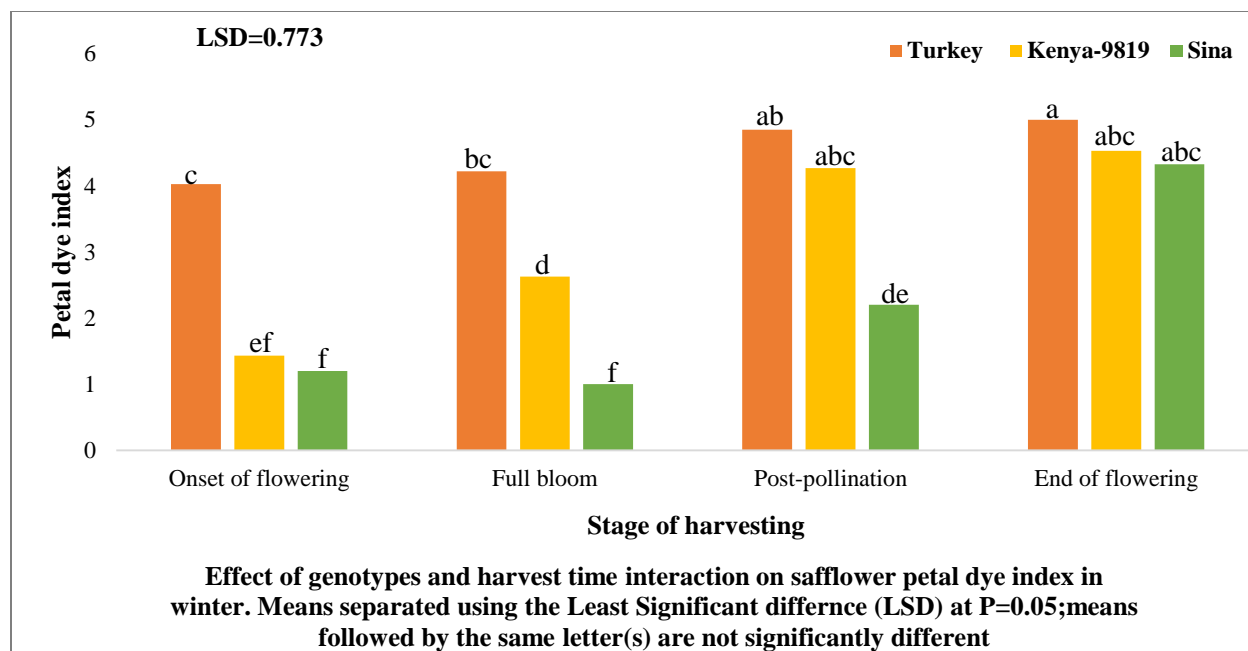
Figure 12: Effect of genotypes and harvest time interaction on safflower petal dye index in winter.

4.5 Effect of genotype and harvest time on mineral composition of safflower petals

4.5.1 Calcium content

There was significant (P<0.05) genotypic variation on petal calcium (Ca) content both in summer and winter (Table 6). The genotype Turkey had significantly (P<0.05) higher petal Ca content of 517.18 and 511.5 mg/100 g in summer and winter, respectively, than other genotypes (Table 6). The genotypes Sina and Kenya-9819 did not significantly (P>0.05) vary in their petal Ca content in both summer and winter grown safflower (Table 6). The Ca petal content was 459.82 and 454.15 mg/100 g in summer and winter, singly, subject on genotype (Table 6). Petal harvest time had a significant (P<0.05) effect on petal Ca content both in summer and winter (Table 6). Petal harvesting at end of flowering had significantly (P<0.05) the highest petal Ca content of 523.91 and 520.19 mg/100 g in summer and winter, respectively, than other petal harvest times (Table 6). However, gathering petals at the onset of flowering had significantly (P<0.05) the lowest petal Ca content of 427.25 and 421.99 mg/100 g in summer and winter, respectively, than other petal

harvest times (Table 6). Generally, the earlier the petal harvest date the lower the petal Ca content (Table 6). Summer grown safflower had significantly (P<0.05) higher petal Ca content of 459.82 mg/100 g than winter planted (454.15 mg/100 g) liable to the petal harvest time (Table 6).

4.5.2 Magnesium content

The petal magnesium (Mg) content did not significantly (P>0.05) vary with genotype both in summer and winter (Table 6). There was also no significant (P>0.05) interaction of genotype and petal harvest time (Table 6). However, petal harvest time had a significant (P<0.05) effect on petal Mg content (Table 6). Petals harvested at full bloom had significantly (P<0.05) the highest Mg content of 305.76 and 298.13 mg/100g in summer and winter, in turn, compared to other petal harvest times (Table 6). On the contrary, petals harvested at the end of flowering had significantly (P<0.05) the lowest Mg content of 260.44 and 257.74 mg/100g in summer and winter, respectively, compared to other petal harvest times (Table 6). The petal Mg content ranged between 257.74-305.76 mg/100g dependent on the harvest time (Table 6).

4.5.3 Potassium content

The main effects of genotype and petal harvest time significantly (P<0.05) influenced petal potassium (K) content (Table 6). The genotype Turkey had significantly (P<0.05) higher petal K content of 2327.88 and 2313.80 mg/100g in summer and winter, respectively, than the genotypes Sina and Kenya-9819 (Table 6). The genotypes Sina and Kenya-9819 did not significantly (P>0.05) differ in their petal K content (Table 6). The petal K contents ranged between 2227.21-2327.88 and 2214.73- 2313.80 mg/100 g in summer and winter grown safflower, respectively, depending on genotype (Table 6). Petals harvested at full bloom had significantly (P<0.05) higher K content of 2327.88 and 2313.80 mg/100 g in summer and winter, respectively, than other petal

harvest times (Table 6). On the contrary, petals harvested at the onset of flowering had significantly (P<0.05) the lowest K content of 2232.40 and 2217.97 mg/100 g in summer and winter, respectively, than other petal harvest times (Table 6). Summer grown safflower had significantly (P<0.05) higher petal K content of 2261.06 mg/100g than winter (2247.64 mg/100 g) grown safflower (Table 6).

Table 6: Effect of genotype and time of petal harvest on calcium, magnesium, and potassium of safflower petals

Main effects and interactions	Calcium (mg/100 g)		Magnesium (mg/100g)		Potassium (mg/100g)	
	Summer	Winter	Summer	Winter	Summer	Winter
Genotype(G)						
Sina	431.37b	427.30b	279.32a	277.16a	2227.21b	2214.46b
Kenya-9819	430.91b	424.10b	278.91a	275.10a	2228.06b	2214.73b
Turkey	517.18a	511.05a	277.52a	273.38b	2327.88a	2313.80a
Significance	***	***	NS	NS	***	***
LSD	3.163	3.898	6.441	3.847	1.582	2.497
Harvest time (H)						
Onset of flowering (H1)	427.25d	421.99d	276.13b	274.30b	2232.40d	2217.97d
Full bloom (H2)	437.99c	430.86c	305.76a	298.13a	2283.16a	2269.29a
Post-pollination (H3)	450.12b	443.55b	271.99c	270.69c	2275.16b	2261.82b
End of flowering (H4)	523.91a	520.19a	260.44d	257.74d	2253.53c	2241.49c
Significance	1.979	2.362	3.946	3.524	1.706	2.494
LSD	***	***	***	***	***	***
Interactions						
G*H	NS	NS	NS	NS	NS	NS

*, **, ***, NS. Significant at 0.05, 0.01, 0.001 or not significantly, respectively. Means separated using the Least Significant Difference (LSD) at P<0.05, means within column followed by the same letter(s) are not significantly different.

4.5.4 Sodium content

The sodium (Na) petal content of genotypes and the interaction of genotypes and petal harvest time did not significantly (P<0.05) differ (Table 7). However, time of petal harvest had a significant (P<0.05) effect on petal Na content (Table 7). Petals harvested at full bloom had significantly (P<0.05) higher Na content of 249.84 and 246.45 mg/100 g in summer and winter, respectively, than other petal harvest times (Table 7). Petals harvested at the onset of flowering had lower Na content of 199.16 and 195.13 mg/100 g in summer and winter grown safflower, respectively, than other petal harvest times (Table 7). Petals harvested at post-pollination had significantly (P<0.05) higher Na content both in summer and winter than petals harvested at the end of flowering (Table 7).

