BOTSWANA UNIVERSITY OF AGRICULTURE AND NATURAL RESOURCES

THE INFLUENCE OF NITROGEN AND PHOSPHORUS NUTRITION ON GROWTH AND YIELD COMPONENTS OF SAFFLOWER (*Carthamus tinctorius* L.)

A research proposal submitted in partial fulfilment of the requirements for the Degree of Master of Science in Crop Science (Horticulture)

By

Maduo Opelo Kolanyane (ID 201100183)

AUGUST 2022

MAIN SUPERVISOR

Prof. V.E. Emongor

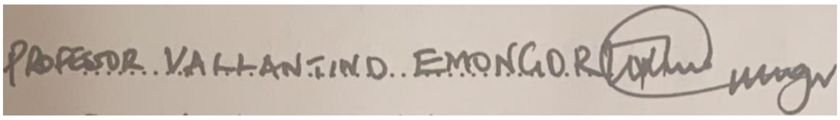
CO-SUPERVISOR

Dr. S. Moroke

Botswana University of Agriculture and Natural Resources

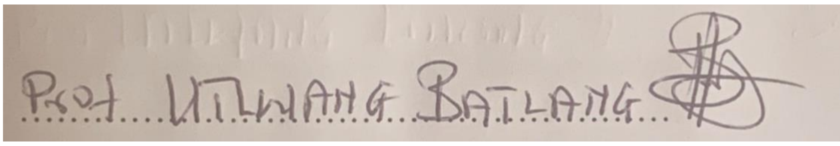
Department of Crops and Soil Sciences

CERTIFICATION



22/08/2022

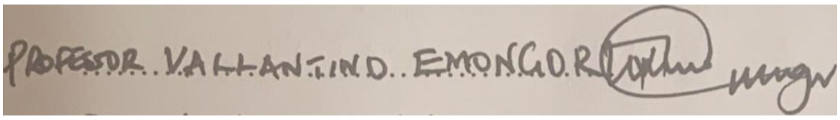
Supervisor’s name and signature Date



22/08/2022

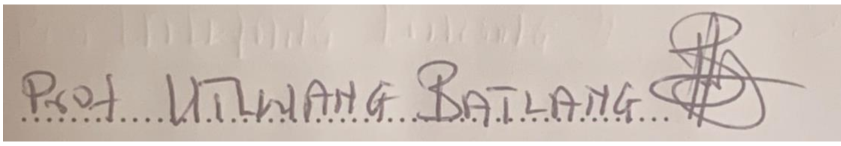
Head of Department’s name and signature Date

APPROVAL



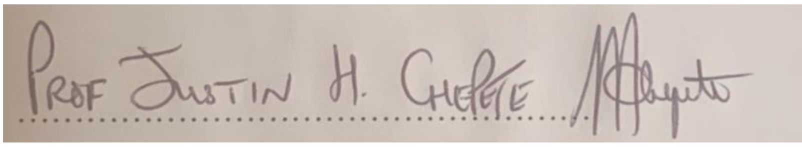
22/08/2022

Supervisor's name and signature Date



22/08/2022

Head of Department's name and signature Date

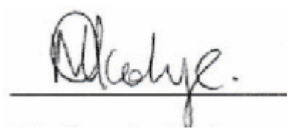


22/08/2022

Dean of Faculty's name and signature Date

STATEMENT OF ORIGINALITY

The work contained in this dissertation was compiled by the author at the Botswana University of Agriculture and Natural Resources between 2020 and 2022. To the best of my knowledge, this work contains no material previously published by another person or material which has been accepted by any other degree, certificate, or diploma of any other University except where due acknowledgement and reference has been made in the text.



Author's signature

22/08/2022
Date

DEDICATION

I dedicate this dissertation to my mothers, Kabo Kolanyane and Boitshepiso Kolanyane, my brothers, sisters and friends who were always supportive throughout this journey.

ACKNOWLEDGEMENTS

I would like to first and foremost thank God for the strength He always afforded me amidst difficulties I encountered throughout this journey. Through His grace and mercies, I was able to triumph every challenge I came across.

I extend my sincere gratitude to my supervisor Prof. V.E. Emongor who has been a father and a mentor. I fall short of words to describe how much he means to me and what he has done for me. My heart is full of gratitude. To my Co Supervisor, Dr. S. Moroke, I extend my gratitude.

I would like to give thanks to RUFORUM/MasterCard Foundation for funding this research. They gave me an opportunity that I will forever be grateful for. To my field assistants, all these would not have been possible without your help. I appreciate every one of you.

I would also like to thank BUAN lab technicians from Crop Science and Production, Animal Science and Production and Basic Science Departments for their technical assistance. I give thanks to the Department of Agriculture Research for allowing me to use their equipment. Fellow graduate students especially Marang Mosupiemang and Keheng Basiame, thank you for every word of advice and encouragement throughout this journey.

Finally, I would like to thank the rest of my family members and friends for their endless support and prayers during my study period.

THANK YOU!

Table of Contents

ABSTRACT

Safflower (*Carthamus tinctorius* L.) is a versatile crop used for edible oil, vegetable, cut flower production, colouring foods, cosmetics, pharmaceuticals, livestock feed, biofuels, textile, and medicinal purposes. Soil fertility is one of the factors that limit crop production in Botswana. The objective of this study was to evaluate the influence of N and P fertilizer application on the growth, seed yield, and oil content of safflower. The experimental design was a split-plot laid down in randomized complete blocks with three replications. The treatments were N application at 0, 40, 80 and 120 kg/ha allocated to main-plots and P application at 0, 25, 50 and 50 kg/ha allocated to sub-plots. The results revealed that N and P significantly ($P < 0.05$) influenced vegetative growth, yield components, seed yield, and oil content of safflower. Application of 40 N + 50 P kg/ha to safflower plants produced maximum first branching height, leaf area, and chlorophyll content. Maximum safflower plant dry matter was obtained from plants applied with 120 N + 75 P kg/ha. While N and P independently significantly ($P < 0.05$) increased plant height (40 N, 50 P kg/ha), primary branch number (80 N, 50 P kg/ha) and stem diameter (40 N, 75 P kg/ha) compared to control plants. The interaction of N and P also significantly ($P < 0.05$) affected NUpE, NUtE, NUE, PUpE, PUtE, and PUE in both summer and winter. Application of 40 N + 50 P and 40 N + 25 P kg/ha, optimised NUpE, NUtE and NUE, and PUpE, PUtE and PUE, respectively. The interaction of N and P significantly ($P < 0.05$) influenced safflower yield components. Maximum capitula number/plant, capitula diameter, seed number/capitulum, and 1000-seed weight were produced by safflower plants applied with 40 N + 50 P and 40 N + 25 P kg/ha, respectively. Application of 40 N + 50 P and 80 N + 75 P kg/ha to safflower plants significantly ($P < 0.0001$) increased the seed yield to 1356 and 3528 kg/ha in summer and winter, respectively. There was no significant interaction of N and P on seed oil content in summer, but the application of N and P independently significantly ($P < 0.05$) increased safflower seed oil content with a maximum oil content of 46.4 and 46.1% obtained through the application of 120 and 25 kg/ha of N and P, respectively. In winter, N and P significantly ($P < 0.0001$) interacted at 0 kg N/ha + 25 kg P/ha to produce the maximum oil content of 71.8%. The results revealed that winter grown safflower produced seed with high oil content than summer grown safflower. From the results based on the performance of safflower as N and P influenced vegetative, yield components, seed yield, and seed oil content it was concluded that application of 40 N + 50 P kg/ha was recommended to maximize safflower production. However, it was also recommended that this study be repeated in Southern Botswana for confirmation of the current results. Also, fertilizer trials to be done in other parts of the country where farmers are currently growing safflower.

CHAPTER 1

INTRODUCTION

1.1 General introduction

Climate is changing, why are we not? With the world's climate continually changing, humanity must develop and adapt to crops that can tolerate the new climatic conditions while remaining food sustainable and secure. Most crops that currently contribute significantly to the world's food supply will be unable to endure the new growing conditions induced by climate change (Bernstein et al., 2007; Schlenker & Roberts, 2009; Challinor et al., 2014; Zhao et al., 2017). Therefore, new crops or crops currently classified as minor crops but adapted to the marginal climatic conditions should be developed and evaluated. Safflower is a minor, underutilized, and neglected crop that is adapted to varied climatic conditions (Ekin, 2005; Emongor, 2010; Emongor & Oagile, 2017).

Safflower (*Carthamus tinctorius* L.) is a multipurpose crop belonging to the Compositae or Asteraceae family that has shown potential value in medical, pharmaceutical, food, livestock feed, and cosmetic applications worldwide (Emongor, 2010; Mündel & Bergman, 2010). Safflower is currently farmed mostly for edible oil, which is considered one of the healthy oils for human consumption due to its high content of polyunsaturated essential fatty acid linoleic acid (70-87%) and monounsaturated fatty acid oleic acid (11-80%) (Murthy & Anjani, 2008; Aghamohammadreza et al., 2013; Kumar et al., 2015; Moatshe, 2019; Moatshe et al., 2020). Linoleic acid has been shown to offer nutritional and therapeutic benefits such as the prevention of coronary heart disease, arteriosclerosis, high blood pressure, and hyper lipaemia (Wang & Li, 1985; Cosge et al., 2007; Li et al., 2016; Liao et al., 2019). The seeds of safflower are also a rich source of minerals (Zn, Cu, Mn, and Fe), vitamins (thiamine and β-carotene), and tocopherols α, β, and γ (Velasco et al., 2005). United States of America (USA) is the world's largest producer of safflower oil followed by India and Mexico, respectively (NationMaster, 2021).

Safflower is adapted to regions of low rainfall and humidity throughout anthesis and seed maturation stages (Knowles, 1976; McPherson, 2004). Safflower is more drought tolerant than other oilseed crops such as sunflower, canola, sesame, and groundnuts, owing to its deep root structure, which allows it to reach water from depths of up to 2.5-3 m (Burgener et al., 2004; Berglund et al., 2007; Emongor, 2010; Emongor & Oagile, 2017). However, for optimal seed production, safflower plants require adequate water supply at the grain filling stage (Dordas & Sioulas, 2008; Emongor, 2010).

1.2 Plant nutrition

With Botswana's population growing, the requirement for greater and intensified food production to meet the population's needs is also increasing. To ensure the success of food production, favourable conditions must exist. Plants, in general, require mineral elements provided by the soil for growth and development. However, Botswana's soils are poor and deficient in nitrogen and phosphorus, thus limiting crop production (Botswana Agriculture Sector Policy Brief, 2012; Emongor & Mabe, 2012; Malikongwa, 2015; Emongor et al., 2017a). The amount of nutrients in the soil must therefore be maintained at a sufficient level throughout the crop's growth stages, as fertilizer treatment is a critical measure for correcting nutrient deficits by replacing components eliminated from harvested products (Abbadi, 2007). Kubsad et al. (2001) demonstrated that safflower requires an adequate supply of nutrients even when moisture is scarce. Increased fertilizer dose efficiency is one of the most critical techniques for enhancing agricultural productivity in crop management practices (Golzarfar et al., 2012). Golzarfar et al. (2012) identified nitrogen (N) and phosphorus (P) as the two most critical essential elements for safflower growth and development. As a result, improving their application rates can significantly boost safflower seed yield and oil content. According to Mündel et al. (2004), nutrition management is a fundamental cultural practice for achieving high safflower output.

Nitrogen fertilizer application significantly improves safflower production (Golzarfar et al., 2016). However, N recovery is often as low as 50%, resulting in significant ecological consequences. Nitrogen is a macronutrient that forms part of proteins, enzymes, chlorophyll, and nucleic acids (Raven et al., 1999; Marschner, 2005). It also plays a role in the synthesis of hormones (Raven et al., 1999; Marschner, 2005; Taiz and Zeiger, 2013). Bonfim-Silva et al. (2015) reported that the application of N fertilizers significantly improved safflower's chlorophyll index and biometric features. Seadh et al. (2012) reported that N influenced dry matter production by affecting leaf area development and maintenance, as well as photosynthetic efficiency. A good supply of N has been shown to enhance root growth and development, uptake of other nutrients, and stimulate vegetative growth with a deep green colour (Leghari et al., 2016).

Golzarfar et al. (2016) reported that the application of P to safflower plants significantly increased seed yield. Not only is P necessary for yield production, but it also plays a critical function in a variety of cellular processes, including membrane structure maintenance, biomolecule synthesis, and the generation of high-energy molecules (Marschner, 2005; Hasanuzzaman et al., 2018). Phosphorus also aids in cell division, activation/inactivation of enzymes, and carbohydrate metabolism (Raven et al., 1999; Mengel & Kirkby, 2001; Marschner, 2005; Razaq et al., 2017). Phosphorus is important for root growth and development (Hasanuzzaman et al., 2018), which may contribute to drought tolerance promotion. After N, P is the most critical inorganic nutrient for plant growth and, unless given as fertilizer, inhibits primary productivity in natural and cropping systems (Vance et al., 2003;

Akanbi et al., 2010). While many soils contain significant amounts of total P, only a small fraction is readily available, leaving many agricultural areas low in P (Mengel &Kirkby, 2001; Marschner, 2005; Pachauri & Meyer, 2014). While the quantity of P in the soil is greater than that in the plant, its fixation in the form of aluminum/iron or calcium/magnesium phosphates renders it unavailable to plants (Mengel & Kirkby, 2001; Marschner, 2005; Hasanuzzaman et al., 2018).

While increasing yield output through fertilizer applications is desirable, there is also a need to increase nutrient efficiency through enhanced nutrient uptake and utilization, which could result in lower fertilizer input costs while maintaining yield (Chen & Liao, 2017). Nutrient efficiency quantifies a plant's capacity to absorb nutrients either natural or provided as fertilizer and use them to generate biomass (Chen & Liao, 2017). Nutrient use efficiency is a vital term in agricultural production system evaluation since it can be significantly influenced by fertilizer management as well as soil and plant-water management (Fixen et al., 2015; Chen & Liao, 2017). The purpose of nutrient use is to improve the overall performance of cropping systems by supplying the crop with the most economically advantageous nutrition possible while reducing nutrient losses from the field (Fixen et al., 2015).

Nutrient-efficient plants will play a significant role in increasing crop yields in the twenty-first century, owing to limited land and water resources available for crop production, the increased cost of inorganic fertilizer inputs, global crop yield decline trends, and growing environmental concerns (Fageria et al., 2008). Additionally, at least 60% of the world's arable land suffers from mineral deficiency or elemental toxicity, making fertilizers and lime amendments critical for increasing crop yields on such soils. Fertilizer inputs are raising farmers' costs of output, and environmental degradation caused by excessive fertilizer inputs is a big concern. Increased global population demands for food and fibre emphasize the importance of nutrient-efficient cultivars that also produce more (Fageria et al., 2008). Baligar & Fageria (2015) reported that nutrient utilization in crop production was influenced by the environment, soil, plant, and farmer's socio-economic status. They further reported that agricultural plants' overall nutrient usage efficiency was less than 50% under all agro-ecological situations, implying that a significant portion of delivered nutrients was lost in the soil-plant system. The inefficient use of nutrients not only increases crop production costs but also contributes to environmental damage. The most critical measures for increasing nutrient use efficiency are the use of an adequate rate, an effective source, appropriate timing, and application methods (Baligar & Fageria, 2015). Reducing abiotic and biotic stressors and utilizing nutrient-efficient crop species and genotypes within species are critical for improving nutrient usage efficiency (Baligar & Fageria, 2015; Fixen et al., 2015; Chen & Liao, 2017).

1.3 Problem statement

The impact of N and P on safflower growth, seed production, yield components, and oil content has been emphasized in various works of literature. However, there is no information on the application rates of these nutrients in Botswana except the research work done by Mazhani (2017), highlighting the significance of this work. The purpose of this study was to expand understanding of the optimal N and P application rates required for optimal safflower growth, development, and yield.

1.4 Justification

When Botswana gained independence in 1966, agriculture was the primary economic activity, accounting for 40% of total Gross Domestic Product (GDP) but has declined to 1.6% (Ministry of Agriculture [MoA], 2010). Mineral discovery and importance in the 1970s led to this fall, relegating it to the bottom tier of GDP contributors (Seleka, 2005). For more than two decades, the Botswana government has vigorously promoted economic diversification as a developmental strategy aimed at reducing the country's reliance on the mining sector through the promotion of other industries (Seleka, 2005). Botswana's policy frameworks emphasize the need for increased opportunities for citizens through rain-fed agricultural production (Food and Agriculture Organization [FAO], 2014), hence assuring crop diversification and food security.