4.5.5 Iron content

Safflower genotypes and petal harvest time had significant (P<0.05) effect on iron (Fe) petal content, but there was significant interaction of genotype and petal harvest time on Fe content (Table 7). The genotype Turkey had significantly (P<0.05) higher petal Fe content of 16.93 and 14.65 mg/100 g than other genotypes (Table 7). However, the genotypes Sina and Kenya-9819 did not significantly (P>0.05) vary in their petal Fe contents in summer and winter (Table 7). Summer grown safflower had significantly (P<0.05) higher petal Fe content of 15.57 mg/100 g than winter grown safflower which had Fe petal content of 12.86 mg/100 g (Table 7).

Petals harvested at the end of flowering had significantly (P<0.05) higher Fe content of 20.23 and 15.98 mg/100 g in summer and winter, respectively, than other petal harvest times (Table 7). Petals harvested at the onset of flowering had significantly (P<0.05) lower petal Fe content of 11.87 and

10.32 mg/100 g in summer and winter, respectively, than other petal harvest times (Table 7). Petals harvested at post-pollination had significantly (P<0.05) higher petal Fe content than petals harvested at full bloom (Table 7).

4.5.6 Zinc content

Genotypes and petal harvest time had a significant (P< 0.05) influence on petal zinc (Zn) content, but the interaction of genotype and harvest time had no effect on petal Zn content (Table 7). Summer grown safflower had a significant (P<0.05) genotype effect on petal Zn content, but winter grown safflower (Table 7). The genotype Kenya-9819 had a petal Zn content of 2.65 mg/100 g which was significantly (P<0.05) higher than that of other genotypes (Table 7). The genotypes Sina and Turkey had no significant (P>0.05) variation in their petal Zn content (Table 7). Safflower grown in summer had significantly (P<0.05) high petal Zn content of 2.2 mg/100g than summer grown which had petal Zn content of 1.81 mg/100g (Table 7).

Petals harvested at the onset of flowering had significantly (P<0.05) higher petal Zn content of 2.66 and 2.05 mg/100g in summer and winter, respectively, than other harvest times (Table 7). While petals harvested at the end of flowering had significantly (P<0.05) the lowest petal Zn content of 1.74 and 1.41 mg/100g in summer and winter, respectively, compared to other harvest times (Table 7). In general, delayed petal harvesting significantly (P<0.05) decreased petal Zn content (Table 7).

Table 7: Effect of genotype and time of petal harvest on sodium, iron, and zinc of safflower petals

Main effects and interactions	Sodium (mg/100g)		Iron (mg/100g)		Zinc(mg/100g)	
	Summer	Winter	Summer	Winter	Summer	Winter
Genotype(G)						
Sina	227.17a	224.96a	15.00b	11.82b	2.01b	1.81a
Kenya -9819	228.06a	225.23a	14.79b	12.10b	2.65a	1.82a
Turkey	227.97a	224.30a	16.93a	14.65a	1.94b	1.81a
Significance	NS	NS	***	***	***	NS
LSD	1.373	2.496	0.602	0.833	0.127	0.026
Harvest time (H)						
onset of flowering (H1)	199.16d	195.13d	11.87d	10.32d	2.66a	2.05a
Full bloom (H2)	249.84a	246.45a	14.22c	11.48c	2.30b	1.94b
Post-pollination (H3)	241.86b	238.99b	15.99b	13.60b	2.09c	1.85c
End of flowering (H4)	220.08c	218.75c	20.23a	15.98a	1.743d	1.413d
Significance	1.687	2.494	0.488	0.581	0.130	0.025
LSD	***	***	***	***	***	***
Interactions						
G*H	NS	NS	NS	NS	NS	NS

*, **, ***, NS. Significant at 0.05, 0.01, 0.001 or not significantly, respectively. Means separated using the Least Significant Difference (LSD) at P<0.05, means within column followed by the same letter(s) are not significantly different.

4.6 Effect of genotype and harvest time on proximate analysis of safflower petals

4.6.1 Moisture content

Safflower genotypes had a significant (P<0.05) influence on moisture content for safflower plants grown both in summer and winter, but there was no significant (P>0.05) interaction of genotype and harvest times on safflower petals' moisture content (Table 8).

Moisture content was significantly (P<0.05) lower in summer by 1.74% than during winter reliant on the genotype (Table 8). Turkey had the highest percentage moisture content of 78.66 and 80.28% during summer and winter individually (Table 8) Genotype Sina had the second highest moisture content (75.47 and 77.09%), conversely, was significantly (P>0.05) not different from genotype Kenya-9819 for the duration of summer and winter. For this study harvest time significantly (P<0.05) influence moisture content during summer but was significantly (P<0.01) affecting moisture content during winter (Table 8). Regardless of the season at post pollination the moisture content was higher than all other petal harvest times (Table 8).

4.6.2 Crude fibre content

Genotype significantly (P<0.05) affected crude fibre for safflower petals harvested from plants grown both in summer and winter, however, there was no significant (P>0.05) interaction of genotype and harvest times on safflower petals crude fibre (Table 8). The crude fibre during summer was significantly (P<0.05) lower by 0.11% compared to winter conditional on the genotype (Table 8). The genotype Turkey had the highest percentage of crude fibre (4.54 and 4.64%) over summer and winter separately (Table 8). Genotype Kenya-9819 had the lowest crude fibre (2.87 and 2.98%) in summer and winter, nevertheless significantly (P>0.05) did not differ

from genotype Sina. Under this investigation harvest time significantly (P<0.05) influence moisture content during summer and winter (Table 8). At post-pollination, the crude fibre percentage was at its highest in both seasons (Table 8).

4.6.3 Petal crude protein content

Genotype significantly (P<0.05) influenced crude protein for safflower petals harvested from plants grown both in summer and winter, but there was no significant (P>0.05) interaction of genotype and harvest times on safflower petals crude protein (Table 8). In summer crude protein was significantly (P<0.05) lower by 1.01% in contrast to winter contingent on the genotype (Table 8). The highest percentage of crude protein was with genotype Turkey at 1.79 and 2.80 for both summer and winter, respectively (Table 8). The lowest crude protein % of (1.00 and 2.00% for summer and winter) was by genotype Kenya-9819, but significantly (P>0.05) did not differ from genotype Sina. In this study harvest time significantly (P<0.05) affected safflower petal crude protein during summer and winter (Table 8). Crude protein from safflower petals was highest when collected at post-pollination and at the end of flowering than the other harvest time in both seasons (Table 8).

Table 8: Effect of genotype and time of petal harvest on moisture content, crude fibre, and crude protein of safflower petals

Main effects and interactions	Moisture (%)		Crude Fibre (%)		Crude protein (%)	
	Summer	Winter	Summer	Winter	Summer	Winter
Genotype(G)						
Sina	75.47b	77.09b	2.95b	3.06b	1.21b	2.21b
Kenya-9819	74.94b	76.94b	2.87b	2.98b	1.00b	2.00b
Turkey	78.66a	80.28a	4.54a	4.64a	1.79a	2.80a
Significance	*	*	*	**	*	*
LSD	2.111	2.147	0.789	0.792	0.488	0.483
Harvest time (H)						
Onset of flowering (H1)	75.06b	77.05c	3.13b	3.24b	1.04b	2.05b
Full bloom (H2)	75.78b	77.53bc	3.30b	3.41b	1.21ab	2.22ab
Post-pollination (H3)	77.49a	79.40a	3.82a	3.93a	1.50a	2.60a
End of flowering (H4)	76.56ab	78.42ab	3.56ab	3.66ab	1.47a	2.47a
Significance	*	**	*	*	*	*
LSD	1.597	1.151	0.431	0.431	0.408	0.410
Interactions						
G*H	NS	NS	NS	NS	NS	NS

*, **, ***, NS. Significant at 0.05, 0.01, 0.001 or not significantly, respectively. Means separated using the Least Significant Difference (LSD) at P<0.05, means within column followed by the same letter(s) are not significantly different

4.6.4 Fat content

During summer and winter, the genotype significantly (P<0.01) affected the fat content of safflower petals (Table 9). There was no significant (P>0.05) interaction between genotype and harvest times on safflower petals fat content (Table 9). During summer fat content was significantly (P<0.05) lower by 0.04% equated to winter depending on the genotype (Table 9). The highest percentage of fat content was with genotype Turkey at 3.35 and 3.39 for both summer and winter, respectively (Table 9). The second highest fat content % of (2.89 and 2.93 % in summer and winter) was produced by genotype Sina, but statistically (P>0.05) was not different from genotype Kenya-9819. Harvest time significantly (P<0.01) affected safflower petal fat content during summer and winter (Table 9). Fat content from safflower petals was higher when collected at post-pollination and followed by the end of flowering in comparison to the other harvest times notwithstanding of the season (Table 9).

4.6.5 Ash content

In both summer and winter, genotypes had significantly (P<0.05) influenced ash content of safflower petals and there was no significant (P>0.05) interaction of genotype and harvest times on safflower petals ash content (Table 9). In summer ash was significantly (P<0.05) higher by 0.95% compared to winter depending on the genotype (Table 9). The highest percentage of ash was with genotype Kenya-9819 at 8.63% for summer but during winter genotype Sina had the highest ash content of 7.17% (Table 9). Irrespective of the season genotype Turkey had the lowest ash of (5.36 and 5.16% in summer and winter). Harvest time had a significant (P<0.05) effect on safflower petal ash during winter (Table 9). Ash content from safflower petals was greater when harvested at full bloom followed by onset flowering compared to the other harvest times (Table 9).

4.6.6 Total carbohydrates content

For summer and winter, genotypes significantly (P<0.05) affected the total carbohydrates of safflower petals. Moreover, there was no significant (P>0.05) interaction between genotype and harvest times on safflower petals carbohydrates (Table 9). Total carbohydrates were significantly (P<0.05) higher by 2.07 in summer contrast to winter depending on the genotype (Table 9). The total carbohydrates were recorded at their highest with genotype Kenya-9819 at 10.07% for summer and 8.74% during winter (Table 9). Irrespective of the season genotype Turkey had the lowest total carbohydrates of 3.75% (Table 9). In summer, harvest time had a significant (P<0.05) effect on safflower petal total carbohydrates while during winter harvest time significantly (P<0.05) influenced the safflower total carbohydrates (Table 9). When harvested at onset flowering and full bloom the total carbohydrates are higher compared to the other harvest times (Table 9).

Table 9: Effect of genotype and time of petal harvest on fat content, ash and carbohydrates of safflower petals

Main effects and interactions	Fat content (%)		Ash (%)		Carbohydrates (%)	
	Summer	Winter	Summer	Winter	Summer	Winter
Genotype(G)						
Sina	2.89b	2.93b	8.00a	7.17a	9.48a	7.54a
Kenya -9819	2.50c	2.54c	8.63a	6.81a	10.07a	8.74a
Turkey	3.35a	3.39a	5.36b	5.16b	6.71b	3.75b
Significance	**	**	*	*	*	*
LSD	0.300	0.300	1.821	1.026	2.055	2.644
Harvest time (H)						
Onset of flowering (H1)	2.62b	2.66b	8.02a	6.75ab	10.12a	8.24a
Full bloom (H2)	2.61b	2.65b	7.75a	6.86a	9.34ab	7.33a
Post-pollination (H3)	3.31a	3.35a	6.39a	5.85b	7.38c	4.87b
End of flowering (H4)	3.09a	3.13a	7.16a	6.06b	8.17bc	6.26ab
Significance	**	**	NS	*	*	**
LSD	0.452	0.452	1.692	0.797	1.757	1.898
Interactions						
G*H	NS	NS	NS	NS	NS	NS

*, **, ***, NS. Significant at 0.05, 0.01, 0.001 or not significantly, respectively. Means separated using the Least Significant Difference (LSD) at P<0.05, means within column followed by the same letter(s) are not significantly different

CHAPTER 5

5.0 DISCUSSION

5.1 Effect of genotype on phenological variables

In the present study, genotype had a significant influence on the phenological stages of safflower in both summer and winter. The phenological traits investigated were days to emergence, days to flowering, days to end of flowering, and days to physiological maturity had significant genotypic variation in the current study. In the present investigation, there was variation in genes in phenological attributes which is important in the collection of safflower varieties for breeding purposes because hybrids derived from lines possess greater genetic variety will demonstrate high level of heterosis than convergent races (Moatshe et al., 2020c; Ojaq et al., 2020; Golkar et al., 2017). Genetic diversity has been discovered by several writers in phenological traits of safflower (Ali et al., 2020; Moatshe et al., 2020c; Zhao et al., 2020b; Bahmankar et al., 2016; Golkar, 2014; Golkar et al., 2011; Singh et al., 2008; Mündel et al., 2004; Kotecha, 1979). The genotype Sina had early maturation compared genotypes Turkey and Kenya-9819 in both summer and winter. Breeding of early maturation cultivars is a foremost goal in several breeding plans of crops mainly for regions with water deficit or that experience chilling temperatures towards the end of the season of growth (Mosupiemang et al., 2022; Weiss, 2000; Dajue & Mündel, 1996). Days to emergence and physiological maturity are important in earliness in plants because it's a mechanism of drought escapers and a strategy of avoiding insect infestation and disease infections (Farooq & Siddique, 2022; Mosupiemang et al., 2022; Emongor et al., 2017; Oarabile, 2017; Golkar, 2014; Farooq et al., 2009; Weiss, 2000). Duration to flowering and physiological maturity are important phenological variables that influencing yield of safflower (Emongor & Emongor, 2022; Moatshe

et al., 2020c; Moatshe, 2019; Emongor et a*l*., 2017; Oarabile, 2017; Golkar, 2014; Weiss, 2000). The current study's outcomes are consistent with those of Moatshe, (2019) who stated that safflower genotypes took 9-10 and 9-10, 30-37 and 35-41, 40-50 and 50-59, 59-67 and 67-71, 82-99 and 110-116, and 100-116 and 135-147 days after sowing to emerge, rosette stage, elongation stage, branching, flowering, and physiological development in summer and winter of southern Botswana, respectively. The duration of safflower to reach flowering varies with genotype and environmental conditions and ranged between 113-121 days after sowing (DAS) in Egypt (Shabana et al., 2013), 103-130 DAS in southern Australia (Wachsmann et al., 2001), 55-77 DAS in Iran (Bahmankar et al., 2016), and 100-147 DAS in southern Botswana (Moatshe, 2019).

The phenological stages of safflower are under genetic control (Golkar et al., 2012a, 2012b, 2017; Golkar, 2011, 2014; Pahlavani et al., 2012; Shahbazi & Seaidi, 2007; Gupta & Singh, 1988; Kotecha, 1979). For example, duration of rosette, elongation, and flowering stages are controlled by additive gene effects (Golkar, 2011). Additive (Shahbazi & Seaidi, 2007; Kotecha, 1979) and over dominant of gene effects (Gupta and Singh, 1988) have been disclosed to govern days to maturation of safflower. While days to flowering of safflower has been reported to be under the control of dominant (Golkar, 2011), over dominant (Shivani et al., 2012), partial dominant (Singh & Gupta, 1988), and both additive and dominant (Singh et al., 2008) gene effects. The variation in findings reported above could be credited to dissimilarities in genotypes and environmental settings in the various studies.

The phenological factors investigated in the present study were significantly influenced by the growing season. The winter season safflower cultivation resulted in noticeably longer phenological phases (emergence, flowering, end of flowering, and physiological maturity) compared to summer. The genotypes of safflower utilized throughout this investigation took 8.3-9.1 and 9.5-11.5, 74-80

and 111-117, 84-90 and 119-126, and 99-109 and 140-157 days after sowing to emerge, flower, end of flowering, and physiological maturity in summer and winter, respectively. During the current study period, the monthly mean minimum and maximum temperatures in summer and winter was 13-20.1°C and 29.5-31.8°C, and 4.8-15.4°C and 20.8 -28.9°C, respectively (Appendix 2, 3). Therefore, the significantly longer duration of the phenological stages of safflower grown in winter was credited to cooler air temperature that favoured growth and developing of safflower than in summer. The most favourable temperature for seed germination and growth of safflower is 15.6°C and 20-32°C, respectively (Torabi et al., 2013, 2015; Emongor & Oagile, 2017; Kaffka & Kearney, 1998; Mündel, 1969). For root development and rosette formation, safflower seedlings need low temperatures of 15-20°C, whereas temperatures of 20-30°C are needed for stem elongation and flowering to occur (El-Bassam, 2010; Li, 1989; Mündel et al., 1992; Emongor & Oagile, 2017; Li et al., 1997; Emongor & Emongor, 2022; Afzal et al., 2022 ; Carapetian, 2001). The latest study's result support the previously published findings in literature (Emongor, 2010; Torabi et al., 2013, 2015; GRDC, 2017; Moatshe, 2019; Abou Chehade et al., 2022; Moatshe et al., 2020c; Kolanyane, 2022). Wachsmann et al. (2001) attributed to the interval of the phenological stages of safflower in relation to temperatures along with photoperiod in southern Australia. While Ahadi et al. (2011) credited the length of phenological phases of safflower to sowing date and temperature in Iran. In general, temperature affects the growth and development of all plants (Slafer & Rawson, 1994). Temperature determines how quickly plants develop, and the rate of growth of plants in the physiological range (10–30°C) correlates positively with temperature, with growth hastened when temperature is increased (Ritche & Ne Smith, 1991; Goudriaan & Laar, 1994; Raven et al., 1999).