Safflower is one of the crops that could be researched for economic diversification. Safflower is a versatile crop that is used for edible oil, as a vegetable, cut flower, food colourant, livestock feed, cosmetics, herbal tea, dyes, and paints (Emongor, 2010). It is a drought-tolerant crop capable of extracting water at soil moisture levels that most crops cannot reach (Weiss, 2000; McPherson, 2004; Khalili et al., 2014). This crop's deep rooting system (2-3 m) contributes to its drought tolerance, as the roots may reach deep soil volumes to take water (Bassil & Kaffka, 2002; Emongor, 2010; Khalili et al., 2014). Safflower is also tolerant to heat, cold (-7 to 40°C), and salinity (Berglund et al., 2007; Khalili et al., 2014). Despite its numerous uses and adaptability to various environments, safflower has remained an underutilized and neglected crop in several countries (Emongor, 2010). Safflower has remained neglected and marginalized, most likely due to a lack of information about its cultivation and farmers' general reluctance to adopt a new crop (Emongor, 2010; Moatshe et al., 2016). However, when a novel crop is introduced into a regional cropping system, data on its performance under local agronomic and environmental conditions are required (Moatshe et al., 2016).

Botswana's location on the southern African plateau contributes to its semi-arid climate, which is very variable in terms of rainfall incidence, both geographically and temporally, with frequent dry spells during the agricultural season, making drought a recurring feature of the country's climate (FAO, 2014). This condition has resulted in crop failures periodically, resulting in famine and food shortages (FAO, 2014). Due to inconsistent and poorly distributed rainfall combined with high temperatures, water is the biggest constraining element to agricultural production in Botswana (Emongor, 2009). Growing a drought, saline, cold, and heat-tolerant high-value crop such as safflower will reduce reliance on food imports such as cooking oil, livestock feeds, vegetables, and cut flowers, thereby bridging the import gap, diversifying the economy, and improving the livelihoods of people in Botswana through increased income levels.

While there is a plethora of studies on safflower globally, information on its fertilizer requirements in Botswana is scarce, yet it is crucial for its adoption and optimal production. Nitrogen and P are deficient in Botswana soils (Pule-Meulenberg & Batisani, 2003; Emongor & Mabe, 2012). Nitrogen is scarce due to leaching and low organic matter in the soil (Pule-Meulenberg & Batisani, 2003; Emongor & Mabe, 2012). Phosphorus availability is limited in most Botswana soils due to poor soil organic carbon and its fixation in the form of aluminum/iron or calcium/magnesium phosphates (Pule-Meulenberg & Batisani, 2003; Emongor & Mabe, 2012; Hasanuzzaman et al., 2018), rendering it unavailable for root absorption. An external supply of these mineral nutrients is required to ensure the safflower crops' success. This study will add to the existing literature on safflower in Botswana by determining the N and P rates required for optimal safflower production.

1.5 Objectives

The overall purpose of this study was to determine the N and P fertilizer requirements of safflower to maximize growth, seed yield, and oil content.

The specific objective of this study was to:

I. Evaluate the influence of N and P fertilizer application on growth, seed yield, and oil content of safflower.

1.6 Hypothesis

H_o: Nitrogen and P fertilizer application have no effect on the growth, seed yield, and oil content of safflower.

H_a: Nitrogen and P fertilizer application have an effect on the growth, seed yield, and oil content of safflower.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This review has been based on the general overview of the response of safflower to mineral nutrition. The emphasis is on how N and P fertilizer application influences growth, development, yield components, yield, and oil content of safflower. The review ends with the identification of the knowledge gap that exists within the Southern African Development Community (SADC) in relation to N and P fertilizer application on how they influence the growth and development of safflower.

2.2 General nutrient requirement of safflower

Crops require light, carbon dioxide, water, and mineral nutrients for growth and development. For example, safflower yield has been reported to respond to water application (Omidi et al., 2012; Santos et al., 2018). Omidi et al. (2012) reported safflower yields of 550 and 4,500 kg/ha, under rainfed and irrigated conditions, respectively. They also concluded that the amount of available water was an important factor in determining safflower yield. While Omidi & Sharifmogadas (2010) reported that good water management allowed safflower plants to maintain soil moisture, which enhanced grain yield due to the increase in the number of capitula and secondary branches. Crop yields are significantly influenced by interactions of mineral nutrients and other factors essential for growth and development (Marschner, 2005). The interaction of water availability and N under field conditions is significant (Marschner, 2005). Yield reduction due to high application of N and other nutrients in combination with low water availability may be attributed to many factors such as delay in stomatal response to water deficit, high water consumption of vegetative biomass, and the corresponding high risk of drought stress in critical periods of economic yield formation (Hocking and Meyer, 1991). Mineral nutrition of crops affects vegetative and reproductive organs, yield components, and economic yield (Marschner, 2005; Emongor & Mabe, 2012). In the majority of crops, both quantity and quality are important yield components. For example, with nitrate accumulation in spinach and sucrose accumulation in sugar beet with increasing levels of N application or with an increase in mineral nutrient supply, the number of reproductive sinks or vegetative storage organs are increased (Marschner, 2005).

Nutrient management is one of the key inputs in achieving the high productivity of safflower and other crops (Weiss, 2000; Mündel et al., 2004). One of the ways of increasing crop productivity is to increase the efficiency of fertilizer application rates. Optimum fertilizer application rates, fertilizer content, nutritional requirements of the plant during the growing season, and the amounts of nutrients present in the soil should be determined (Dong et al., 2005; Alivelu et al., 2006). Nitrogen and P are the two essential nutrients for safflower growth and development; therefore, optimization of their rates can significantly increase the seed yield and oil content in safflower. Suggestions for fertilizers with N should be applied to crops to ensure a high-quality product, optimum yield, high profit, and lesser environmental pollution risks (Belanger et al., 2000; Antoniadou & Wallach, 2002; Henke et al., 2007). Excessive or insufficient N fertilizer application results in economic losses and environmental problems over time, respectively (Grant, 2006). Therefore, to obtain optimum yields and quality produce, adequate nutrients at the right time and form should be applied to the plant.

2.3 Effects of nitrogen on vegetative growth of safflower

In most crops, plant development during the vegetative stage controls both the biological and economic yields (Mengel & Kirkby, 2001; Marschner, 2005). The dependence of biological and economic yields on the vegetative growth stage is because during this period green plant tissues are biosynthesized which provide photosynthates for seeds or storage tissues (Mengel & Kirkby, 2001; Marschner, 2005; Moatshe et al., 2016). Nitrogen nutrition influences the growth and development of many crops (Mengel & Kirkby, 2001; Marschner, 2005; Taiz & Zeiger, 2013). Weiss (2000) and Sabbagh et al. (2012) reported that safflower was more responsive to N application than other nutrients because it's required in large quantities for vegetative growth. Nitrogen application resulted in the production of a greater number of branches, leaves, and leaf area than in control plants (Sabbagh et al., 2012). Nitrogen fertilizer application was reported to be crucial and limiting safflower grain yield due to its multi-dimensional effect on growth and development (Sabbagh et al., 2012). While Al-Zubaidy & Al-Mohammad (2021) in Iraq reported that N application at 90, 120, and 150 kg/ha increased safflower plant height, the number of branches, leaf area, total leaf chlorophyll content, and plant dry matter compared to control plants. The best N rate for increasing vegetative growth of safflower was 150 kg/ha (Al-Zubaidy & Al-Mohammad, 2021). Abbadi et al. (2008) investigated the effect of N supply on growth, yield, and yield components of safflower and sunflower. They reported that increased N supply significantly enhanced the growth and morphology of both safflower and sunflower. Plant height and stem diameter of safflower plants was increased by application of 0.25, 0.5, and 1.0 g N/pot when grown in a potting medium consisting of sand, nutrient-poor limed soil, and perlite in equal proportions compared to control plants (Abbadi et al., 2008). Application of N above 1.0 g N/pot, resulted in a decrease in safflower plant height and stem diameter (Abbadi et al., 2008). While Santos et al. (2018) in Brazil, reported that application of 50, 100, 150, 200, 250, 300, and 350 kg N/ha to safflower plants under irrigation increased plant height compared to control plants, beyond which plant height decreased. However, under rainfed conditions, maximum plant height was obtained in safflower plants applied with 200 kg N/ha (Santos et al., 2018). Tunçtürk & Yildirim (2004) reported that application of N at 40, 80, or 120 kg/ha significantly enhanced safflower plant height compared to control plants, with 120 kg N/ha yielding the tallest plants. They further reported that N fertilizer application in the form of ammonium nitrate enhanced safflower plant height substantially more than ammonium sulphate or urea.

While Siddiqui & Oad (2006) in Pakistan, found that applying N to safflower increased plant height and primary branch number per plant compared to control plants. However, both safflower plant height and primary branch number per plant reached a maximum with the application of 120 kg N/ha (Siddiqui & Oad, 2006). Increasing N application from 30 to 180 kg/ha delayed the maturity of safflower (Siddiqui & Oad, 2006). More recently, Haliloglu & Beyyavas (2019) in Turkey reported that the application of 50 kg N/ha was the best rate for increasing safflower plant height under dryland conditions. Bitarafan et al. (2011), Katar et al. (2012), Khalil et al. (2013), and Eryigit et al. (2015) had earlier reported similar results with N application increasing safflower plant height.

Mohamed et al. (2012) observed that increasing the N (0, 25, 50, 75, 100, 125, 150, 175 kg N/ha) application rate enhanced above-ground biomass by an average of 42-46% compared to control plants. Similar findings are reported in the literature that high N application increased the dry mass of safflower (Dordas & Sioulas, 2008; Golzafar et al., 2011; de Anicésio et al., 2015). These findings may be explained by the role of N in enhancing the photosynthetic active area, which is responsible for the absorption of solar energy and so promoted crop development (Megda & Monteiro, 2010). Increased N supplies resulted in an improved leaf area index (LAI) and thus enhanced light absorption, resulting in increased total dry matter production and accumulation in various plant parts, which accounts for the increase in safflower vegetative development (Weinberg et al., 2007).

Safflower leaf assimilation rate at anthesis increased with increasing levels of N fertilizer (Mohamed et al., 2012). At 100 kg N/ha, the assimilation rate was found to be increased by

42%, compared to control safflower plants where no significant increase in plant photosynthesis above 100 kg N/ha was observed. Dordas & Sioulas (2008) observed a comparable maximal assimilation rate during anthesis at 100 kg N/ha under rainfed conditions. The stomatal conductance and water usage efficiency increased by 27% and 60%, respectively, when N was applied during anthesis (Dordas & Sioulas, 2008). Stomatal conductance was up to twofold greater at the greatest N level than at the lowest N level during anthesis (Dordas & Sioulas, 2008). Increased assimilation rate due to N fertilizer application was reported also in other crops (Ciompi et al., 1996) and it is considered an essential aspect of the crop's physiological response to limiting factor improvement. Nitrogen availability tends to increase the photosynthetic capacity of C_3 plants because of increased investment in the amounts of Rubisco and thylakoids, which are directly correlated to an increase in leaf N content (Evans, 1989).

Strasil & Vorlicek (2002) found no significant effect of N fertilizer application (40 and 80 kg/ha) on safflower growth. Malek & Ferri (2014) reported similar findings, stating that applying 30, 60, or 90 kg N/ha had no significant effect on safflower growth in dryland farming in Iran. Safflower plants' lack of responsiveness to N fertilizer application in dryland farming in Iran was linked to a lack of available water during growth.

2.4 Effect of nitrogen on yield components

The reported yield components of safflower are seed weight, plant height, first branch height, number of branches per plant, capitula diameter, number of seeds per capitulum, and number of capitula per plant (Chaundry, 1990; Gonzalez et al., 1994; Omidi-Tabrizi, 2000; Bagheri et al., 2001; Camas & Esendal, 2007; Kedikanetswe, 2012; Emongor et al.,2015; Emongor & Oagile, 2017; Haliloglu & Beyyavas, 2019; Moatshe, 2020). Al-Zubaidy & Al-Mohammad (2021) in Iraq reported that application of N fertilizer at 90, 120 and 150 kg/ha significantly increased seed number per plant, seed weight per plant, and 200-seed weight compared to control plants. Application of 150 kg N/ha resulted in safflower plants with the highest seed number per plant, seed weight per plant, and 200-seed weight (Al-Zubaidy & Al-Mohammad, 2021). While Haliloglu & Beyyavas (2019) in Turkey reported that application of 50, 100, and 150 kg N/ha to safflower plants significantly increased capitula number per plant, seed number per capitulum, and 1000-seed weight compared to control plants. Application of 150 kg N/ha gave the highest yield components determined (Haliloglu & Beyyavas, 2019). Whereas in Brazil, Santos et al. (2018) reported that application of 50, 100, 150, 200, 250, 300, 350, and 400 kg N/ha to safflower significantly increased capitula number per plant, 1000-seed weight, and seed weight per plant compared to control plants. Application of N above 400 kg/ha significantly decreased yield components due to N toxicity (Santos et al., 2018). While Singh & Singh (2013) reported that N fertilizer application at 40 and 80 kg/ha increased safflower capitula number per plant, seed number per capitulum, and 1000-seed weight compared to control plants. Nitrogen application above 40 kg/ha resulted in an increase in yield components, but the increase was not statistically significant (Singh & Singh, 2013). The increase in safflower yield components caused by N treatment was attributed to the role of N in promoting cell division and elongation, as well as leaf chlorophyll synthesis (Singh & Singh, 2013).

An increase in safflower yield components induced by N fertilizer application ranging from 30-400 kg/ha depending on the country, water availability, and time of application is reported (Siddiqui & Oad, 2006; Dordas & Sioulas, 2008; Golzarfar et al., 2012; Seadh et al., 2012; Santos et al., 2018). However, Elfadl et al. (2009) reported that application of N at 40 and 80 kg/ha had no effect on the number of seeds per plant, harvest index, the number of capitula per plant, and the number of seeds per capitulum of safflower.

2.5 Effect of nitrogen on seed yield

Al-Zubaidy & Al-Mohammad (2021) in Iraq reported that application of N fertilizer to safflower plants at 90, 120, and 150 kg/ha significantly increased seed and biological yields, and harvest index compared to control plants. Safflower plants applied with 90, 120, and 150 kg N/ha produced seed yields of 1364, 1587, and 1762 kg/ha, respectively (Al-Zubaidy & Al-Mohammad, 2021), while the control plants produced 1074 kg/ha of seed (Al-Zubaidy & Al-Mohammad, 2021). Meanwhile, Santos et al. (2018) in Brazil reported that N fertilizer application at 50, 100, 150, 200, 250, 300, 350, and 400 kg/ha significantly increased safflower seed yield under irrigated and rainfed conditions. However, optimum safflower seed yields of 4216 and 2048 kg/ha were obtained by application of 200 kg N/ha under irrigated and rainfed conditions, respectively (Santos et al., 2018). Dordas & Sioulas (2008) reported that N fertilizer application to safflower plants increased seed yield by 19% compared to control plants. Maximum seed yield under rainfed conditions was achieved by application of 150 kg N/ha. Strasil & Vorlicek (2002), also achieved maximum safflower seed yield with an application of 150 kg N/ha. While Rastigou et al. (2013) reported positive effects of N fertilizer application on safflower seed yield, with maximum yield achieved by application of 200 kg N/ha.

Eryiğit et al. (2015) in Turkey reported that the application of N to safflower plants increased seed yield in a two-year study in Turkey. The application of 150 kg N/ha produced an optimal seed yield of 2,152 kg/ha (Eryíğít et al., 2015). Golzarfar et al. (2012) found in an Iranian study that applying N at 75 and 150 kg/ha enhanced safflower seed output from 984 kg/ha (control) to 2758 and 3228 kg/ha, respectively. While Malek & Ferri (2014) observed that applying 30, 60, or 90 kg N/ha increased safflower seed output significantly regardless of location or cultivar. The control plants produced 679 kg of seed/ha, whereas those treated with 30, 60, or 90 kg N/ha produced 770, 780, and 837 kg of seed/ha, respectively. In India, Singh & Singh (2013) reported that application of N at 40 or 80 kg/ha to safflower plants significantly increased seed yield compared to plants that did not receive N. Safflower plants applied with 40 and 80 kg N/ha produced 2,270 and 2,520 kg seed/ha, respectively, while control plants produced 1,570 kg seed/ha (Singh & Singh, 2013). While Abd El-Mohsen & Mahmoud (2013) reported that application of N fertilizer at 40, 80, and 120 kg/ha increased safflower seed yield and the response was quadratic with increasing N application rate. Nitrogen fertilizer application of 40, 80, and 120 kg increased safflower seed yield by 38, 52, and 50%, respectively, when compared to control plants (Abd El-Mohsen & Mahmoud, 2013). Similar studies in which N fertilizer application in the range of 25-175 kg/ha increased safflower yield are reported in the literature (Haghighati, 2010; Mohamed et al., 2012; Taleshi et al., 2012; Mazhani, 2017). However, Haliloglu & Beyyavas (2019) in Turkey reported application of 50, 100, or 150 kg N/ha had no significant effect on safflower seed yield.