Safflower is a day-neutral crop (Dajue & Mündel, 1996) however, long days promote flowering (Dadashi & Khajehpour, 2004; Gilbert, 2008). Safflower summer cultivars in temperate zones, grown as winter crops in sub-tropical and tropical locations with seasonally short days have extended the phases of phenology (Dajue and Mündel, 1996; Gilbert, 2008; Emongor et al., 2017; Moatshe, 2019; Emongor & Emongor, 2022). Cold temperatures (less than 20°C) and short days (fewer than 10 hours) experienced in Botswana during winter delays all the phenological stages of safflowers regardless of population of plants or genotype (Emongor et al., 2015, 2017; Moatshe, 2019). Interaction of temperature and day length is also reported to contribute to safflower phenological phases differing in duration (Dadashi & Khajehpour, 2004).

5.2 Effect of genotype on vegetative growth

In the current study, genotype had a significant effect on safflower vegetative growth (height to first branching, plant height, and number of primary branches/plant) in both summer and the winter. The genotype Turkey had significantly the highest height to first branching, plant height, and number of primary branches/plant compared to genotypes Kenya-9819 and Sina. The genetic deviation in vegetative progression of safflower observed in the recent investigation was due to genetic and environment (summer and winter conditions) interaction. Safflower plant height has been reported to be governed by additive gene effects (Kotecha, 1979; Shahbazi & Saeidi, 2007; Golkar et al., 2012). Mandal and Banerjee (1997) reported that safflower plant height was not influenced by extra nuclear (genes present in cytoplasmic organelles such as mitochondria and chloroplasts) genes. Gupta and Singh (1988) found number of primary branches/plant of safflower are controlled by additive gene effects. While Narkhede and Patil (1987) reported that number of primary branches/plant of safflower to be controlled by epistasis gene effects. However, Golkar et al. (2012a) stated that there was no significant effect of epistasis in number of primary

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branches/plant of safflower. Branching pattern in safflower is meticulous regulated by both digenic genes and environmental conditions (Deokar & Patil, 1975). While Kose (2019) reported that height of first branch development in safflower was due to low narrow sense heritability and it's under the control of non-additive gene effects. Genotypic variant in safflower vegetative development has been reported in literature (Moatshe et al., 2020c, La Bella et al., 2019; Moatshe, 2019; Oarabile, 2017; Killi et al., 2016; Hamza, 2015; Zareie et al., 2013; Ada 2013; Abd El-Lattief, 2012; Camas et al., 2007; Carapetian, 2001; Bratuleanu, 1997; Dajue & Mündel, 1996). Bratuleanu (1997) reported that safflower plant height was determined by genotype x environment x cultural practices interaction (Dajue et al., 1993; Dajue & Mündel, 1996; Weiss, 2000; Camas et al., 2007). Significant genotype variations for safflower vegetative growth (initial branching height, height of plant, and sum branches/plant) of safflower have been accounted for in writings (Camas et al., 2007; Killi et al., 2016; Emongor et al., 2017, Arzu, 2019; Moatshe, 2019; Moatshe et al., 2020c, Koç,2021). Moatshe (2019) indicated that genotypes Sina had the tallest plants with the highest primary branch number/plant. On the contrary, the genotype Gila had the shortest plants with the fewest primary branch number/plant (Moatshe, 2019).

In the current study, the average height to first branching, plant height, and number of primary branches/plant in summer was 13.59, 73.43, and 8.19 cm, respectively. While in winter, the average height to first branching, plant height, and number of primary branches/plant was 31.18, 91.21, and 10.60 cm, respectively. The significant variation in vegetative growth (height to first branching, plant height, and number of primary branches/plant) between summer and winter grown safflower was attributed to temperature (Appendix 2, 3). The reason for the disparity in safflower plant height and height to first branching was due to the higher change among night and day temperatures (DIF) in winter (average minimum and maximum temperatures 4.8-15.4 and

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20.8-28.9°C).In summer the mean minimum and maximum temperatures during the experimentation period was 13-20.1 and 29.5-31.8°C. Positive DIF in some plants such as safflower and other ornamental plants such as poinsettia, lilies, chrysanthemums, and roses promotes stem elongation due to induced biosynthesis of gibberellins hence explaining the taller plants and higher first branching height in winter grown safflower than summer plants (Went, 1944; Erwin et al., 1989; Berghage & Heins, 1991; Karlsson et al., 1989; Myster & Moe, 1995; Dole & Wilkins, 2005). Temperature differences throughout day and night have physiological impact on stem elongation (Went, 1944; Emongor et al., 2013; 2015). The use of DIF to manage plant height has been shown in many plant species (Myster & Moe, 1995). When DIF is positive and higher, the higher the stem elongation due to increased internode elongation induced by gibberellins (Berghage & Heins, 1991; Erwin et al., 1989; Myster & Moe, 1995; Taiz & Zeiger, 2002; Dole & Wilkins, 2005; Emongor et al., 2015). Optimal level of day and night temperatures is important for the balance of photosynthetic and respiration rates which both are key contributors to growth and yield of crops through dry matter accumulation. The conducive cool temperatures in winter promoted development of more primary branches of safflower than summer in the current study. In accordance with the literature, the best temperature for safflower to develop and grow was 20-32°C (Mündel, 1969; Dajue & Mündel, 1996; Torabi et al., 2015; OGTR, 2019; OECD, 2020; Emongor & Emongor, 2022; Kaffka & Kearney, 1998). Better vegetative growth of safflower grown in winter than summer has been recounted in literature (Kedikanetswe, 2012; Emongor et al., 2013, 2015,2017; Moatshe et al., 2016, 2020b; Sarkees & Tahir, 2016; Oarabile, 2017; Sampaio et al., 2017; Moatshe, 2019). Emongor et al. (2017) reported that winter grown safflower had 70 more primary branches/plant than summer grown plants. While Sampaio et al. (2017) observed that winter grown safflower had significantly higher number of primary

branches/plant than summer grown plants. Sarkees and Tahir (2016) also reported that there were significantly fewer number of primary branches/plant in summer grown safflower than winter. They attributed this to shortened safflower growth cycle in summer than winter which resulted in short duration of vegetative phase which was essential in promoting lateral growth (Sarkees & Tahir, 2016; Emongor et al., 2015).

5.3 Effect of genotype and harvest time on yield components

The yield components of safflower are branch number/plant, number of capitula/plant, number of achenes (seeds)/capitulum, and 1000-seed weight (Kolanyane, 2022; Moatshe et al., 2020b, Moatshe, 2019; Emongor & Oagile, 2017; Emongor et al., 2015, 2017; Oarabile, 2017; Ahmadzadeh et al., 2012; Amini et al., 2008; Camas & Esendal, 2006; Bagheri et al., 2001; Gonzalez et al., 1994; Chaundry, 1990). The safflower yield components determined in the current study included capitula diameter, number of capitula/plant, number of seeds (achenes)/capitulum, and 1000-seed weight. There was significant genotypic variation in yield components of safflower. The genotypes Turkey and Sina had significantly the highest and lowest yield components both summer and winter grown safflower with exception of 1000-seed weight in which the genotype Kenya-9819 had the lowest weight. The significant genotypic variation observed in the current study was explained by different genetic expressions among the genotypes evaluated on these traits. Number of capitula/plant is reported to be governed by dominant gene effects (Pahlavani et al., 2007). While Deshmakh et al. (1991) reported a high heterosis for number of capitula/plant. However, other researchers have reported additive×additive and dominance×dominance epistasis gene effects were accountable for the genetic control of number of capitula/plant (Shahbazi & Saeidi (2007). While Sahu and Tewari (1993) described that additive-dominance gene effects were responsible for the genetic regulation of number of capitula/plant in safflower. While

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Ramachandram and Goud (1981) demonstrated that the function of maternal impact a significant part in the inheritance of number of capitula/plant and 1000-seed weight. Number of seeds/capitulum has been reported under the influence of additive gene impact (Mandal & Banerjee, 1997; Singh & Pawar, 2005). This infers that enhanced performance might be achieved through selected breeding of number of seeds/capitulum. Golkar et al. (2012b) reported that the genetic regulation of 1000-seed weight of safflower was brought about by additive gene effects. Furthermore, additive-dominance (digenic model) has been reported to have a significant role in 1000-seed weight of safflower (Shahbazi & Saeidi, 2007). According to Golkar (2014) and Golkar et al. (2012b) safflower seed yield and its components are under the control of additive gene effects with exception of number of capitula/plant. Also, Golkar, (2014) and Golkar et al. (2012b) reported that safflower capitula diameter (size) was under the control of dominant gene effects. Also, safflower capitula diameter is reported to have a low broad-sense heritability (Camas & Esendal, 2006). Genetic variation of safflower yield components is reported in literature (Moatshe et al., 2020b; Moatshe, 2019; Oarabile, 2017; Killi et al., 2016; Hamza, 2015; Zareie et al., 2013; Kedikanestwe, 2012; Emongor et al., 2013, 2015, 2017; Camas et al., 2007; Singh & Nimbkar, 2006; Gonzalez et al., 1994). Moatshe (2019) reported significant genotypic variation in safflower yield components. The genotypes 'Sina' and 'Gila' had significantly the highest and lowest yield components in Safflower cultivated throughout the height of summer and wintertime. Emongor et al., (2017) and Oarabile (2017) found genotypic variation of number of capitula/plant, capitula diameter, number of seed/ capitulum, and 1000-seed weight. Genotypic variation of safflower is reported also to depend on the environmental conditions, inherited traits, and cultural practices (Singh & Nimbkar, 2006; Jonchike et al., 2002; Rahamatalla et al., 2001). Mahasi et al. (2006) noted significant genotypic x environment interaction for number of capitula number/plant,

capitula size, number of seeds/capitulum, and 1000-seed weight. The significant genetic x environment interaction demonstrated the presence of large distinctions among genotypes and growing seasons, and that different cultivars responded differently to different seasons (Singh & Pawar, 2005; Mahasi et al., 2006). Also, in the current study all the safflower genotypes under investigation had higher yield components (capitula diameter, number of capitula/plant, number of seeds (achenes)/capitulum, and 1000-seed weight) in winter than summer. Ashri et al., (1974) reported of significant genotype x environment (G x E) interaction for number of capitula/plant, seed number/capitulum, and 1000-seed weight. The existence G x E interaction in safflower yield components breeding is needed to suit regional circumstances. Elfadl et al., (2010) reported that excellent diversity existed among safflower genotypes which provides opportunities for breeding initiatives decision for local growing conditions.

Also, in the current study petal harvest time had a substantial impact on capitula diameter, number of seeds/capitulum, and 1000-seed weight. Harvesting safflower petals at post-pollination and end of flowering promoted capitula diameter and number of seeds/capitulum. While harvesting petals at the onset of flowering increased 1000-seed weight. The increase in capitula diameter due to harvesting petals at post-pollination and end of flowering was attributed to the induce increase in number of seeds/capitulum. While the increase in 1000-seed weight induced by petal harvesting at the onset of flowering was attributed to effective pollination which might have taken place in the process of picking the petals since harvesting is done manually with fingers.

5.4 Effect of genotype on safflower seed yield

Genotypic variation on safflower seed yield was observed in the current study irrespective of growing season. The genotype Turkey had considerably more seed yield of 1479 and 2697 kg/ha in summertime and winter, correspondingly, than other genotypes. The genotypes Kenya-9819 and

Sina did not vary in their seed yield in summer and winter. The genetic variation in seed yield of safflower in the existing study was partly explained by genetic variation in the yield components capitula diameter, number of capitula/plant, number of seeds (achenes)/capitulum, and 1000-seed weight but higher in the genotype Turkey than other genotypes in the study. The genetic variation in seed yield of safflower was also attributed to genetic expression of the different genotypes with respect to seed yield. Golkar et al. (2012b) and Golkar (2014, 2017) recounted that seed yield of safflower is under the control of additive gene effects. Several scientists have reported high approximations of broad-sense heritability for safflower seed harvest (Mather & Jinks, 1982; Falconer & Mackay, 1996; Camas & Esendal, 2006; Golkar et al., 2012a, b; Golkar, 2014). It's further reported that epistasis may also be involved in the genetic variation of safflower seed produce (Mather and Jinks, 1982; Golkar *et al*., 2012; Golkar, 2014). Genetic variation in safflower yield is reported in literature (Moatshe et al., 2020b; Moatshe, 2019; Oarabile, 2017; Killi et al., 2016; Hamza, 2015; Zareie et al., 2013; Kedikanestwe, 2012; Emongor et al., 2013, 2015, 2017; Beyyava et al., 2011; Kizil et al., 2008; Camas et al., 2007; Singh & Nimbkar, 2006; Azari and Khajehpour, 2005; Dadashi & Khajehpour, 2004; Gonzalez et al., 1994).

Also, in the existing study winter grown safflower had an average seed yield of 2511 kg/ha which was significantly higher than the seed yield of 1241 kg/ha produced by summer plants. Moatshe, (2019) described significant seasonal variation of genotypes on seed yield of safflower in Botswana. Wintertime including summer grown safflower produced an average seed yield of 4205 and 4005 kg/ha, respectively, independent of genotype and plant density (Moatshe, 2019). Moatshe (2019) attributed this seasonal variation in safflower seed yield to the longer growing period in winter of 135-147 days to physiological maturity, and higher LAI, LAD, and NAR than summer in which the plants had a short growth period of 100-116 days to physiological maturity,

and lower LAI, LAD, and NAR. The seasonal variations in safflower seed yield have also been attributed to differences in climatic conditions in winter and summer (Tahmasebpour et al., 2016; ulHassan et al., 2015; Emongor et al., 2013, 2015, 2017). Also, the seasonal variation of different yield components of safflower in winter and summer observed in the current study had an impact on seed yield and was more dependent on temperature compared to other abiotic factors. The conducive cool temperatures in winter observed in the current study promoted the development of more primary branches and yield components of safflower than summer in the current study. The results of the current study agree with those stated in literature (OECD, 2020; Mündel, 1969; Dajue & Mündel, 1996; Kaffka & Kearney, 1998; Torabi et al., 2015; Emongor & Oagile, 2017; GRDC, 2017; OGTR, 2019; Emongor & Emongor, 2022). Rasul et al. (2016) reported that temperature fluctuations affected the seed yield of many crops and an increase in temperature of 2-4°C above optimal, caused in a noteworthy drop in seed yield.

5.5 Effect of genotype and stage of harvesting on petal yield

In the current study, in both summer and winter, safflower genotypes and harvest time interacted significantly to influence on petal yield. In summer, the genotype Turkey and petals harvested at the onset of flowering had significantly the maximum petal yield of 98.3 kg/ha likened to other genotypes and harvest times with exception of the genotypes Turkey harvested at full bloom and post-pollination, and Sina harvested at onset of flowering and full bloom in summer grown safflower. While genotype Sina harvested at end of flowering significantly generates the least petal yield of 90.3 kg/ha in juxtaposition with the rest of the genotypes and harvest times aside the genotype Kenya-9819 harvested at post-pollination and end of flowering in summer. In winter the genotype Turkey and petals harvested at full bloom had significantly the highest petal yield of 117 kg/ha in distinction to other genotypes and harvest times besides of the genotypes Turkey

harvested at onset of flowering and post-pollination, and Sina harvested at onset of flowering and full bloom. The genotype Sina harvested at end of flowering substantially produced the least petal yield of 108 kg/ha compared to other genotypes and harvest times with exception of the genotype Kenya-9819 harvested at end of flowering in winter. The significant interaction among genotype and time of petal harvest on petal yield indicated genotype x agronomic practices interaction suggesting that safflower genotypes will produce different petals yields subject to time of petal harvest. The genotype Turkey had the highest petal yield in both summer and wintertime because of the high number of capitula/plant it had in the current study. The number of capitula/plant in safflower are reported to determine petal yield (Steberl et al., 2020b; Singh et al., 2008; Zheng et al., 1993). Omidi and Sharifmoghaddasi (2010) found that height to first branching of safflower was positively correlated with petal yield and the variables plant height, branching height, number of capitula/plant, and number of seed/capitulum directly influenced petal yield (Omidi & Sharifmoghaddasi, 2010). High yielding varieties of safflower tend to be tall with many branches, amount of capitula per plant, and longer flowering period (Johnson et al., 2001; Choulwar et al., 2005; Omidi, 2006; Omidi & Sharifmoghhaddasi, 2010; Kedikanetswe, 2012; Karimi et al., 2013; Emongor & Oagile, 2017; Emongor et al., 2017; Moatshe et al., 2016, 2020b). Due to the positive correlation of number of capitula/plant and safflower petal yield it's hypothesized that safflower petal may be under additive×additive and dominance×dominance epistasis (Shahbazi & Saeidi, 2007; Golkar et al., 2012b; Golkar, 2014) and additive-dominance (Sahu & Tewari, 1993) gene effects since these genes control number of capitula/plant.