2.6 Effect of nitrogen on oil content

Safflower plants applied with N at 40, 80, or 120 kg/ha produced seed with higher seed oil content than seed from control plants (Abd El-Mohsen & Mahmoud, 2013). Increased N application rate resulted in a quadratic response in safflower seed oil content, with a maximum seed oil content of 33.9 % attained with an application of 80 kg N/ha (Abd El-Mohsen & Mahmoud, 2013). While Singh & Singh (2013) reported that safflower plants applied with 40 and 80 kg N/ha produced seed with 33.3 and 33.5 % oil content, respectively, compared to control plants that produced seed with 32.4 % oil content. However, the difference in oilseed content between 40 and 80 kg N/ha was not statistically significant (Singh & Singh, 2013). Also, Bitarafan et al. (2011) reported that the application of 50, 100, and 150 kg N/ha increased safflower seed oil content compared to control plants. Increasing N fertilizer application from 50 to 100 kg/ha to safflower plants increased seed oil content, however, application of 150 kg N/ha decreased seed oil content (Bitarafan et al., 2011). Soleimani (2010) found that the application of safflower plants with 50, 75, 100, 125, and 150 kg N/ha increased seed oil content. The maximum seed oil content of 29.9 % was in safflower plants applied with 100 kg N/ha (Soleimani, 2010). While Tunçtürk &Yildirim (2004) found that the application of N at 40 and 80 kg/ha improved the seed oil content of safflower compared to control plants.

However, when 120 kg N/ha was applied, the seed oil content reduced in comparison to control plants or plants applied with 40 or 80 kg N/ha (Tunçtürk & Yildirim, 2004). However, no statistically significant variation occurred in seed oil content of safflower plants applied with either 40, 80, or 120 kg N/ha (Tunçtürk & Yildirim, 2004).

El-Nakhawy (1991) showed that application of N at 46, 92, and 138 kg/ha reduced the oil content of safflower seeds by 1.0, 7.8, and 3.9 percent, respectively, as compared to control plants. Elfadl et al. (2009) observed that application of either 40 or 80 kg N/ha had no effect on the seed oil content of safflower. More recently, Haliloglu & Beyyavas (2019) and Santos et al. (2018) reported that N fertilizer application to safflower plants had no significant effect on seed oil content. Whereas Dordas & Sioulas (2008) found no correlation between N application rates and safflower seed oil content.

2.7 Nitrogen use efficiency

While nitrogen fertilizer application is very effective in increasing safflower yields, N availability is difficult to increase in organic farming, where synthetic N fertilizers are prohibited (Abbadi & Gerendás, 2009). Additionally, in developing countries with a significant proportion of less fertile soils, it may be challenging to supply the nutritional requirements of high-yielding crops (Marschner, 2005). Furthermore, even with inorganic N fertilizers, N recovery is frequently as low as 50% or less, posing major ecological effects (Scheiner et al., 2002; Baligar et al., 2001). Thus, it is beneficial to strive for effective N usage because plants that are efficient in absorbing and utilizing nutrients significantly improve the efficiency of applied fertilizers, lowering input costs and limiting nutrient losses to ecosystems (Baligar et al., 2001). Abbadi & Gerendás (2009) revealed that in pot trials with five levels of N at 0.25, 0.5, 1.0, 1.5, and 2.0 g per pot, safflower out-yielded sunflower at low N supplies, but sunflower out-yielded safflower at high N supplies. Additionally, they showed that both species gathered comparable amounts of N per pot when supplied with equivalent amounts of

N, but safflower accumulated more N because of its reduced dry matter output. They showed that safflower more efficiently utilizes absorbed N than sunflower to produce seed yield at suboptimal N supply in terms of efficiency ratio and utilization index, whereas the opposite was true at optimal and high N supply. Functional investigation of dry matter and seed production efficiency validated safflower's superior efficiency (Abbadi & Gerendás, 2009). As a result, it was found that safflower is a low-input crop that outperforms sunflower in terms of seed yield on soils deficient in accessible N.

2.8 Effects of phosphorus on vegetative growth of safflower

Sofy et al. (2020) reported that application of P in Iraq at 50 and 100 kg P_2O_5/ha significantly increased safflower leaf number per plant by 17.4 and 39.1%, respectively compared to control plants. However, P application had no significant effect on safflower plant height (Sofy et al., 2020). Abbadi & Gerendas (2011) reported that safflower crop responded positively to increasing levels of P supply in 2004 (0.04, 0.08, 0.024, and 0.72 P g/pot) and 2005 (0.25, 0.5, 1 and 2 P g/pot). These significant positive responses were related to an increase in total plant dry matter (leaf, capitula, and stem), which peaked at a P supply of 1g/pot. However, low P levels were observed to reduce the stem diameter of safflower. While Arani et al. (2011) reported that applying 50, 75, or 100 kg P_2O_5/ha improved the plant biomass of safflower from 2.8 to 9.4 %, depending on the irrigation regime and stage of growth and development. Application of 100 kg P_2O_5/ha resulted in maximum plant biomass, regardless of irrigation regime or stage of growth and development (Arani et al., 2011). Golzarfar et al. (2011) observed similar results. Application of 50 or 100 kg P_2O_5/ha to safflower plants enhanced plant height, stem diameter, primary branch number per plant, and plant biomass when compared to control plants. The optimal rate of application was 100 kg P_2O_5/ha (Golzarfar et al., 2011). Singh & Singh (2013) observed that applying 40 or 80 kg P_2O_5/ha enhanced safflower plant biomass and P uptake by 24.6-31.2 % and 17.9-35.3 %, respectively, compared to control plants. Application of P at 40 and 80 kg P_2O_5/ha increased N and S uptake by 17.0-24.1 % and 11.5-23.8 %, respectively, compared to control plants (Singh & Singh, 2013). However, Mazhani (2017) and Haghighati (2010) observed that the application of P at 25- 60 kg/ha had no effect on safflower vegetative growth.

2.9 Effect of phosphorus on yield components

The application of P fertilizer to safflower plants has been reported in literature to improve yield components (Golzarfar et al., 2012; Singh & Singh, 2013; Mazhani, 2017). Singh & Singh (2013) reported that the application of 40 or 80 kg P_2O_5/ha significantly increased safflower capitula number per plant, 1000-seed weight, and harvest index. Maximum yield components were obtained by application of 80 kg P_2O_5/ha (Singh & Singh, 2013). Application of P at 50 or 100 kg P_2O_5/ha significantly increased safflower capitula number per plant, seed number per capitulum, and 1000-seed weight compared to control (Golzarfar et al., 2012). Phosphorus application of 50 and 100 kg P_2O_5/ha increased capitula number per plant, seed number per capitulum, and 1000-seed weight by 7.9-11.8, 15.6-23.7, and 11.8-18.9%, respectively, compared to control (Golzarfar et al., 2012). Maximum capitula number per plant, seed number per capitulum, and 1000-seed weight were achieved by application of 100 kg P_2O_5/ha (Golzarfar et al., 2012). More recently, Mazhani (2017) reported that application of P at 25, 50, and 75 kg/ha significantly increased safflower capitula number per plant by 36.5, 37.2, and 30.8% compared to control plants. The maximum capitulum number per plant was obtained by application of 50 kg P/ha (Mazhani, 2017). However, Mazhani (2017) reported that P fertilizer application at 25, 50, and 75 kg/ha had no significant effect on capitula diameter, seed number per capitulum, and 100-seed weight. While Sofy et al. (2020) in Iraq reported that application of P at 50 or 100 kg P_2O_5/ha to safflower plants significantly increased plant dry matter, biological yield, and 100-seed weight compared to control plants. Maximum plant dry matter, biological yield, and 100-seed weight were obtained by application of 100 kg P_2O_5/ha (Sofy et al., 2020). Application of P at 50, 75, or 100 kg P_2O_5/ha enhanced the 1000-seed weight and harvest index of safflower (Arani et al., 2011). Abbadi & Gerendás (2011) reported that adding P at concentrations of 0.25, 0.5, 1.0, and 2.0 g/pot increased capitula dry matter and capitula number/pot significantly, increasing from 15.4 to 26.5 g and 8.7 to 16.0, respectively.

2.10 Effect of phosphorus on seed yield

Several researchers have reported a positive influence of P application on safflower seed yield (Arani et al., 2011; Haghighati et al., 2011; Golzarfar et al., 2011, 2012; Singh & Singh, 2013; Malek & Ferri, 2014; Mazhani, 2017; Sofy et al., 2020). Sofy et al. (2020) reported that application of P at 50 or 100 kg P_2O_5/ha to safflower plants resulted in a significant seed yield of 1673 and 1972 kg/ha, while control plants yielded 1470 kg/ha. Mazhani (2017) reported that application of P at 25, 50, and 75 kg/ha to safflower plants significantly increased seed yield by 21.2, 37.2, and 15.8%, respectively, compared to control plants. Golzarfar et al. (2011) found that applying P at 50 and 100 kg P_2O_5/ha enhanced safflower seed production by 23 and 38%, respectively, as compared to control plants. Golzarfar et al. (2012) reported comparable results, when they found that safflower plants applied with 50 and 100 kg P_2O_5 kg/ha produced 2,392 and 2,642 kg/ha of seed, respectively, compared to control plants that produced 1936 kg/ha. While Arani et al. (2011) found that application of P at 50, 75, or 100 kg P/ha enhanced safflower seed yield by 7, 13, and 15.8 %, respectively, compared to control plants. While Singh & Singh (2013) reported that application of 40 and 80 kg P_2O_5/ha to safflower plants increased seed yield when compared to control plants. However, there was no significant difference in seed yield of safflower plants applied with 40 or 80 kg P_2O_5/ha (Singh & Singh,2013). Safflower plants applied with 40 and 80 kg P_2O_5/ha produced 2.22 and 2.36 tons of seed/ha, respectively, whereas control plants produced 1.77 tons of seed/ha (Singh & Singh, 2013). Malek & Ferri (2014) found that treatment of safflower plants with 30 and 60 kg P/ha significantly improved seed yield. However, Haghighati (2010) observed that applying P between 30 and 60 kg/ha had no significant effect on safflower seed yield, suggesting that applying P at a rate greater than 60 kg P_2O_5/ha would have been optimal for increasing seed yield.

2.11 Effect of phosphorus on oil content

Sofy et al. (2020) in Iraq reported that safflower plants applied with 50 and 100 kg P_2O_5/ha significantly produced seed with a higher oil content of 30.0 and 32.6%, respectively, than control plants that produced 28.7% of oil content. While Singh & Singh (2013) reported that application of 40 and 80 kg P_2O_5/ha to safflower plants increased seed oil content by 6.0 and 6.6%, respectively, compared to seed oil content from control plants. Arani et al. (2011) similarly reported that safflower plants applied with 50, 75, and 100 kg P/ha produced seed with significantly higher oil content by 2.1, 3.8, and 4.6%, respectively, than seed oil content from control plants.

2.12 Phosphorus use efficiency

Phosphorus is a necessary and irreplaceable component of all living cells, plants, and animals alike (Roberts & Johnston, 2015). According to Marschner (2005) and Roberts & Johnston (2015), plants extract large amounts of phosphorus from the soil solution in the form of phosphate ions, primarily $H_2PO_4^-$, but the concentration in the soil solution is extremely low. That is why there must be a supply of readily available P in the soil to maintain its concentration in the soil solution. Most of the P in the soil is taken away during harvesting (economic yield), and phosphatic fertilizers must be applied to replenish it (Mengel & Kirkby, 2001; Marschner, 2005; Roberts & Johnston, 2015).

Different plant species and cultivars have varying capacities for growth and output under poor P availability (Abbadi & Gerendás, 2015). Thus, one strategy for achieving sustainable land use that maximizes output with minimal input, resource conservation, and protects the environment could be the cultivation of nutrient-efficient plant species (Place et al., 2013). Abbadi (2017) investigated the P utilization efficiency of alternative oil crops, safflower, and sunflower, which were grown under semi-controlled conditions in sandy and loamy soils with three P treatments (0, 0.2, and 1.0 g P per pot). Both safflower and sunflower responded strongly to increased P availability in both soils but performed better in loamy soil (Abbadi, 2017). Furthermore, both safflower and sunflower accumulated similar amounts of P in the shoots at low P supply in both sandy and sandy loam soils (Abbadi, 2017). In general, safflower accumulated less P in shoots than sunflower at all P levels. Abbadi (2017) observed that sunflower required less external P than safflower in both soil types, although safflower had a higher efficiency ratio in sandy soils and lower values in loamy soils. At all P levels, safflower showed a poorer utilization index than sunflower in both soils. In both soils, safflower recovered less external P (added P, extractable P, and soil solution P) than sunflower. Crop efficiency in terms of P use was determined by a variety of competing factors. Safflower was shown to be inferior to sunflower in most qualities evaluated. As a result, safflower cannot be compared to sunflower as a low-input species in terms of P uptake and utilization efficiency. These findings are consistent with those of Abbadi & Gerendás (2015), who reported that safflower cannot be deemed as a low-input crop for P application when compared to sunflower.

2.13 Effect of N and P interaction on growth, yield components, seed yield, and oil content of safflower

2.13.1 Vegetative growth

The critical deficiency of N increases when P increases, and vice versa (Marschner, 2005). When their contents approach the deficit status range, interactions between these two mineral nutrients are critical (Marschner, 2005). Increased application of either N or P alone results in reduced vegetative growth than when both N and P are added together (Mengel & Kirkby, 2001; Marschner, 2005). Mirzakhani et al. (2009) researching Azobacter and Mycorrhiza bacteria in safflower, reported that there was a significant interaction of N and P on root dry weight. Application of 50 N + 25 P, 100 N + 50 P and 150 N+ 75 P kg/ha, increased safflower root dry matter by 28.1, 48.3 and 67.4% compared to control plants, respectively (Mirzakhani et al., 2009). While Haghighati (2010) observed that interactions of 60 N + 30 P or 60 N + 60 P kg/ha increased safflower plant height but had no effect on plant biomass. Recently, Sreekanth et al. (2021) reported significant interactions of N+P when applied at rates between 30-50 N + 40-60 P kg/ha concerning safflower plant height. The interaction that resulted with the tallest (102.93 cm) safflower plants was 50 N + 60 P kg/ha but was not significantly different from plants applied with 40 N + 60 P or 50 N + 50 P kg/ha (Sreekanth et al., 2021).

2.13.2 Yield Components

Golzarfar et al. (2012) reported that application of 75 N + 50 P_2O_5, 75 N + 100 P_2O_5, 150 N + 50 P_2O_5, and 150 N + 100 P_2O_5 kg/ha significantly increased capitula number/primary branch, capitula number/plant, capitula number/secondary branch, seed number/secondary capitulum, and 1000-seed weight when compared to safflower plants applied with N and P alone. El-Nakhawy (1991) reported that increased application of N + P at 46N + 46P, 92N + 46P, 138N + 46P increases the 100 seed weight by 8%, 18.7%, and 19.2%, respectively. The same N + P interactions increased capitulum per plant by 10.6, 43.2, and 35.7%, respectively, and weight of seed/head by 17.6%, 54.4%, and 32.4%, compared to control plants. Sreekanth et al. (2021) reported significant interactions of N+P at application rates between 30-50 N + 40-60 P kg/ha with respect to safflower plant dry matter, capitula number/plant, seed number/capitulum, and biological yield. The best interaction that resulted in high safflower yield components was 50 N + 60 P kg/ha (Sreenkanth et al., 2021).

2.13.3 Yield

Sreekanth et al. (2021) reported significant interactions of N+P at application rates between 30-50 N + 40-60 P kg/ha concerning safflower seed yield. The best interaction that resulted in a high safflower seed yield was 50 N + 60 P kg/ha (Sreenkanth et al., 2021). Sreekanth et al. (2021) concluded that application of 50 N + 60 P kg/ha was the best fertilizer combination for achieving maximum safflower seed yield of 1.57 t/ha, and the highest benefit-cost ratio of 2.29 in safflower plant with variety PBNS-12. While Golzarfar et al. (2012) reported that interaction of N + P at 75 N + 50 P_2O_5, 75 N + 100 P_2O_5, 150 N + 50 P_2O_5, and 150 N + 100 P_2O_5 kg/ha increased safflower seed yield. Application of 150 N + 100 P_2O_5 kg/ha resulted in the highest seed yield (Golzarfar et al., 2012). While Mirzakhani et al. (2009) had earlier reported that application of 50 N + 25 P, 100 N + 50 P, or 150 N + 75 P kg/ha to safflower plants significantly increased seed yield by 16.4, 55.4, and 28.3 %, respectively when compared to control plants.