In the current study, the petal yield ranged between 90.3-98.3 and 108-117 kg/ha correspondingly, in the summer and the winter, depending on genotype and petal harvest time. Steberl et al. (2020b) reported that safflower petal yield ranged from 2.30 to 469 kg/ha depending on cultivar, year, and

planting density. The results of Steberl et al. (2020b) suggested genotype x environment x agronomic practices interaction. While Omidi and Sharifmoghaddasi (2010) reported that safflower grown for petal production ranged between 120 to 150 kg/ha under rain-fed schemes and 180 to 250 kg/ha using irrigation. The results of the present research are analogous to those published in the scientific literature, which vary from 2 to 648 kg/ha contingent upon the year., cultivar, time of petal and plant density (Steberl et al., 2020b; Hamza, 2015; Mohammadi & Tavakoli, 2015; Koutroubas et al., 2009; Nagaraj, 2009; Kizil et al., 2008; Azari et al., 2005; Armah-Agyeman et al., 2002; Weiss, 2000; Knowles, 1972). Omidi and Sharifmoghaddasi (2010) stated that harvesting safflower petals every three days from the onset of flowering increased petal yield and prolonged flowering time by 22 days. Mohammadi and Tavakoli (2015) observed that the period of petal harvest influenced petal yield. The maximum petal yield was observed when petals were collected at the onset of flowering but lessened afterwards (Omidi & Sharifmoghaddasi, 2010). The recent study's findings indicated that the highest and lowest petal yields of 117 and 90.3 kg/ha were obtained when petals were harvested at the full-bloom and end of flowering every three days, respectively agreeing with the findings of Omidi and Sharifmoghaddasi (2010). While Steberl et al. (2020b) reported that harvesting safflower petals post-pollination resulted in significantly the highest petal yield of 469 kg/ha. Also in the current study, the highest petal yield of 117 kg/ha was obtained from the spineless genotype Turkey. Spiny cultivars of safflower are presumed to produce fewer petals than varieties without spines. (Patil et al., 2005; Pushpa et al.,2023, Kizil et al., 2008). Red flowering spineless safflower varieties were selected for decorative/cut flower and dyeing purposes (Pascual-Villalobos & Alburquerque, 1995; Mohammadi & Tavakoli, 2015).

5.6 Effect of genotype and harvest time on carthamidin and carthamin contents and dye index

In the current study, safflower genotypes and harvest time interacted significantly to influence the petal carthamidin and carthamin contents, and dye index in both summer and winter. The genotypes Sina and Kenya-9819 petals harvested at inception of flowering and completion of flowering, had the highest (7.0-7.5%) and lowest (1.0-1.6%) petal carthamidin content, respectively pending on season of growth. The genotypes Turkey and Kenya-9819 petals harvested at post-pollination and onset of flowering, had the highest (0.044-0.049%) and lowest (0.0018-0.024%) petal carthamin content, respectively pending on season of growth. The genotypes Turkey and Sina petals harvested at post-pollination and full bloom, had the highest (5.0) and lowest (1.0-1.82) petal dye index, respectively pending on season of growth. The significant interaction between genotype and time of petal harvest on carthamidin and carthamin contents, and dye index suggested existence of genotype x agronomic practices interaction implying that safflower genotypes will produce different contents of carthamidin and carthamin, and dye index depending on time of petal harvest. Steberl et al. (2020b) found that the Chinese cultivar had higher carthamidin content in 2018 (6.98–8.12%) than 2017 (5.91–7.29%). Furthermore, throughout all petal harvests periods and in both of the years 2017, as well as 2018, the Chinese cultivar displayed larger carthamidin content (6.45-7.71%) over the German cultivar (2.94-4.56%) (Steberl et al., 2020b). The results of Steberl et al. (2020b) agree with the findings of the current study existence of genotype x agronomic practices interaction with respect to petal carthamidin content. Mohammadi and Tavakoli (2015) found significant interaction of safflower cultivar and petal harvest time on carthamidin and carthamin contents. The safflower cultivar Zendehrood petals

harvested at the onset of flowering had the highest carthamidin content of 5.933% while the cultivar Sina had the lowest carthamidin content of 4.618% (Mohammadi & Tavakoli, 2015). Mohammadi and Tavakoli (2015), further found that the safflower cultivar Zendehrood petals harvested when petals are wilting (after full bloom) had the highest carthamin content of 0.041% while the cultivar MEC88 had the lowest carthamin content of 0.018%. Carthamin content was less in the petals at the initial stages of flowering and elevated subsequent pollination at the outset of petal succumbing to wilting (Mohammadi & Tavakoli, 2015). Other studies have shown differences in petal carthamidin and carthamin contents involving harvest time frames, varieties, and their interactions with one another (Steberl et al., 2020b; Mohammadi & Tavakoli, 2015; Omidi & Sharifmoghaddasi, 2010; Kizil et al., 2008; Trabriz, 2002). The carthamidin content is also reported in literature to be influenced by environmental weather conditions and harvest date (Steberl et al., 2020b; Ghorbani et al., 2015; Mohammadi & Tavakoli, 2015; Fatahi & Heidari, 2009; Mündel et al., 2004; Tabriz, 2002). Steberl et al. (2020b) found that the carthamidin content in safflower petals ranged between 4.60-5.93%. While Mohammadi and Tavakoli (2015) found that the carthamidin content of safflower petals ranged between 4.604-5.933% depending on cultivar and time of harvest. The petal carthamidin content in the current study ranged between 1-7.5% which is comparable to the findings stated in literature (Steberl et al., 2020b; Ghorbani et al., 2015; Mohammadi & Tavakoli, 2015; Mündel et al., 2004; Tabriz, 2002). The genetic variation in petal carthamidin content were ascribed to differences in gene expression of this trait by different genotypes in the present investigation's result. The petal carthamidin content of safflower petals were reported under the control of epistasis gene effects (Golkar, 2018; Rampure et al., 2014; Pahlavani et al., 2004). Furthermore, these particular the research's findings demonstrated that carthamidin content was highest when petals were reaped at the start of flowering and decreased

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thereafter. This was attributed to the existence of the β-glucose oxidase is a specific type of enzyme which oxidizes the carthamidin (yellow) to carthamin (red) progressively (Cho et al., 2000; Ghorbani et al., 2015). Carthamin is biosynthesized from carthamidin via oxidation which explains why carthamin content is low at the onset of flowering but increases as the petals senesce (Cho et al., 2000; Ghorbani et al., 2015; Steberl et al., 2020b). Additionally, according to Mohammadi and Tavakoli (2015), the amount of carthamidin in the safflower florets varied regarding when they were initially taken at the start of flowering, florets had a greater carthamidin level, whereas when peals were picked shortly after pollination towards the start of petal withering had lesser carthamidin amount. Safflower petals had a higher carthamidin content when harvested at the onset of flowering, but petals harvested after pollination at the onset of petal wilting had lower carthamidin content. Safflower petal dye index is related to the ratio of carthamidin to carthamin contents in the petals (Chen et al., 2022; Zhao et al., 2020a). The higher the carthamin content the higher the dye index since carthamin is red in colour. Given that carthamin is biosynthesized by its yellow precursors (precarthamin), the safflower's flower colour begins as yellow and progressively transitions to red as it reaches its peak of flowering. (Chen et al., 2022; Zhao et al., 2020a; Ghorbani., et al., 2015; Cho et al., 2000; Kazuma et al., 1995; Kumazawa et al., 1994).

5.7 Effect of genotype and stage of harvesting on the mineral composition of petals

The present study showed that there was genotypic variation with respect to safflower petal mineral nutritional content (Ca, Mg, K, Na, Fe, and Zn). The genotypes Turkey and Sina had significantly the highest and lowest petal mineral nutritional content of all the elements analysed, respectively. The genotypic variation with respect to mineral nutritional content of safflower petals was attributed to different genetic expressions of this trait by the genotypes being studied in relation to environmental surroundings between summertime and winter. Safflower genotypes

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vary in phenology, morphological makeup and physiology, this variation influences the source-sink strength resulting in variations in the availability of minerals and partition among various plant parts (Dajue & Mündel, 1996; Golkar, 2014; Emongor et al., 2017; Moatshe et al., 2020d). In the current study the safflower petal mineral nutritional contents of Ca, Mg, K, Na, Fe, and Zn ranged between 424.10-517.18, 273.38-279.32, 2214.73-2327.88, 224.3-228.06, 11.82-16.93, and 1.81-2.65 mg/100 g, respectively depending on genotype and growing season. Nagaraj et al. (2001) in India found that Ca, Mg, and Fe petal contents was 530, 287, and 7.3 mg/100 g, respectively. While Barashovets et al. (2016) in Ukraine reported that safflower petals showed a pattern for the contents of minerals, as shown below: K >Ca > Si > Mg > P > Na > Fe > Al > Zn > Sr > Mn. Barashovets et al. (2016) found that safflower petal mineral nutritional content was as follows Na, K, Ca, Fe, Fe, and Zn 54, 2040, 680, 24, and 6.8 mg/100g, respectively. The outcome of the current investigation concurs with those findings highlighted by Nagaraj et al. (2001) and Barashovets et al. (2016). It can be concluded that safflower petals are an excellent source of potassium that aids in decreasing the body's elevated blood pressure readings by counteracting the effects of sodium (Grillo et al., 2019; Mente et al., 2014; Denton et al., 1995). Hence explaining why safflower petals are used in lowering high blood pressure (Lee et al., 2020; Sun et al., 2018; Tan et al., 2020).

Also, in the current study the mineral nutritional content of safflower petals varied with time of harvest. Whenever petals were collected at the ending of flowering, calcium and iron amounts were substantial.While Mg, K, and Na were high when petals were harvested at the full bloom. When petals were taken at the starting point of flowering, levels of zinc were high. Li et al., (2020) reported that mineral elements content of peony flower petals (*Paeonia lactiflora*) was Na, Mg, K, Ca, Mn, Fe, Ni, and Zn 20.96–65.51, 810.85–1342.36, 6723.68–12253.63, 848.67–3038.46, 1.14–8.44, 48.70–149.72, 1.20–2.17 and 16.69–25.50 µg/g dry weight, respectively depending on

cultivar and time of petal harvest. In all the three cultivars of peony, petals harvested at the flower bud stage had the highest Zn content and the lowest Na, Mg, and Fe content (Li et al., 2020). Petals collected at full blossom had the most minimal Mg content and the most elevated Mn content. The level of Ca, Fe, and Ni in blossom petals of peony were greatest when harvested at the end of flowering when the petals are senescing (Li et al., 2020). The K petal content of peony was not affected by the time of petal harvest (Li et al., 2020). Ghimire et al. (2021) uncovered that accumulation of macro and micronutrients in *Atractylodes japonica* Koidz was influenced by time of harvest. The macro elements Ca and Mg levels were considerably rose by delayed harvesting time, while the microelements Fe, Cu, Al, and As levels were high when early harvesting was done (Ghimire et al., 2021). Mineral concentration in plant tissues and organs is influenced by several variables, including the soil's type and level of fertility, root-soil interactions, features of the absorption system, and translocation inside the plant (Welch & Graham, 2004; Imenšek et al., 2021). The translocation of mineral substances, which varies depending on plant species, tissues, development phases, and environmental factors throughout growth, also affects the mineral element composition of distinct plant components (Mengel & Kirkby, 2001; Martínez-Ballesta et al., 2010; Marschner, 2005; Imenšek et al., 2021; Welch, 1999; Pandey, 2015). Mineral elements are the foundation blocks of plant organs and tissues, and are important in several metabolic processes (Weiss, 2000; Mündel et al., 2004; Marschner, 2005; Emongor & Mabe, 2012). They are also important in the maintenance of osmotic pressure and acid-base in plants (Marschner, 2005; Mengel & Kirkby, 2001; Taiz & Zeiger, 2013). Magnesium is an important component of chlorophyll and influences interception and capture of solar energy absorption by plants (Mengel & Kirkby, 2001; Tränkner et al., 2018; Marschner, 2005; Gardner et al., 2017; Taiz & Zeiger, 2013). In plants, the minerals zinc, copper, magnesium, and Iron are used in the formation of

secondary metabolites (Mengel & Kirkby, 2001; Valmorbida et al., 2007; Taiz & Zeiger, 2013). The recent study's findings indicated that safflower petals are an excellent source of K, Ca, Mg, Fe, and Zn for human nutrition. For example, K is essential for human health since it lowers the risk of coronary artery disease (Mironenko & Eliseeva, 2020; Mani et al., 2020). In order to build stronger, denser bones as well as to maintain bone health and strength as we age, calcium is vital. (Ramya & Patel, 2019).

5.8 Effect of genotype and harvest time on proximate analysis of petals

The current study revealed that there was genotypic variation in safflower petals proximate analysis components irrespective of growing season. Genotypes of safflower varies significantly in the petal proximate content of crude protein (1.00-2.80%), crude fibre (2.89-4.64%), moisture content (74.94-80.28%), fat (2.50-3.39%), ash (5.36-8.63%), and carbohydrates (3.75-10.07%) depending on season of growth. The genotype Turkey had significantly the highest moisture content, crude fibre, crude protein, and fat content in the petals. While the genotype Kenya-9819 was high in ash and carbohydrates contents. The genetic variation in the proximate variables was ascribed to genetic dissimilarities between the investigated genotypes in response to influences from the environment. Similarly in the current study the time of safflower petal harvest affected proximate variables. When petals were gathered after pollination, they had the highest levels of moisture, crude fibre, crude protein, and crude fat; however, they contained the least at the beginning of flowering. When petals had been collected near the onset of flowering, the ash content reached its highest level. The angiosperms flower's function is to reproduce sexually, and after pollination is complete or its stigma is no more receptive to the pollination process the corolla dies. (Jones, 2002, 2012; Marschner, 2005; Taiz & Zeiger, 2013). The penultimate phase of flower growth, known as senescence, is when nutrients are reabsorbed to growing tissues before the

flower's petals dies (Bieleski, 1995; Verlinden, 2003; Chapin & Jones 2007; Trivellini et al., 2011; Jones, 2012; Taiz & Zeiger, 2013). The recycling of nutrients during senescence explains the variation in safflower petal mineral nutritional content and proximate variables with time of petal harvest. Flower senescence is a genetically programmed process that permits plants to methodically segregate cellular macromolecules and organelles before cell death and remobilize micronutrients into its petals (Jones, 2012; Taiz & Zeiger, 2013). Hormones produced by plants regulate floral senescence (Jones, 2012; Taiz & Zeiger, 2013).

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The results from this study showed that safflower genotypes influenced phenological stages, vegetative growth, seed yield and yield components, petal yield, dye content, and petal mineral nutrient composition. Genotype and petal harvest time interacted to influence petal yield, carthamidin and carthamin contents, and dye index. From the results it was concluded that the best genotype to maximise safflower seed and petal yield with high carthamidin and carthamin contents, dye index, mineral nutritional content and proximate variables was Turkey (spineless). It was further concluded that the best time to harvest safflower petals to maximise seed and petal yield with high carthamidin content which has many health benefits was either at the onset of flowering or full bloom. However, the best time to harvest safflower petals to maximise mineral nutritional content and proximate variables was variable and not conclusive.

6.2 Recommendations

It was recommended that current study be repeated with more genotypes (spineless and spiny) and various districts of Botswana. It was further recommended that research be done to evaluate how mineral nutrients (NPK) could influence safflower petal yield, mineral nutritional content, and proximate variables.