2.13.4 Oil content

El-Nakhawy (1991) reported that application of 46 N + 46 P, 92 N + 46 P, or 138 N + 46 P kg/ha decreased the seed oil content by 2.8, 3.3, and 4.9 %, respectively, compared to seed oil content from control plants. Nitrogen and P fertilizer application significantly interacted to increase the safflower seed oil content (Mazhani, 2017). Increasing both N and P application rates significantly increased safflower seed oil content compared to control plants and plants applied with N or P alone (Mazhani, 2017). The maximum seed oil content of 39.2% was obtained with the application of a combination of 100 kg N/ha and 50 kg P/ha (Mazhani, 2017).

From the above literature review, N and P fertilizer application rates for optimum safflower growth, yield, and seed oil content varied significantly depending on location, environmental conditions, cultural practices, irrigation, or farming system. Nitrogen and P application rates ranged between 25-600 and 25-100 kg/ha. These large variations in N and P application rates reported in regions of the world growing safflower imply recommendations of N and P should be based on specific climatic conditions, soil type, cultivar, and farming system (irrigated or rainfed) which justifies the current study. The current study will contribute to the filling of the knowledge gap of N and P recommendations for optimizing safflower yield in Southern Botswana.

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental site

The study was conducted at a farmer's field at Molepolole (24.3966 S, 25.4970 E at an elevation of 1189 m above sea level). The climate is semi-arid with an average annual rainfall of 376.2 mm. Most rain falls in summer which generally starts in late October to February or March. During the summer trial (October 2020 to February 2021), rainfall started in early November to late February. The minimum and maximum rainfall recorded were 2.7 and 35 mm, respectively. The minimum and maximum temperature recorded was 16 and 34 ℃, respectively. During winter which starts from April to October 2021, the minimum and maximum rainfall recorded were 4 and 29.7 mm respectively. The minimum and maximum temperature recorded was -0.1 and 38.8 ℃, respectively. The soil in the experiment site was sandy clay loam or sandy loam depending on the section of the field where the study was implemented.

3.2 Experimental design

The experiment consisted of two trials, one from October 2020 to February 2021 and the other from April to September 2021. The experimental design was a split-plot with three replications arranged into randomized complete blocks. The treatments were N (main plots) applied as calcium ammonium nitrate (28 % N), while P (sub-plots) was applied as single super phosphate (8.3 % P). Prior to planting, the soil was sampled at a depth of 30 cm to determine the physicochemical properties and total N and P. Nitrogen was applied at 0, 40, 80, and 120 kg N/ha in two splits. The first split was applied two weeks after emergence and the second split was applied at the bolling stage of safflower . Phosphorus was applied at 0, 25, 50, and 75 kg/ha P_2O_5 two weeks after emergence. . At the end of each trial, total N and P were determined in the experimental plots. The main plots and sub-plots were 14 m x 4 m and 14 m x 1 m, respectively.

3.3 Soil sampling and analysis

Soil samples were collected using a zigzag pattern from five different locations in the field, and subsamples were combined to create a composite sample before and after the experiment. The pH, total N, P, organic carbon (OC), texture, electrical conductivity (EC), and cation exchange capacity (CEC) of the soil were determined. With exception of OC, all the soil variables were determined using the method of Estefan et al. (2013). The Walkley Black method was used to determine the soil's organic carbon content (Walkley, 1947).

3.4 Crop husbandry

The safflower cultivar, Sina, was used for the study. The seeds were planted at a 25 cm intra-row spacing on drip lines spaced 1 m apart. The fertilizer was incorporated into the soil by side banding about 5 cm away from the plants. The plants were irrigated when there was no rain twice a week at 6 mm per irrigation interval. Weed control was done manually by hoeing between rows and plants. Routine scouting of pests and diseases was done twice a week, where neither was found throughout the experiment.

3.5 Data collection

The dependent variables determined were height to first branching, plant height, stem diameter, leaf area, leaf chlorophyll content, number of primary branches per plant, number of capitula per plant, capitula diameter, seed number per capitulum, 1000-seed weight, plant biomass, seed yield, oil content (%), oil yield and leaf N and P. **3.5.1 Height to first branching**

The height to the first branch was determined using fifteen randomly selected plants per treatment per replication. The height to the first branch was determined by measuring from the ground level to the first branch using a measuring tape.

3.5.2 Plant height

Plant height was determined using fifteen randomly selected plants per treatment per replication. The height of the plant was determined by measuring from the ground level to the tallest point of the plant using a measuring tape at physiological maturity.

3.5.3 Stem diameter

The stem diameter was determined using fifteen randomly selected plants per treatment per replication. The stem diameter of the plant was determined by measuring the diameter at the bottom of the stem using a digital vernier caliper.

3.5.4 Leaf area

Leaf area was determined using fifteen randomly selected plants per treatment per replication. The leaf area was determined using a digital leaf area meter.

3.5.5 Chlorophyll content

The chlorophyll content of 15 randomly selected leaves per treatment per replication was determined at the end of the elongation phase of safflower using a Minolta SPAD-502 chlorophyll meter.

3.5.6 Number of primary branches

The number of primary branches on each plant was determined from 15 randomly tagged plants per treatment per replication. Primary branches were counted.

3.5.7 Number of capitula

The number of capitula produced per plant was determined by counting the heads of 15 randomly tagged plants per treatment per replication at the end of the flowering period.

3.5.8 Capitula diameter

The capitulum diameter of 15 randomly tagged plants per treatment per replication was determined using an electronic vernier caliper at the end of the flowering period.

3.5.9 Seed number per capitulum

The number of seeds was counted from a set of 75 capitula collected from 15 randomly selected plants within each plot using a counter.

3.5.10 Thousand-seed weight

The weight of 1000 representative seeds per treatment per replication was determined using a Mettler PM 400 digital balance.

3.5.11 Seed yield

The seed yield was determined from a 4m^2 area in the plot centre. The capitula were threshed and winnowed to separate the seeds from the chaff. Weighing the seeds was accomplished with a Mettler PM 400 digital balance.

3.5.12 Plant Biomass

The dry mass of the complete plants was determined by randomly harvesting ten plants per sub-plot, putting them in paper bags, and allowing them to dry for 72 hours at a constant weight in an oven set to 66°C. The dried samples were weighed on a Mettler PM 400 digital balance.

3.5.13 Determination of leaf mineral content

Leaf and seed samples were oven-dried at 66°C for 72 hours to constant weight. The dried samples were ground and sieved using a size two sieve. A 1.25 g composite sample was digested for 7 hours at 330°C in a BD block with 20 mL sulphuric acid (98%) and 4 mL hydrogen peroxide (30%). The micro Kjeldahl method was used to determine the amount of N in the sample (AOAC, 1995). Phosphorus from leaf and seed samples was determined calorimetrically using sodium phenol and ammonium molybdate plus ascorbic acid (AOAC, 1996). The UV spectrophotometer was used to determine the absorbance (UV-1602, IPC, Shimadzu, RSA).

3.5.14 Determination of seed oil content

The Soxhlet extraction method was used to extract oil from achenes (SOXTHERM 2000, S 206 AK/S2006A, Automatic from Gerhardt, Bonn, Germany). A sample of 0.5 g of the ground dried safflower seed was weighed and placed into pre-weighed extraction bags, which were sealed and immersed in petroleum ether for one hour in the XT10 Ankom Extraction System (Ankom Technology). Following extraction, the bags were dried in an oven for 15 minutes and then chilled for another 15 minutes. The bag and sample were weighed at the conclusion. The difference between the starting and end weights represented the oil content expressed as a percentage of the ground dried safflower seed's initial weight.

3.5.15 Determination of nutrient use efficiency

Determination of nitrogen use efficiency (NUE)

The nitrogen use efficiency was calculated according to the method of Moll et al. (1982). The nitrogen uptake (NUpE) and nitrogen utilization efficiency (NUtE) were calculated.

NUpE = N(plant)/N(applied)

NUtE = SDM/N(plant), where SDM is shoot dry mass

NUE = SDM/N(applied)

Determination of phosphorus use efficiency (PUE)

Phosphorus uptake efficiency (PUpE), P utilization efficiency (PUtE), and PUE were calculated as follows according to the methods of Moll et al. (1982) and Gerloff & Gabelman (1983).

PUpE = (P (plant) * Dry matter) / P(applied)

PUtE = Dry matter / (P plant * Dry matter)

PUE = Dry matter / P plant

3.6 Statistical Analysis

Data was subjected to analysis of variance (ANOVA) using the SAS (Statistical Analysis System, 2016) program's general linear models (Proc GLM) algorithm. The Least Significant Difference (LSD) was used to separate treatment means at a 5% level of significance.

CHAPTER 4

RESULTS

4.1 Introduction

This chapter will commence by presenting the analysed data which will answer the objective questions of this study. The objective of this study was to find the effect of N and P application on the growth, yield components, seed yield, and oil content of safflower. The results of the vegetative growth, yield components, seed yield, and oil content will be outlined.

4.2 Soil analysis

The soil physico-chemical properties of the experimental site at the start of the study, both summer and winter are shown in Table 4.1. The soil textural class of the experimental site in summer was sandy clay loam while that of the winter trial was sandy loam. For both soils, soil organic carbon content and soil total N were low while total P and EC were high. The soil CEC in the sandy clay loam soil was medium but in the sandy loam soil was low (Table 4.1). The pH of both soils was acidic (5.08). The application of N and P fertilizers had a significant ($P \leq 0.05$) effect on all soil physico-properties at the end of the experiment (Table 4.2, 4.3, 4.4). Application of N as calcium ammonium nitrate significantly increased the soil residual N and P, pH, EC, and CEC, but had no significant ($P > 0.05$) effect on the soil OC at the end of the experiment both in summer and winter (Table 4.2, 4.3). Application of P in the form of single super phosphate had no significant ($P > 0.05$) effect on the soil total N and OC, but significantly ($P < 0.05$) increased soil total P, pH, EC, and CEC both in summer and winter at end of the trials (Table 4.4).

Table 4.1. The physico-chemical properties of the soil before the start of the study

Soil characteristics	Summer	Winter
pH ($CaCl_2$)	5.07	5.08
OC (%)	0.408	0.264
CEC (cmol/kg)	19.11	6.80
EC (µs/cm)	35.3	58.7
Total N (%)	0.11	0.11
Total P (mg/kg)	39.59	38.8
Sand (%)	70.72	76.72
Silt (%)	29.28	23.28
Clay (%)	21.28	11.28
Textural Class	Sandy clay loam	Sandy loam

Table 4.2. Summarised ANOVA for dependent variables: soil pH, soil nitrogen, soil phosphorus, soil cation exchange capacity (CEC), soil organic carbon (OC), and soil electrical conductivity (EC) after the trials in winter and summer.

Dependent variable		**Summer season**		**Winter season**	
	Effects	**F-value**	**P-value**	**F-value**	**P-value**
Soil pH	N	2.95	0.1205	23.95	0.0010
	P	5.00	0.0078	19.23	<0.0001
	N * P	16.95	<0.0001	2.40	0.0417
Soil N	N	9.54	0.0106	49.73	0.0001
	P	4.59	0.0612	23.78	<0.0001
	N * P	1.68	0.1497	55.42	<0.0001
Soil P	N	6.14	0.0293	36.84	0.0003
	P	2.33	0.0997	32.11	<0.0001
	N * P	3.08	0.0134	23.35	<0.0001
CEC	N	330.00	<0.0001	44.78	0.0002
	P	29.35	<0.0001	1.11	0.3654
	N * P	26.25	<0.0001	2.46	0.0381
OC	N	87.18	<0.0001	3.55	0.0873
	P	14.06	<0.0001	0.39	0.7630
	N * P	15.06	<0.0001	1.41	0.2399
EC	N	197.76	<0.0001	62.55	<0.0001
	P	732.31	<0.0001	4.00	0.0193
	N * P	865.76	<0.0001	66.46	<0.0001

Table 4.3. Effect of N as calcium nitrate on soil physico-chemical properties.

Summer	Kg N/ha	N (%)	P (mg/kg)	pH ($CaCl_2$)	OC (%)	EC (µs/cm)	CEC (cmol/kg)
	0	0.1119d	29.58b	5.13c	0.57b	50.09d	6.47d
	40	0.1146c	36.38a	5.24b	0.62a	55.68c	11.55a
	80	0.1186b	34.11a	5.26ab	0.42c	60.68b	10.38b
	120	0.1248a	34.59a	5.32a	0.40c	65.67a	9.51c
	Significance	*	**	**	***	**	***
	LSD	0.002	4.02	0.07	0.03	1.23	0.59
Winter	**Kg N/ha**	**N (%)**	**P (mg/kg)**	**pH ($CaCl_2$)**	**OC (%)**	**EC (µs/cm)**	**CEC (cmol/kg)**
	0	0.1221c	20.30c	5.61b	0.36a	173.33c	4.81c
	40	0.1236a	37.41a	5.89a	0.42a	210.39a	8.94a
	80	0.1231c	35.90a	5.99a	0.48a	206.33a	6.94b
	120	0.1272a	26.41b	5.94a	0.44a	190.17b	8.86a
	Significance	*	***	*	NS	***	**
	LSD	0.0005	0.5059	0.2134	0.1336	8.6485	0.7686

*, **, ***, NS. Significant at 0.01, 0.05, 0.0001 or not significant, respectively. Means separated using the Least Significant Difference (LSD) at P = 0.05; means with the same letter(s) are not significantly different from each other.

Table 4.4. Effect of application of P as single super phosphate on soil physico-chemical properties.

Summer	Kg P/ha	N (%)	P (mg/kg)	pH(CaCl2)	OC (%)	EC (µs/cm)	CEC (cmol/kg)
	0	0.11a	31.66b	5.25a	0.47b	54.48d	8.89bc
	25	0.11a	32.50ab	5.26a	0.46b	50.08c	8.65c
	50	0.11a	34.23ab	5.28a	0.55a	60.68b	9.30b
	75	0.11a	36.23a	5.26b	0.53a	71.27a	11.07a
	Significance	NS	**	**	***	**	***
	LSD	0.002	4.02	0.07	0.03	1.23	0.59
Winter	Kg N/ha	N (%)	P (mg/kg)	pH ($CaCl_2$)	OC (%)	EC (µs/cm)	CEC (cmol/kg)
	0	0.12b	24.01c	5.23b	0.43a	186.33b	7.06a
	25	0.12b	25.30c	5.28ab	0.40a	197.91a	7.60a
	50	0.12b	31.34b	5.33ab	0.41a	196.59a	7.62a
	75	0.13a	43..30a	5.39a	0.46a	199.38a	7.63a
	Significance	*	***	***	NS	**	NS
	LSD	0.0005	0.5059	0.1434	0.1336	8.6485	0.7686

*, **, ***, NS. Significant at 0.01, 0.05, 0.001 or not significant, respectively. Means separated using the Least Significant Difference (LSD) at P = 0.05; means with the same letter(s) are not significantly different from each other.

4.3 Effect of N and P on Vegetative growth

The effect of N and P on the dependent variables that define vegetative growth in both summer and winter are shown in Table 4.5. The table shows the independent and interaction significance of both N and P on vegetative growth of safflower in summer and winter.

Table 4.5. Summarised ANOVA for dependent variables: height to the first branch, plant height, primary branch numbers, stem diameter, dry matter, leaf area, and chlorophyll content.