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Appendix 1: Total precipitation (mm) for the first trail (October 2021 to January 2022) and second trial (March to August 2022)

	Year 2021: Trial 1 (Summer)				Year 2022: Trial 2(Winter)						
Day	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	Jul	Aug
1	0	0	18.4	8.7	0	0	0	0	0	0	0
2	0	0	0	6.3	0	0	0.6	0	0	0	0
3	0	0	3.6	0	3.5	13.6	32.8	0	0	0	0
4	0	0	8.3	0	0	1.7	0	0	0	0	0
5	0	1.8	0.3	0	0	11.8	0	0	0	0	0
6	13.9	0	8.3	10	0	0	0	0	0	0	0
7	15.8	0	0	0	0	4.8	0	0	0	0	0
8	0	0	0	0	0	8.8	25.4	0	0	0	0
9	0	0	0	0	0	12.6	12.9	0	0	0	0
10	0	0	0	3.8	3.4	0	16.7	0	0	0	0
11	0	0	0	8.3	0	0	0	0	0	0	0
12	0	0	0.3	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	3.3	0	0.1	0	0	0	0	0
15	0	0	53.6	0	0	0	5.2	0	0	0	0
16	0	0	0	1.3	0	6.8	13.4	0	0	0	0
17	0	0	0.5	0	0	13	3	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0
20	0	19	0	0	0	0	0	0	0	0	0
21	9.8	0	0	0	0	0	0	0	0	0	0
22	0	0	4.3	0	0	0	0	0	0	0	0
23	0	0	0	0	2.8	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0
25	0	14.4	6.6	0	5.4	8.6	0	0	0	0	0
26	0	0	0	0	0.8	0	0	0	0	0	0
27	0	0	0	0	12.8	0	0	0	0	0	0
28	0	0	24.6	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0		0	0	0	0	0	0
31	0		0	0		0		0	0	0	0
Total	39.5	35.2	128.8	41.7	29.1	81.8	110	11	–	–	–
Adopted from Department of Meteorological Services											

Appendix 2: Temperature data for trail 1								
	Year 2021 (Summer)							
Day	October		November		December		January	
	Max	Min	Max	Min	Max	Min	Max	Min
1	24.1	17	37.1	17.1	34.4	20.5	31.6	19.2
2	28.7	13.6	34.2	17.2	28	18.7	32.7	20.2
3	29.3	11.1	36.1	17.3	34.5	19.9	32.3	18.8
4	28.5	8.4	37.1	18.1	32.7	22.3	31.3	19.1
5	27.2	15.3	37.8	22	23.5	18.7	32.1	19.9
6	31.7	16.8	37.5	21.4	31.5	17.8	31.5	20.1
7	28.1	17.9	36.6	21.3	32.1	18.7	27	19.6
8	//	//	25.3	20.7	28.7	19.2	29	19.1
9	33.8	17.7	32	14.5	29.8	18.7	//	18
10	32.3	13.2	31.5	15.7	32.6	19.7	28.3	16.4
11	23.4	15	32	18.5	32.4	20.7	26	20.1
12	29.3	10.7	33.3	17.3	26.9	19.1	30.7	18.5
13	33.4	11.2	38.8	19.3	26	19.5	30	20.4
14	36.3	12.9	38	20.4	30.7	17	30.8	19.9
15	37.2	15.3	37.5	20.7	33.7	18.1	//	20.8
16	36.3	17.6	39.1	20.2	24.9	17.1	28.2	//
17	30	15.8	36.7	21	28.6	18.6	//	18.5
18	28.1	14.5	36.7	20.2	20	16.9	27.3	20.3
19	21.2	14.2	//	17.5	23.3	14.2	32.1	17.9
20	26.1	11.7	32	18.5	24.8	16.2	31	19.6

21	28.2	14.2	33.3	17.3	26.7	14.8	32.6	//
22	30.2	16.3	38.8	19.3	30.1	17.2	28.5	15.7
23	35.2	15.2	38	20.4	31	16.8	//	16.3
24	31.6	15.8	37.5	20.7	32.6	13.4	31.5	15.7
25	28.5	16.3	39.1	20.2	32.2	16.9	34.5	17.6
26	29.2	17.6	36.7	21	28.8	17.8	//	19.4
27	28.7	18.6	36.7	20.2	23.7	17.8	31.6	//
28	20.3	19.3	//	//	30.2	14.2	//	21.1
29	30.8	17.3	//	//	34.4	16.4	25.7	19.3
30	32.5	16.7	//	//	33.8	19.2	25.4	19.3
31	32.6	15.8			34.6	19.7	28.1	19.8
Mean	**29.8**	**15.1**	**35.7**	**19.2**	**29.6**	**17.9**	**30.0**	**19.0**

Day	February		March		April		May		June		July		August	
	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min
1	31.5	18.1	31.1	17.3	31.7	12.7	//	//	20.6	-0.3	20.6	6.5	22.4	12.5
2	33.8	20.4	33.8	18	33.2	14.7	23.7	11.6	20.7	1.5	21	5.7	20.3	10.7
3	34.6	21.9	32.3	18.3	34	17.1	24.3	10.5	20.6	1.6	21.8	5	20	13.2
4	32.2	19.9	25.5	19.2	30.4	18.7	25.6	9.9	20.6	1.5	23.3	3.7	27.6	9.2
5	29.8	19.7	28.6	19.5	31.2	17.5	25.6	11.2	23.6	2.6	24.6	3.4	27.9	4.4
6	29.3	19.6	//	//	33.1	17.7	26.1	8.4	20.6	6	24.9	6.6	29.8	4.4
7	30.8	17.3	28.1	19.5	33.1	19.5	25.3	8.6	21.4	2	24.3	1.3	31.6	7.2
8	33.9	16.9	24	17.4	24.6	18.9	24.2	10.7	22.1	2.5	//	1.8	25.7	10.3
9	33.6	20.8	20.1	17.7	22	16.5	24.1	4.9	22.1	4.1	22.1	//	26.3	3.3
10	34.3	17.5	//	17.2	19.7	16.1	23.2	5.7	22.1	2.8	24.7	//	26	5.1
11	33.3	16.9	28.1	19.2	21	16.4	25.1	7	20.3	4.9	22.4	4.3	27	5
12	31.6	15.1	29.7	//	28	14.5	27	7.3	21	1.4	//	2.2	30.4	6.5
13	32.3	17.8	//	15.7	31	15	26.6	7.3	22.8	1.3	24.3	//	31.5	//
14	34.1	16.6	27.2	//	28.5	16.5	28.5	8.1	24	1.8	25	4.1	22.9	5.5
15	32.4	20.7	27.8	16	22.5	15.9	26.9	8.2	17.8	8.8	23.3	2.7	21.8	2.3
16	32.8	18.6	32.8	14.9	27.1	19.6	21.8	10.7	18	1.3	20.8	5.9	24.3	-0.1
17	34.5	19.6	31.3	14.8	20.6	16.1	21	9.2	20.1	1.6	20.5	2.8	30.4	2.9

Appendix 3: Temperature data for trail 2

Year 2022 (Winter)

18	32.6	20.3	22.4	16.1	24.1	15.8	22.4	10.5	23.5	-0.6	//	5.3	30.6	7
19	30.6	20.8	28.3	16.2	22.9	14.4	23.6	5.4	25.3	-2.5	22.1	//	20.6	8.2
20	35.2	19.8	32.4	16.3	23	12.6	24.6	8.7	23.7	8.9	21.5	//	23.7	2
21	36.2	18.1	//	//	20.6	15.2	11	2.8	21	10.1	24.1	10.9	23	2.4
22	35.7	21	26.4	//	25	15.5	17.7	4.7	18.3	8.6	//	8.2	23.1	5.9
23	33.8	19.3	26.7	13.3	28.4	14.4	22.2	5.2	21.9	9.2	23.9	10.8	24.3	5.1
24	34.2	18.8	28.6	14.7	30	15.5	25	7.5	22.3	6.7	25.7	//	24.5	7.5
25	33.6	19.6	32.8	18.7	30.6	16.7	26	6.2	15.7	9.6	22.3	3.9	27.4	4.3
26	30.4	19.3	31.3	17.1	27.6	16.1	26.5	5.9	19.8	6.2	22.5	8.7	29.2	4.6
27	34.6	19.5	22.4	16.1	22.7	17.2	25.1	6.2	19.1	6.4	23.3	8.1	30.9	8.1
28	31.5	19.6	28.3	17.6	22.5	11.5	23.3	9.3	16.4	2.4	//	6.2	31.4	7.9
29			32.4	15.9	26.1	7.6	26.2	8.4	18.2	4.1	23.9	//	31.4	11
30			29.8	13.2	29.6	9.6	22.3	4.5	19.1	0.9	26.7	//	30.4	10.1
31			32.9	15.2			21.4	1.2			//	//	20.6	13.4
Mean	33.0	19.1	28.7	16.7	26.8	15.5	23.9	7.5	20.7	3.8	23.2	5.4	26.4	6.7