Dependent variables	**Effects**	**Summer season**		**Winter season**	
		F-value	**P-value**	**F-value**	**P-value**
Height to 1st branch	N	137.25	<0.0001	0.68	0.5980
	P	103.80	<0.0001	1.40	0.2667
	N * P	18.24	<0.0001	1.91	0.0999
Plant height	N	59.44	<0.0001	0.07	0.9755
	P	23.24	<0.0001	2.96	0.05
	N * P	0.88	0.5549	0.50	0.8573
Primary branch numbers	N	11.02	0.0075	0.52	0.6844
	P	3.97	0.0198	1.42	0.2615
	N * P	1.42	0.2332	1.10	0.4022
Stem diameter	N	2.05	0.2088	0.34	0.7956
	P	79.65	<0.0001	4.88	0.0087
	N * P	1.28	0.2973	1.68	0.1486
	N	85.08	<0.0001	24.08	0.0010

Dry matter	P	183.53	<0.0001	55.44	<0.0001
	N * P	62.94	<0.0001	41.41	<0.0001
Leaf area	N	35.08	0.0003	327.05	<0.0001
	P	25.24	<0.0001	150.98	<0.0001
	N * P	5.38	0.0005	10.54	<0.0001
Chlorophyll	N	273.57	<0.0001	11.15	0.0072
	P	63.67	<0.0001	0.44	0.7235
	N * P	5.03	0.0007	13.59	<0.0001

4.3.1 Height to the first branching

In summer, the interaction of N and P had a significant ($P < 0.0001$) effect on the height to the first branching of safflower (Table 4.5, Figure 4.1a). The interactions of N and P at different levels were all significantly ($P < 0.05$) different from each other as they influenced the first branching height of safflower (Figure 4.1a). An interaction of 40 kg N/ha and 50 kg P/ha resulted in a significant ($P < 0.05$) highest height to first branching (16.8 cm) of safflower compared to any other treatment interactions (Figure 4.1a). The second highest first branching height of safflower plants was produced by the application of 80kg N/ha and 50 kg P/ha in summer (Figure 4.1a). The lowest first branching height (8.8 cm) was produced by safflower plants where neither N nor P were applied (Figure 4.1a). In general, an interaction of 50 kg P/ha plus any amount of N promoted the first branching height of safflower plants in summer, with exception of the interaction of 120 kg N/ha and 75 kg p/ha (Figure 4.1a).

In winter, there was no significant ($P > 0.05$) interactions of N and P, or main effects of N and P independently as they influenced the first branching height of safflower (Table 4.5, Figure

4.1b & c). However, safflower applied with N and P independently at 40 and 50 kg/ha produced the highest first branching height of safflower plants of 31.4 and 30.4 cm respectively, though not significantly (P > 0.05) different (Figure 4.1b & c).

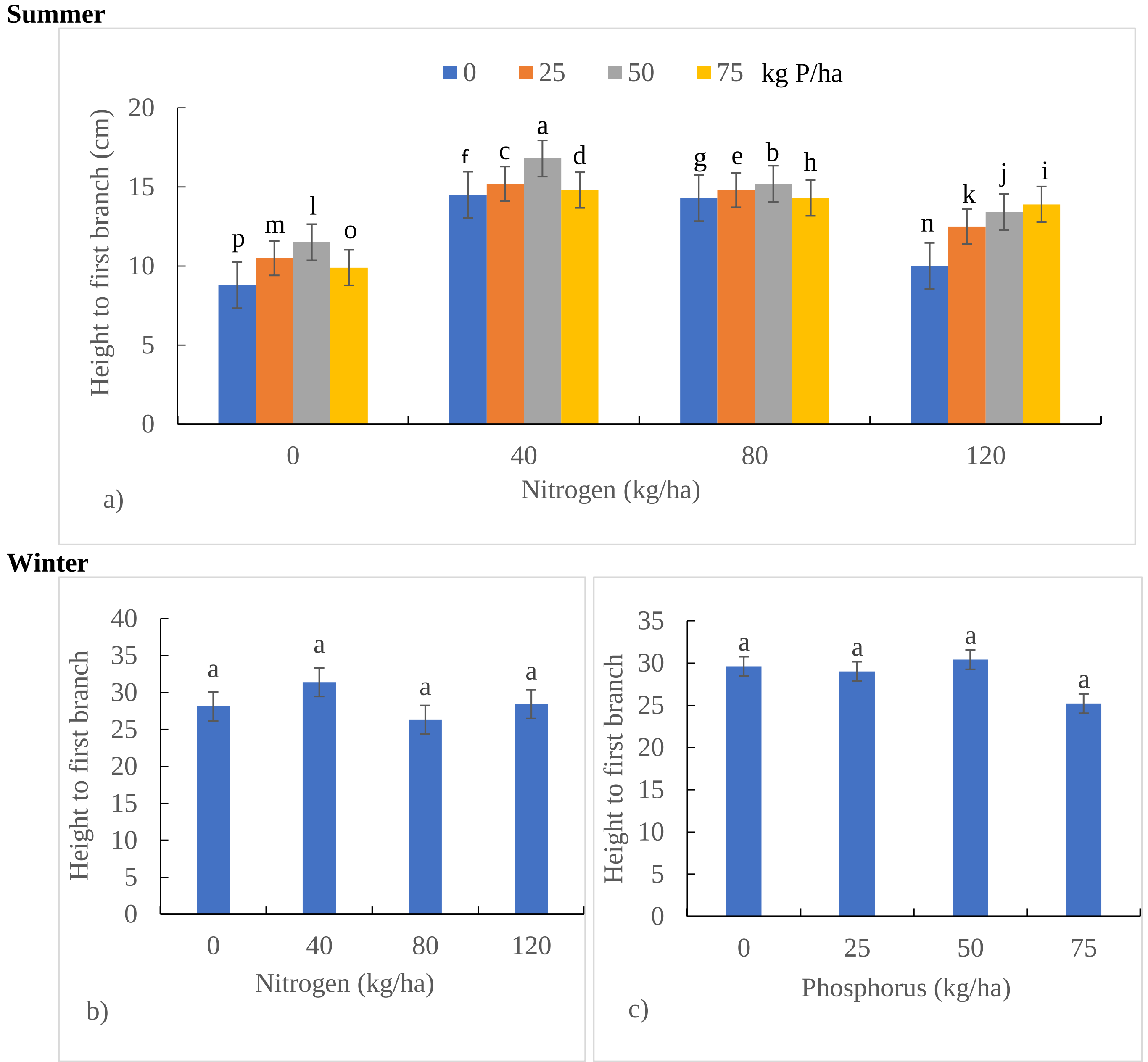


Figure 4.1. Effect of nitrogen and phosphorus on the height to the first branch: a) interaction effect in summer, b) nitrogen effect in winter, and c) phosphorus effect in winter, bars are standard error. Means with the same letter(s) are not significantly different; mean separation by LSD at P = 0.05.

4.3.2 Plant Height

In summer and winter grown safflower, N and P did not significantly ($P > 0.05$) interact to influence plant height, therefore main effects are presented (Table 4.5). In summer, N and P independently significantly ($P < 0.05$) influenced safflower plant height (Table 4.5, Figure 4.2a & b). Safflower plant height increased with increasing application of N fertilizer up to 40 kg/ha (59.5 cm), thereafter plant height decreased (Figure 4.2a). The response of safflower plants to increasing N application was quadratic in summer with maximum plant height obtained with application of 40 kg N/ha (Figure 4.2a). Application of N above 40 kg/ha significantly ($P < 0.05$) decreased safflower plant height in summer (Figure 4.2a).

The response of safflower plants to increasing P application in summer was quadratic with maximum plant height (57.2 cm) produced by plants applied with 50 kg/ha (Figure 4.2b). Application of 25 and 50 kg P/ha significantly ($P < 0.05$) increased safflower plant height compared to plants applied with 0 and 75 kg P/ha in summer (Figure 4.2b).

In winter, application of N fertilizer to safflower plants had no significant ($P > 0.05$) influence on plant height (Table 4.5, Figure 4.2c). However, there was a non-significant ($P > 0.05$) increase in safflower plant height with an increase of N from 40 to 120 kg/ha compared to control plants (Figure 4.2c). The response of safflower plants to increasing N application was quadratic with 40 kg N/ha producing the tallest plants (60.6 cm) though non-significant (Figure 4.2c). On the contrary, the application of P had a significant ($P < 0.05$) effect on plant height (Table 4.5, Figure 4.2d). The response of safflower plants to increasing P application in was also quadratic. Application of 50 kg P/ha significantly ($P < 0.05$) increased safflower plant height compared to plants applied with either 0 or 75 kg P/ha (Figure 4.2d). The plant height of safflower plants applied with either 0 or 75 kg P/ha did not significantly ($P > 0.05$) differ (Figure 4.2d). Similarly, plants applied with either 25 or 50 kg P/ha did not differ in plant height in winter (Figure 4.2d).

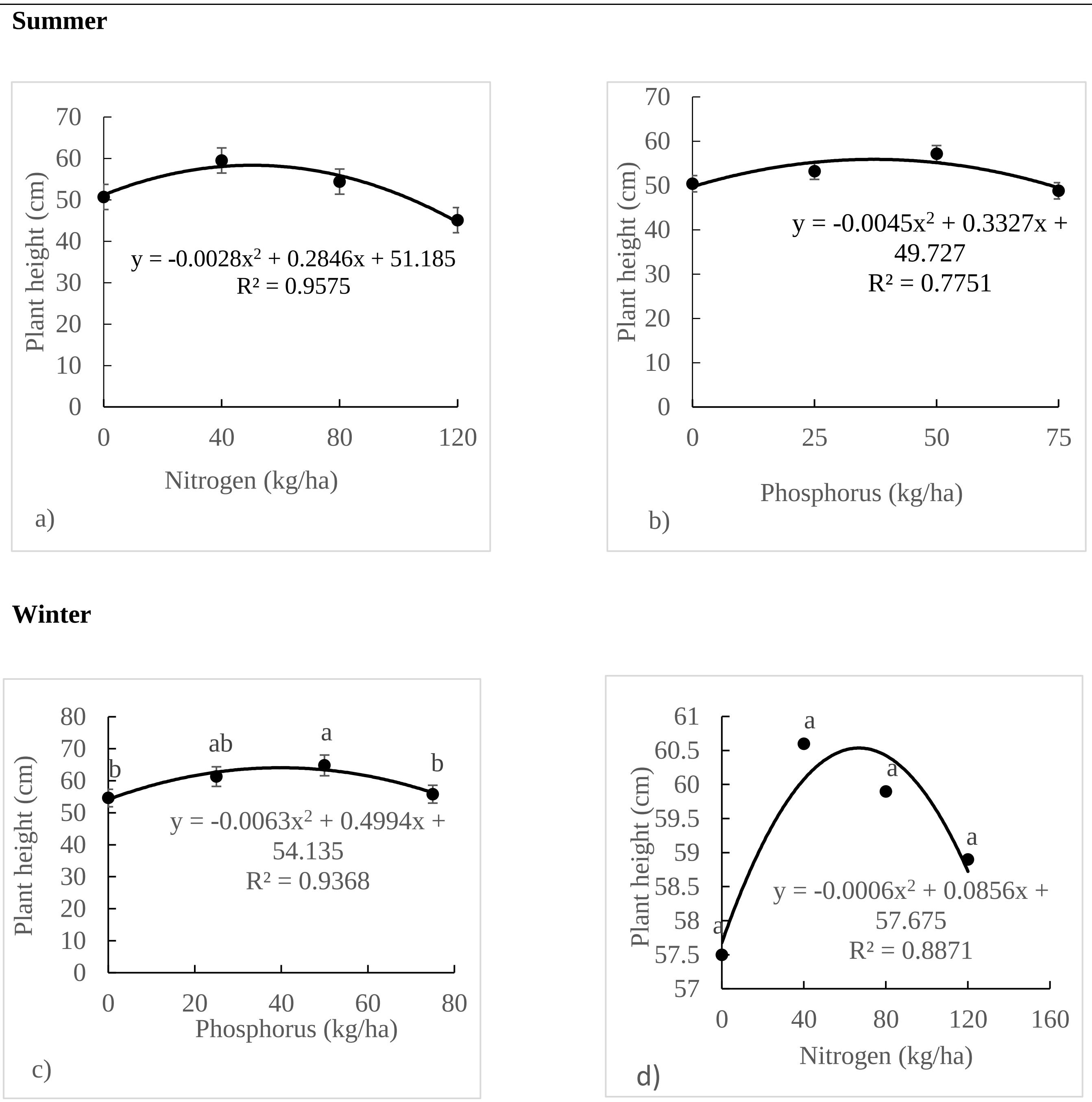


Figure 4.2. Effect of N and P on safflower plant height: a) N effect on plant height in summer, b) P effect on plant height in summer, c) N effect on plant height in winter and d) P effect on plant height in winter; bars are standard error. Means with the same letter(s) are not significantly different; mean separation by LSD at P = 0.05.

4.3.3 Primary branch numbers

In summer, N and P interaction did not significantly ($P > 0.05$) influence the primary branch number of safflower plants, therefore N and P main effects are presented (Table 4.5). However, in winter none of the treatments had a significant influence on safflower primary branch number (Table 4.5). Nitrogen and P independently significantly ($P < 0.05$) influenced primary branch numbers of safflower plants in summer (Table 4.5, Figure 4.3a &b). Application of N at 40, 80, and 120 kg/ha significantly ($P < 0.05$) increased the primary branch number of the safflower plants compared to control plants (Figure 4.3a). The maximum number of primary branches (14.2) was observed on safflower plants applied with 80 kg N/ha, but this was not significantly ($P > 0.05$) different from the primary branch number of plants applied with 40 kg N/ha (Figure 4.3a). Application of 120 kg N/ha to safflower plants in summer significantly reduced the primary branch number of safflower plants compared to plants applied either with 40 or 80 kg N/ha.

Application of P at 25, 50, or 75 kg/ha significantly ($P < 0.05$) increased the primary branch number of safflower plants compared to control plants in summer (Figure 4.3b). However, the primary number of safflower plants applied with 25, 50, or 75 kg P/ha did not significantly ($P > 0.05$) differ from each other (Figure 4.3b). Safflower plants applied with 50 kg P/ha produced the highest (13.1) number of primary branches (Figure 4.3b). The number of primary branches decreased when P was applied at 75 kg/ha (Figure 4.3b). In winter, there was no significant ($P > 0.05$) interaction effect of N and P on the number of primary branches of safflower. Independently N and P also had no significant ($P > 0.05$) effect on the number of primary branches (Figure 4.3c & d).

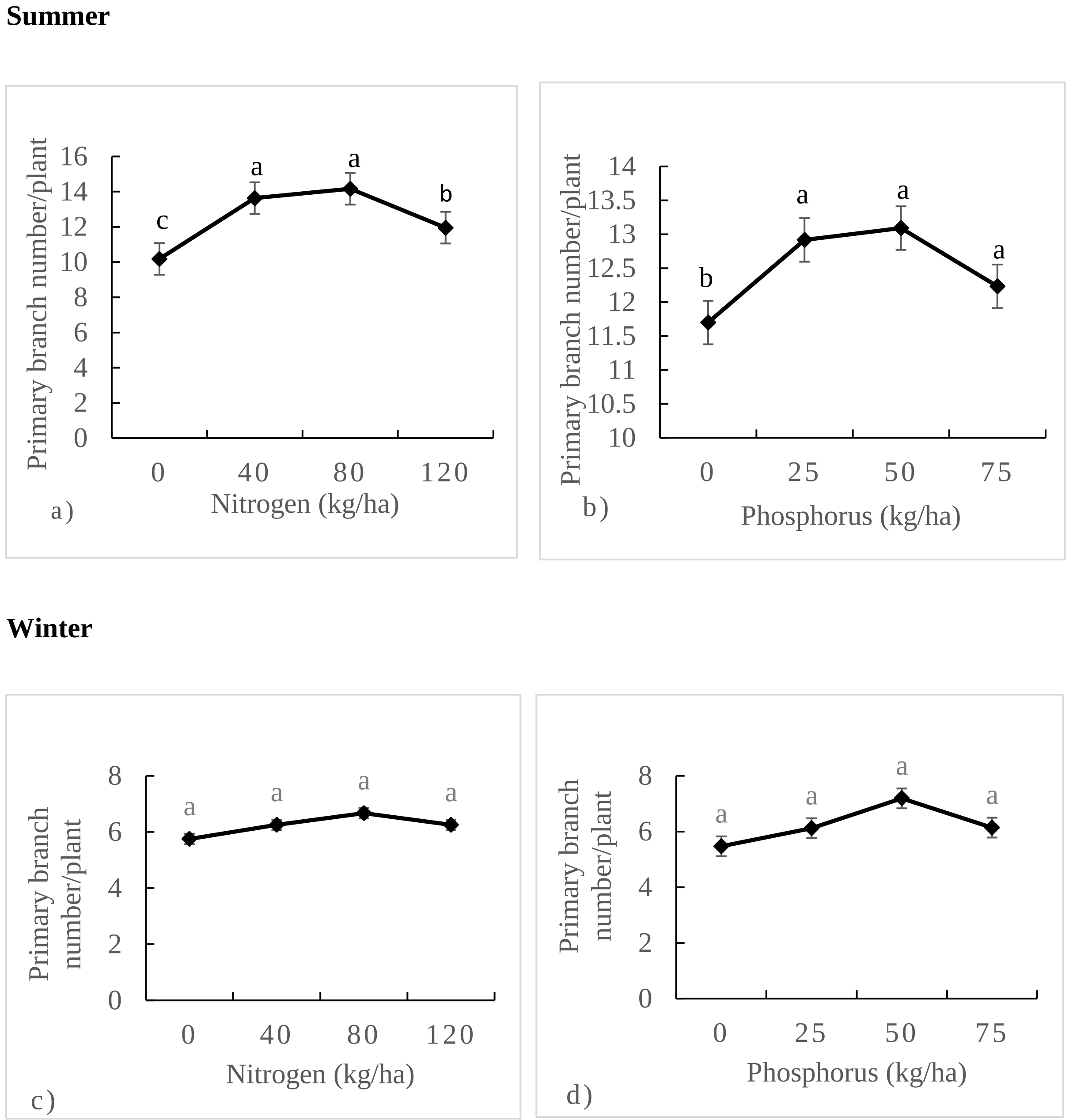


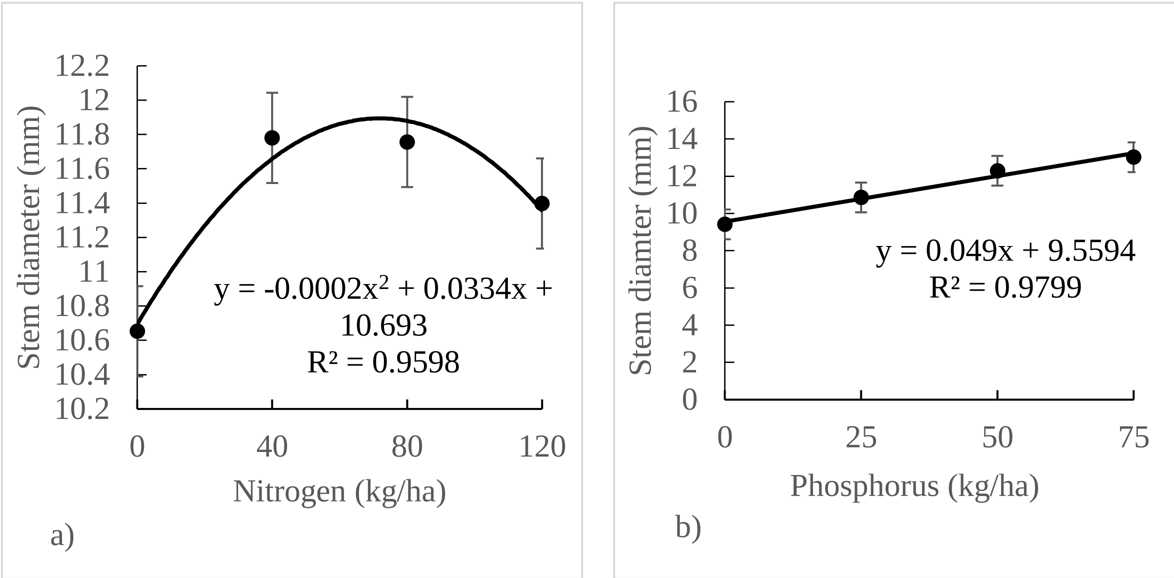
Figure 4.3. Effect of N and P on safflower primary branch number: a) N effect on primary branch number in summer, b) P effect on primary branch number in summer, c) N effect on primary branch number in winter and d) P effect on primary branch number in winter; bars are standard error. Means with the same letter(s) are not significantly different; mean separation by LSD at P = 0.05.

4.3.4 Stem diameter

In both summer and winter the interaction of N and P, and N had no significant ($P > 0.05$) effect on the stem diameter of safflower plants (Table 4.5). However, the application of P fertilizer to safflower plants significantly ($P < 0.05$) influenced stem diameter in both summer and winter (Table 4.5). The response of safflower plants to increasing N application was a non-significant ($P > 0.05$) quadratic with respect to stem diameter (Figure 4.4a). A maximum stem diameter of 11.78 mm was produced by safflower plants applied with 40 kg N/ha in summer. Whilst in summer, application of P fertilizer had a significant ($P < 0.0001$) effect on the stem diameter of safflower (Figure 4.4b). Increasing the P application rate significantly ($P < 0.05$) increased safflower stem diameter in summer and the response was linear (Figure 4.4b). A maximum stem diameter of 13.02 mm was produced by plants applied with 75 kg P/ha (Figure 4.4b). There was a significant ($P < 0.05$) positive linear correlation of $r = 0.99$ between stem diameter and the amount of P applied to safflower plants in summer (Figure 4.4a).

Although the application of N did not significantly ($P > 0.05$) influence stem diameter in winter, there was an increase in stem diameter with an increase in N application rates (Figure 4.4c). There was a positive linear correlation ($r = 0.97$) between the stem diameter and N application rates. The maximum stem diameter of 8.92 mm was produced by safflower plants applied with 120 kg N/ha (Figure 4.4c). In winter application of P to safflower, plants had a significant ($P < 0.05$) effect on stem diameter (Figure 4.4d). The response of safflower plants to increasing P application rates was quadratic, with a maximum stem diameter of 9.48 mm produced by plants applied with 50 kg/ha in winter (Figure 4.4d). Application of P above 50 kg/ha significantly ($P < 0.05$) decreased safflower stem diameter (Figure 4.4d).

Summer



Winter

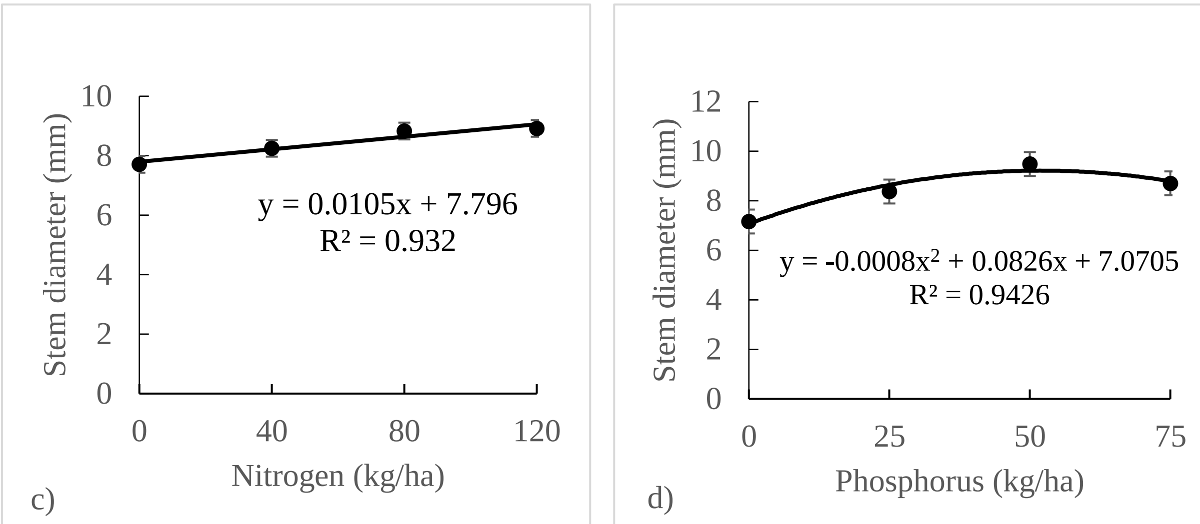


Figure 4.4. Effect of N and P on safflower stem diameter: a) N effect on stem diameter in summer, b) P effect on stem diameter in summer, c) N effect on stem diameter in winter, and d) P effect on stem diameter in winter; bars are standard error. Means with the same letter(s) are not significantly different; mean separation by LSD at P = 0.05.

In winter, the response of safflower plants to applied N and P fertilizers was relatively poor with respect to vegetative growth especially the variables first branching height, plant height, and primary branch number due to extremely low temperatures that occurred in July and late August 2021 during the elongation stage which caused a chilling injury (Figure 4.4 e, f, Appendix 3). However, safflower plants recovered from chilling when the temperatures increased in September 2021 (Figure 4.4 g).

Figure 4.4e. Chilling injury caused by low temperature of -4.2 °C on 14th July 2021

Figure 4.4f. Chilling injury symptoms on safflower caused by low temperature of -6.3 ℃ on 29th August 2021.

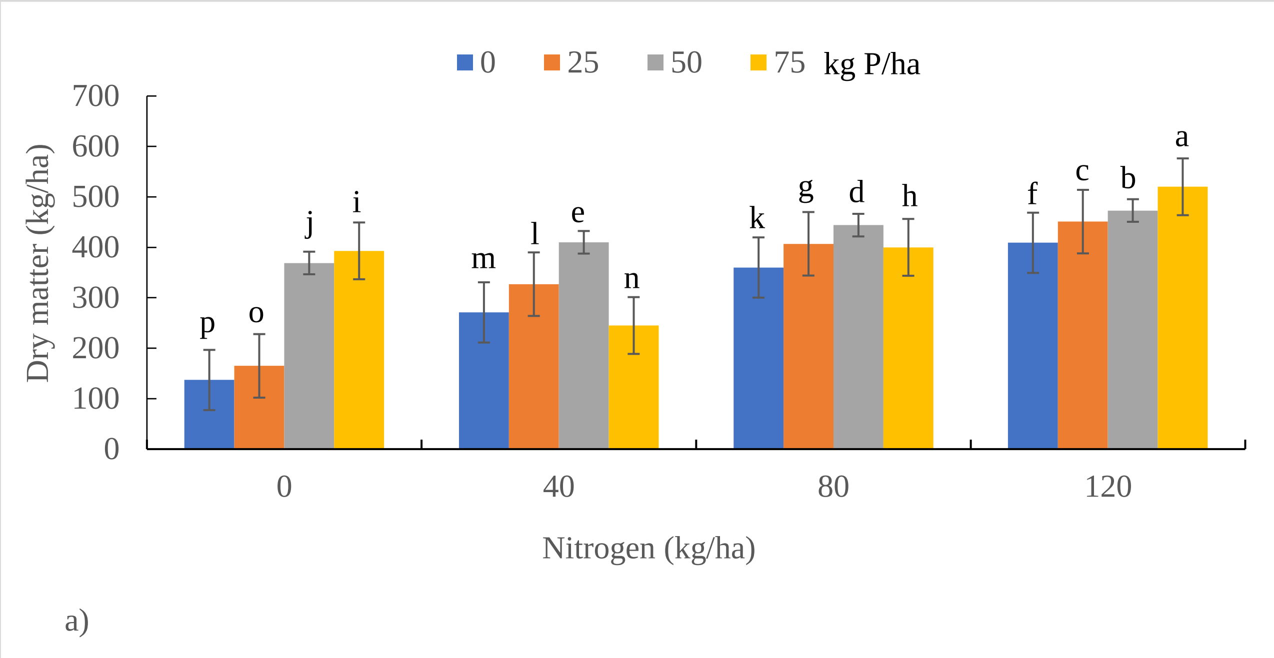
Figure 4.4g. Safflower plants recovered from chilling injury

4.3.5 Dry matter

There were significant ($P < 0.0001$) interactions between N and P on safflower plant dry matter both in summer and winter (Table 4.5). All N and P interaction combinations were significantly ($P < 0.05$) different from each other both in summer and winter grown safflower (Figure 4.5a & b). In summer application of 120 N + 75 P kg/ha to safflower plants significantly ($P < 0.05$) produced the highest plant dry matter of 520 kg/ha (Figure 4.5a). While safflower plants not applied with both N and P in summer produced significantly ($P < 0.05$) lowest plant dry matter of 137 kg/ha (Figure 4.5a). While in winter, safflower applied with 40 N + 25 P kg/ha significantly ($P < 0.05$) produced the highest dry matter of 763 kg/ha (Figure 4.5b). While application of 80 N + 0 P kg/ha significantly ($P < 0.05$) produced the lowest dry matter of 199 kg/ha in winter (Figure 4.5b).

In the winter study, there was also a significant ($P < 0.0001$) interaction effect of N and P on the dry matter of safflower (Figure 4.5b). The application of N improved the dry matter of safflower. Maximum dry matter was obtained through a combination of 40 kg N/ha and 25 kg P/ha. As the rates of N application increased, the dry matter decreased when P was applied at 25 kg/ha. Phosphorus application at 50 kg/ha increased the dry mass when N rates increased up to 80 kg/ha where thereafter there was a decrease in the dry mass. The combination of N and P at 120 kg N/ha yielded the least dry mass compared to other combinations. The treatment combinations were all significantly different from each other.

Summer



Winter

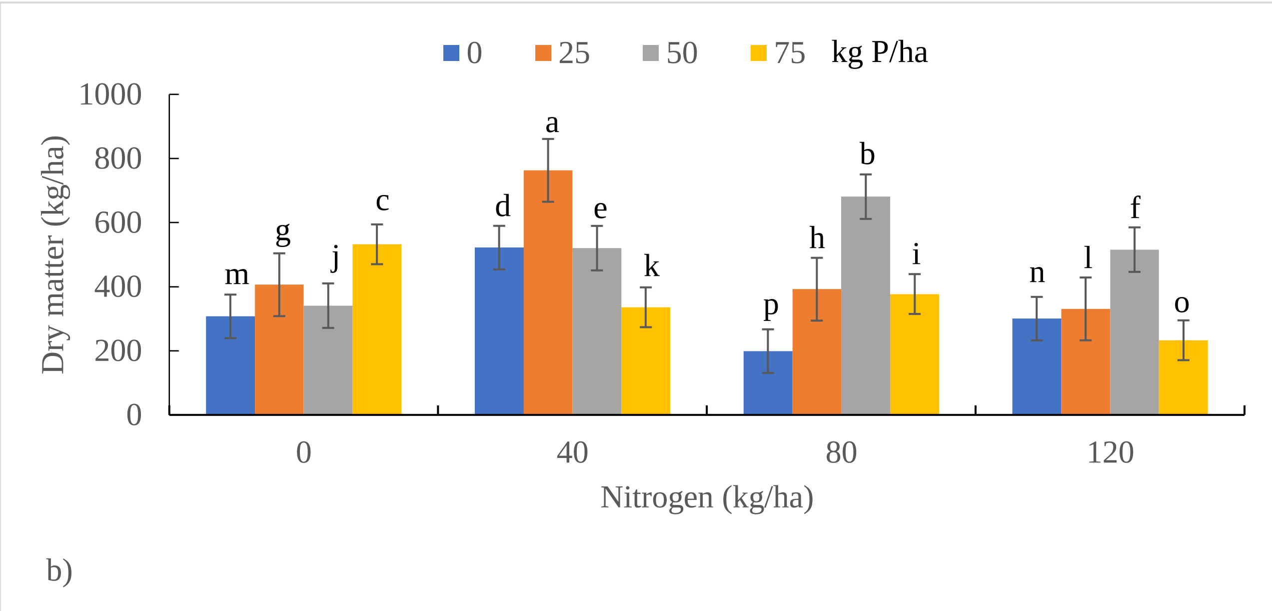


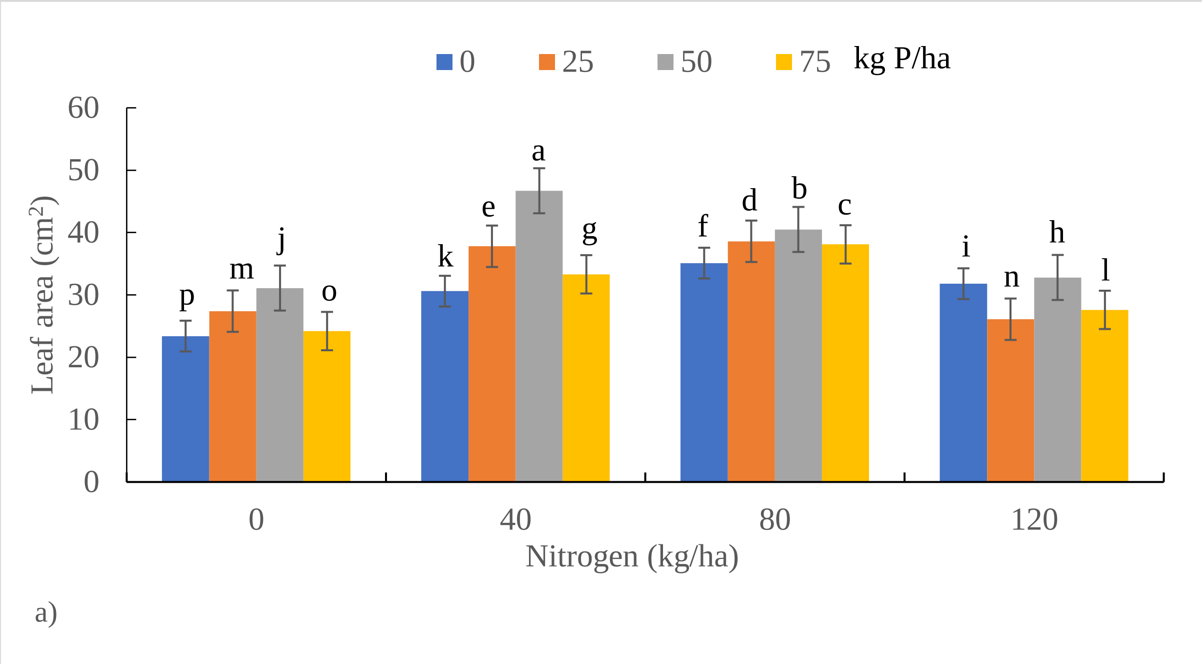
Figure 4.5. Effect of nitrogen and phosphorus on safflower dry matter: a) nitrogen and phosphorus interaction effect on the dry matter in summer, b) nitrogen and phosphorus interaction effect on the dry matter in winter, bars are standard error. Means with the same letter(s) are not significantly different.

4.3.6 Leaf area

The interaction effect of N and P on the leaf area of safflower plants was significant ($P < 0.05$) in summer (Table 4.5). Figure 6a illustrates the results of the leaf area as affected by the interaction of N and P. The N and P interaction combinations were all significantly different from each other (Figure 4.6a). The maximum leaf area of 46.7 cm^2 was significantly ($P < 0.05$) obtained in safflower plants applied with a combination of 40 kg N/ha and 50 kg P/ha in summer (Figure 4.6a). The lowest leaf area of 23.4 cm^2 was significantly ($P < 0.05$) produced by safflower plants not applied with either N or P in summer (Figure 4.6a). In summer, the interactions of 80 kg N/ha with P levels were significantly ($P < 0.05$) higher in the leaf area than the corresponding interactions of 40 kg N/ha with P with exception of 40 N + 50 P kg/ha (Figure 4.6a).

During winter, the leaves of safflower were generally small across all treatment combinations compared to those in summer (Figure 4.6b). There was a significant ($P < 0.05$) interaction of N and P in the leaf area of safflower plants in winter (Table 4.5). When N was not applied, the leaves were relatively smaller compared to when N was applied. The maximum leaf area of 30.5 cm^2 was significantly ($P < 0.05$) obtained in safflower plants applied with 40 N + 50 P kg/ha (Figure 4.6b). Safflower plants applied with 0 N + 75 P kg/ha produced a significant ($P < 0.05$) lower leaf area of 7.82 cm^2. When N was not applied, the leaf area was significantly ($P < 0.05$) smaller compared to when N was applied.

Summer



Winter

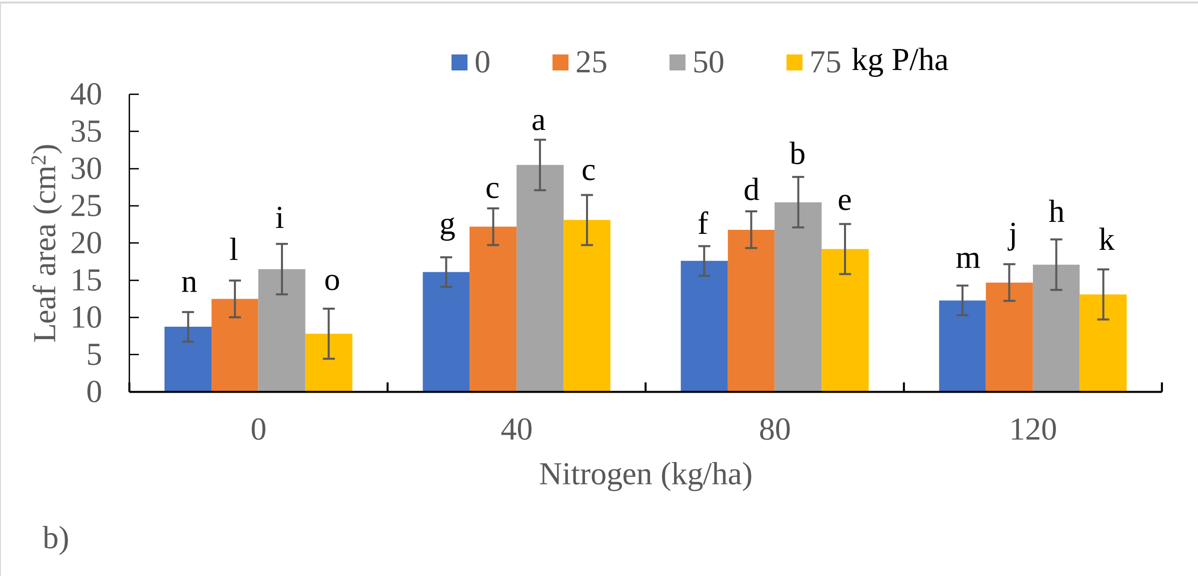


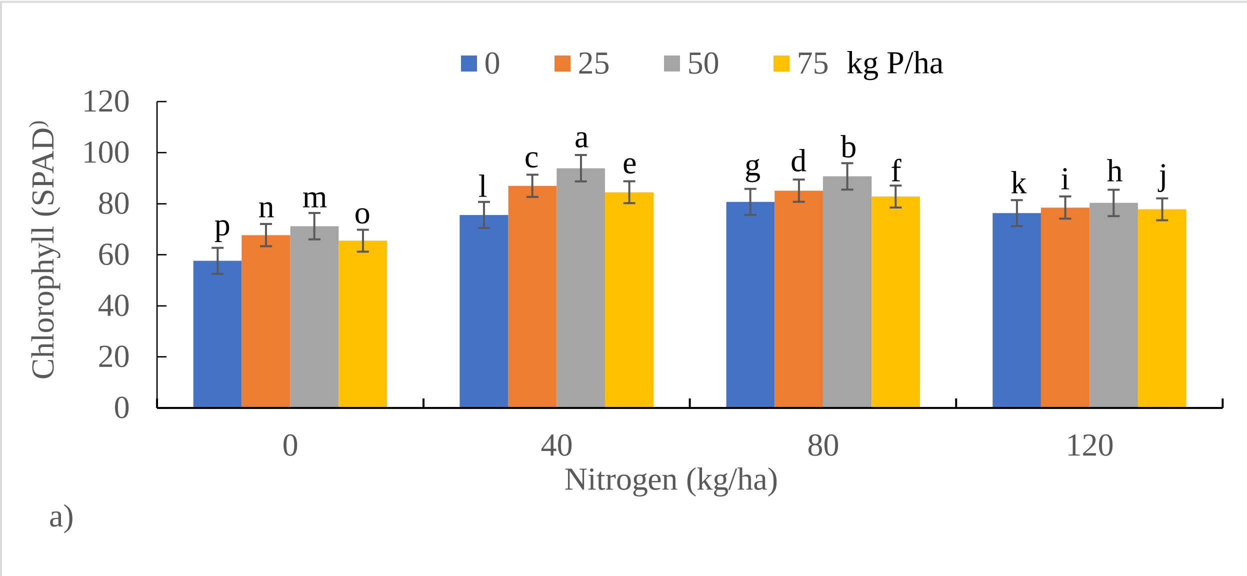
Figure 4.6. Effect of N and Pi interactions on safflower leaf area: a) N and P interaction effect on leaf area in summer, b) N and P interaction effect on leaf area in winter; bars are standard error. Means with the same letter(s) are not significantly different.

4.3.7 Chlorophyll content

There were significant ($P < 0.05$) interactions of N and P in both summer and winter grown safflower (Table 4.5). The interaction of 40 N + 50 P kg/ha significantly ($P < 0.05$) produced the highest (93.9 SPAD) safflower leaf chlorophyll content compared to any other interaction treatment combinations in summer (Figure 4.7a). While the interaction of 0 N + 0 P kg/ha significantly ($P < 0.05$) produced the lowest (57.6 SPAD) safflower leaf chlorophyll content compared to any N and P interaction in summer (Figure 4.7a). Whatever level of N interaction with 50 kg P/ha significantly ($P < 0.05$) produced higher leaf chlorophyll content than the interaction of N with P in summer (Figure 7a). Beyond 40 kg N/ha any interaction the chlorophyll concentration significantly ($P < 0.05$) decreased in summer grown safflower (Figure 4.7a).

In winter, N and P interaction was significant ($P < 0.0001$) with respect to the leaf chlorophyll content of safflower plants (Table 4.5). Application of 120 N + 25 P kg/ha to safflower plants significantly ($P < 0.05$) produced safflower plants with the highest (72.3 SPAD) leaf chlorophyll content than other N + P interactions in winter (Figure 4.7b). While application of 80 N + 75 P kg/ha to safflower plants significantly ($P < 0.05$) produced safflower plants with the lowest (52.6 SPAD) leaf chlorophyll content than N + P interactions in winter (Figure 4.7b).

Summer



Winter

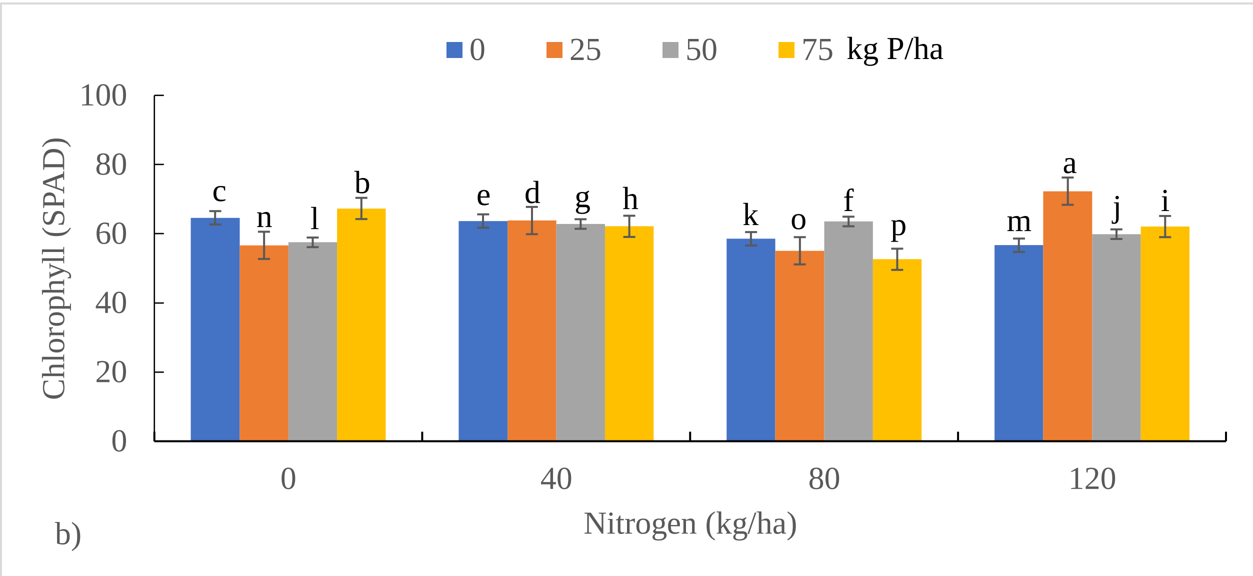


Figure 4.7. Effect of nitrogen and phosphorus on safflower chlorophyll content: a) nitrogen and phosphorus interaction effect on chlorophyll content in summer, b) nitrogen and phosphorus interaction effect on chlorophyll content in winter, bars are standard error. Means with the same letter(s) are not significantly different.

4.4 Nutrient Use Efficiency

The effect of N and P on the nutrient use efficiency of safflower plants in both summer and winter are shown in Table 4.6. The table shows the independent and interaction significance of both N and P on nutrient use efficiency of safflower plants in summer and winter.

Table 4.6. Summarised ANOVA for dependent variables: nitrogen uptake efficiency (NUpE), nitrogen utilization efficiency (NUtE), nitrogen use efficiency (NUE), phosphorus uptake efficiency (PUpE), phosphorus utilization efficiency (PUtE) and phosphorus use efficiency (PUE).

Dependent variable	Effects	Summer season		Winter season	
		F-value	**P-value**	**F-value**	**P-value**
NUpE	N	238.61	<0.0001	18305.1	<0.0001
	P	70.67	<0.0001	159.18	<0.0001
	N * P	29.87	<0.0001	196.26	<0.0001
NUtE	N	58.10	<0.0001	40.96	0.0002
	P	247.56	<0.0001	29.40	<0.0001
	N * P	41.19	<0.0001	49.43	<0.0001
NUE	N	542.21	<0.0001	663.14	<0.0001
	P	75.40	<0.0001	64.55	<0.0001
	N * P	48.62	<0.0001	46.96	<0.0001
PUpE	N	20.03	0.0016	4.39	0.0586
	P	115.54	<0.0001	131.35	<0.0001
	N * P	6.79	<0.0001	10.07	<0.0001
PUtE	N	11.41	0.0068	6.23	0.0284
	P	7.11	0.0014	11.70	<0.0001
	N * P	2.97	0.0159	9.64	<0.0001

PUE	N	23.00	0.0011	50.56	0.0001
	P	12.50	<0.0001	25.35	<0.0001
	N * P	3.17	0.0115	32.69	<0.0001

4.4.1 Nitrogen Use Efficiency

In both summer and winter, NUpE, NUtE, and NUE were significantly (P < 0.0001) influenced by the interaction of N and P fertilizer application in safflower (Table 4.6). Safflower plants were found to be more efficient in N uptake with low N input but in the presence of P in summer (Figure 4.8). Safflower plants were efficient in the uptake of N at the interaction of 40 N + 75 P kg/ha which resulted in maximum N uptake of 12.43 mg in summer (Figure 4.8). Increasing N application above 40 kg/ha in the presence of P significantly (P < 0.05) decreased NUpE in summer (Figure 4.8).

Increasing the N application rate in the presence of P significantly (P < 0.05) increased NUtE (Figure 4.9). Safflower plants applied with 120 N + 75 P kg/ha in summer had significantly (P < 0.05) the highest NUtE of 116.13 kg (Figure 4.9). However, there were no significant differences between the highest NUtE from safflower plants applied with 120 N + 75 P kg/ha compared to plants applied with 120 N + 50 P, 80 N + 50 P, and 40 N + 50 P kg/ha in summer (Figure 4.9). Across all N and P combinations, P at 50 kg/ha resulted in an increased NUtE of safflower in summer (Figure 4.9). When N nor P was not applied, safflower was significantly (P < 0.05) the NUtE of 31.83 kg in summer.

Nitrogen and P fertilizer application in summer significantly (P < 0.0001) interacted to influence NUE (Table 4.6). The NUE of the safflower plant decreased as the rate of N application increased in summer (Figure 4.10). Application of 40 N +50 P kg/ha to safflower plants in summer resulted in significantly (P < 0.05) the highest NUE of 1.02 kg. The lowest

NUE of 0.34 kg was in plants applied with 120 kg N/ha but no phosphorus in summer (Figure 4.10).

In the winter, there was a significant interaction ($P < 0.0001$) of N and P to influence the NUpE, NUtE, and the NUE of safflower (Table 4.6). Increasing N application irrespective of P significantly ($P < 0.05$) decreased NUpE in safflower grown in winter (Figure 4.11). Safflower plants applied with 40 N + 25 P kg/ha in winter had significantly ($P < 0.05$) the highest NUpE of 10.2 mg compared to all other treatments with exception of plants applied with 40 N + 50 P kg/ha that had NUpE of 9.95 mg (Figure 4.11). The lowest NUpE of 3.19 mg was in plants applied with 120 N + 75 P kg/ha in winter (Figure 4.11). However, there were no significant differences in NUpE between plants applied with 120 N + 75 P, 120 N + 50 P, 120 N + 0 P, and 80 N + 75 P kg/ha in winter (Figure 4.11).

Safflower plants applied with 40 N + 50 P kg/ha in winter had significantly ($P < 0.05$) the highest NUtE of 187.3 kg compared to interaction treatments in winter (Figure 4.12). The lowest NUtE of 41.6 kg was recorded with safflower plants applied with 80 N + 0 P kg/ha in winter (Figure 4.12). In general N treatment combination with 50 kg P/ha in winter resulted in high NUtE (Figure 4.12). Application of P enhanced NUtE of safflower in winter (Figure 4.12). The NUE of the safflower plant decreased as the rate of N fertilizer application increased in winter (Figure 4.13). Safflower plants applied with 40 N + 50 P k/ha in winter had significantly ($P < 0.05$) the highest NUE of 1.91 kg (Figure 4.13). On the contrary, in winter plants applied with 120 N + 75 P kg/ha had significantly ($P < 0.05$) the lowest NUE of 0.19 kg (Figure 4.13).

Summer

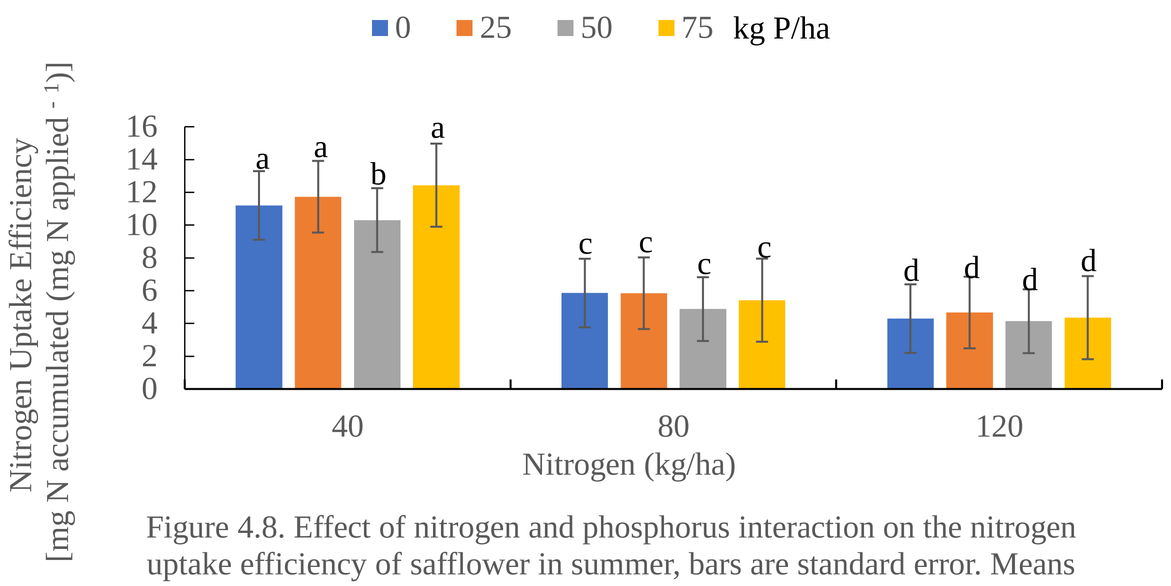


Figure 4.8. Effect of nitrogen and phosphorus interaction on the nitrogen uptake efficiency of safflower in summer, bars are standard error. Means with the same letter(s) are not significantly different.

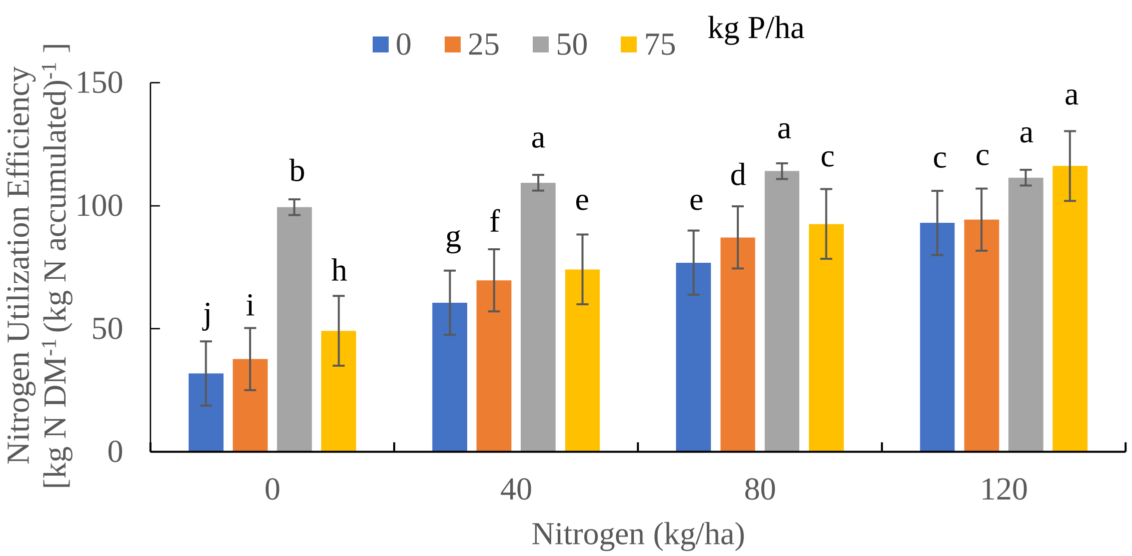


Figure 4.9. Interaction effect of nitrogen and phosphorus on the nitrogen utilization efficiency of safflower in summer, bars are standard error. means with the same letter(s) are not significantly different.

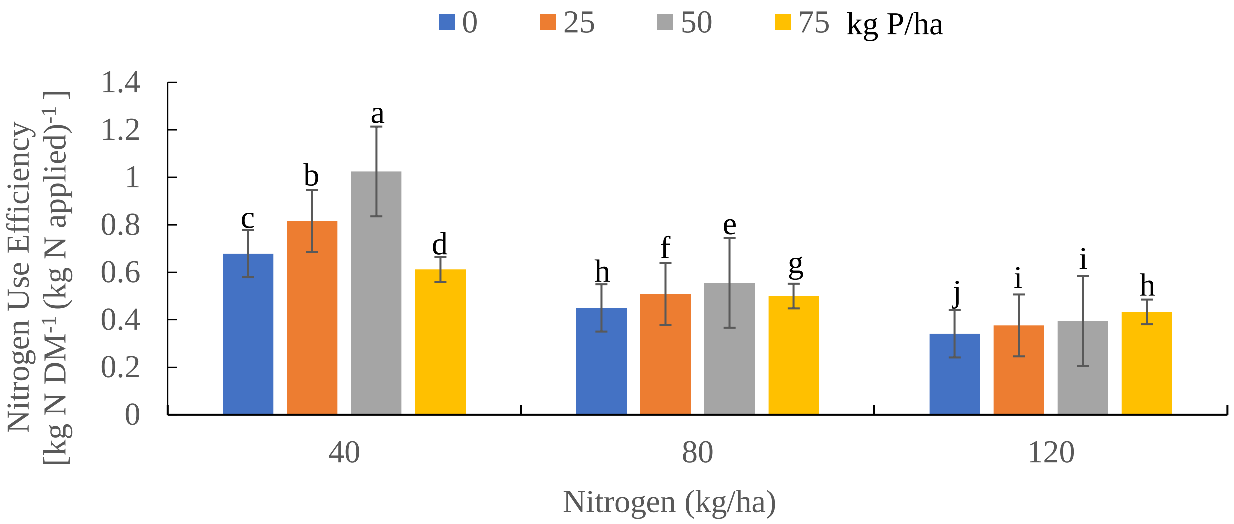


Figure 4.10. Interaction effect of nitrogen and phosphorus on the nitrogen use efficiency of safflower in summer, bars are standard error. Means with the same letter(s) are not significantly different.

Winter

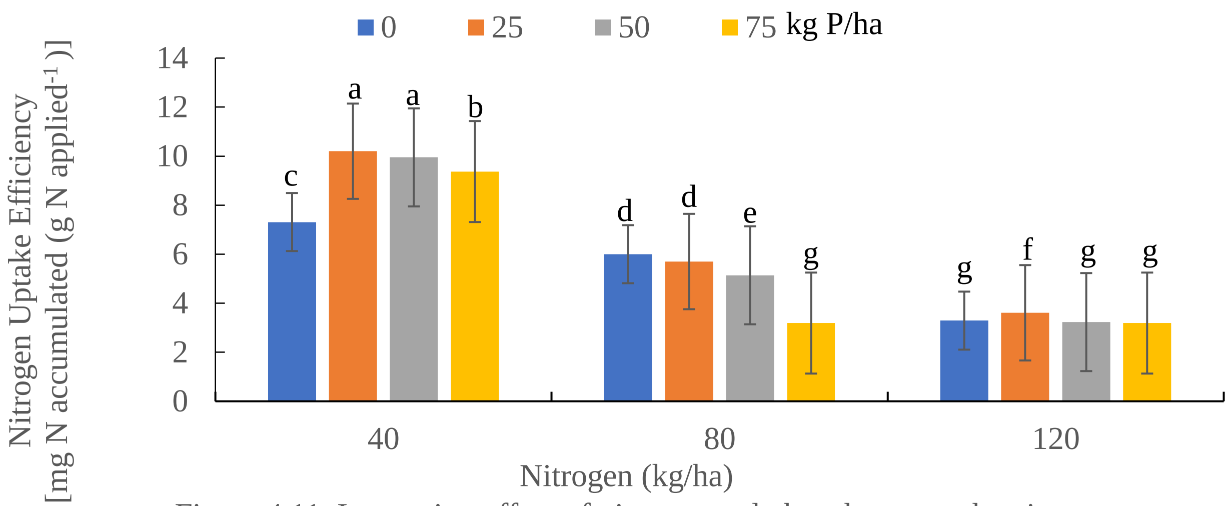


Figure 4.11. Interaction effect of nitrogen and phosphorus on the nitrogen uptake efficiency of safflower in winter, bars are standard error. Means with the same letter(s) are not significantly different.

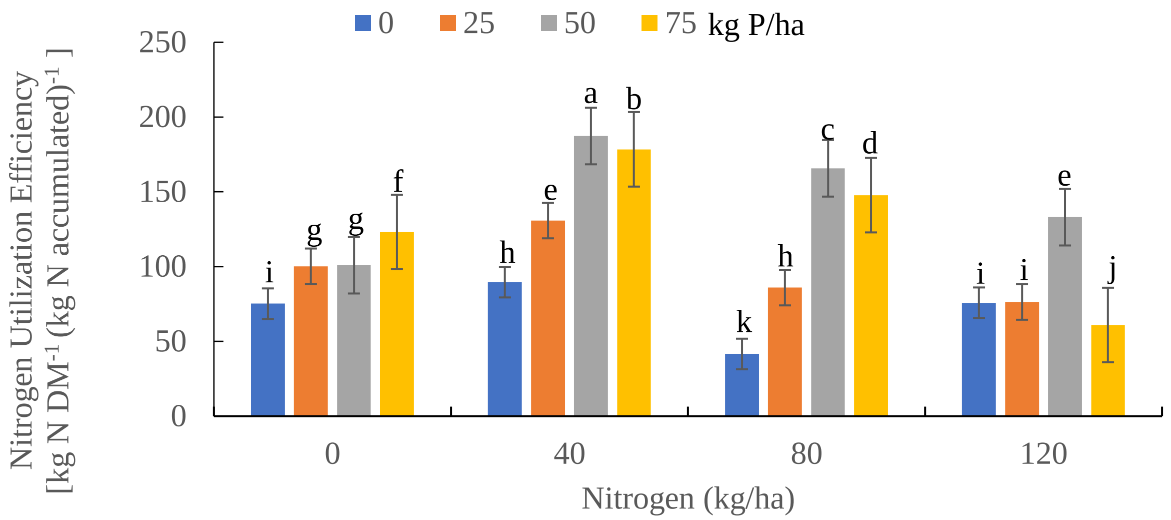


Figure 4.12. Interaction effect of nitrogen and phosphorus on the nitrogen utilization efficiency of safflower in winter, bars are standard error. Means with the same letter(s) are not significantly different.

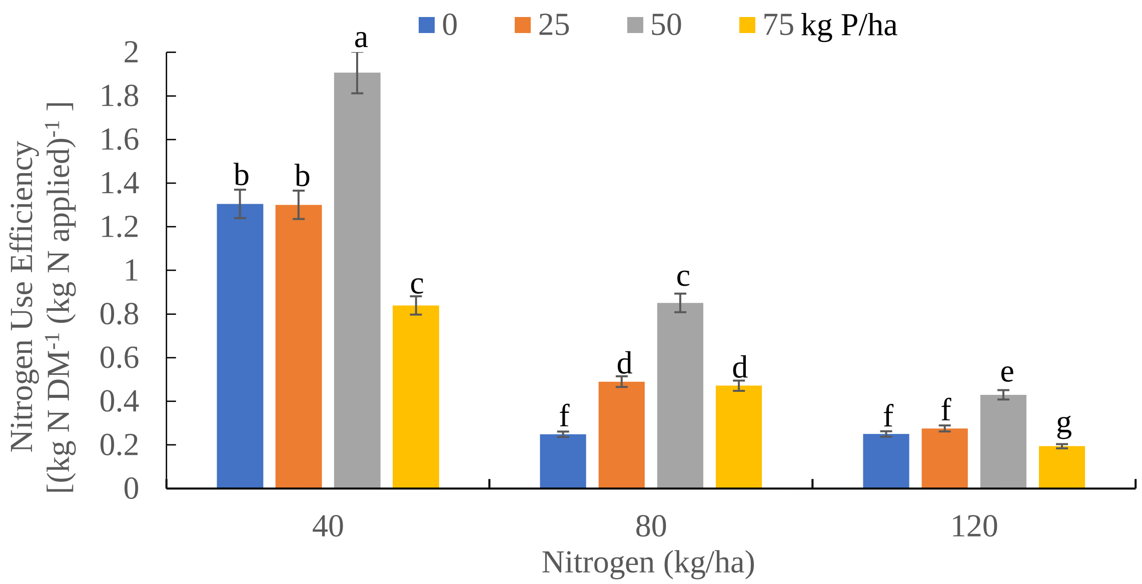


Figure 4.13. Interaction effect of nitrogen and phosphorus on the nitrogen use efficiency of safflower in winter, bars are standard error. Means with the same letter(s) are not significantly different.

4.4.2 Phosphorus Use Efficiency

In both summer and winter, N and P significantly ($P < 0.0001$) interacted to influence safflower PUpE, PUtE, and PUE (Table 4.6). In summer, safflower plants applied with 40 N + 25 P kg/ha had significantly ($P < 0.05$) the highest PUpE of 7.0 g (Figure 4.14). The phosphorus application rate of 25 kg/ha resulted in the highest PUpE across all N rates (Figure 4.14). Safflower plants applied with 0 N + 75 P kg/ha had significantly ($P < 0.05$) the lowest PUpE of 0.68 g compared to other treatments (Figure 4.14).

In summer, safflower plants applied with 0 N + 75 P kg/ha had significantly ($P < 0.05$) the highest PUtE of 0.70 mg compared to other interaction treatments (Figure 4.15). On the contrary in summer, safflower plants applied with 40 N + 0 P kg/ha had significantly ($P < 0.05$) the lowest PUtE of 0.09 mg compared to other treatments (Figure 4.15).

In summer safflower plants applied with 120 N + 75 P kg/ha had significantly ($P < 0.05$) the highest PUE of 26.7 kg compared to all other treatments (Figure 4.16). The lowest PUE of 1.97 kg was recorded in safflower plants applied with no N or P (Figure 4.16). Application of 120 kg N/ha in combination with P resulted in high PUE compared to other treatment combinations (Figure 4.16).

Increasing P application rates beyond 25 kg/ha significantly ($P < 0.05$) decreased the PUpE of safflower plants in winter (Figure 4.17). The treatment combination of N and P at 40 and 25 kg/ha significantly ($P < 0.05$) had the highest PUpE of 34.87 g by safflower plants compared to other treatment combinations in winter (Figure 4.17). Application of 120 N + 75 P kg/ha to safflower plants in winter resulted significantly ($P < 0.05$) in the lowest PUpE of 3.42 g (Figure 4.17).

Safflower plants applied with 80 N + 75 P kg/ha in winter had significantly ($P < 0.05$) the highest PUtE of 0.12 mg compared to any other treatment (Figure 4.18). There were no significant ($P > 0.05$) differences between the PUtE recorded through the application of 50 kg P/ha across all treatment combinations with N in winter (Figure 4.18). Safflower plants applied with 80 N + 25 P kg/ha had significantly ($P < 0.05$) the least PUtE of 0.07 mg compared to other treatments with exception of plants applied with 80 N + 0 P kg/ha (Figure 4.18).

Safflower plants applied with 40 N + 25 P kg/ha in winter had significantly (P < 0.05) the highest PUE of 6.68 kg compared to other interactions of N and P (Figure 4.19). Application of 50 kg P/ha across all treatment combinations except for 40 kg N/ha which resulted in high PUE in winter (Figure 4.19). Safflower plants applied with 80 N + 0 P kg/ha had significantly (P < 0.05) the lowest PUE of 1.38 kg in winter (Figure 4.19).

Summer

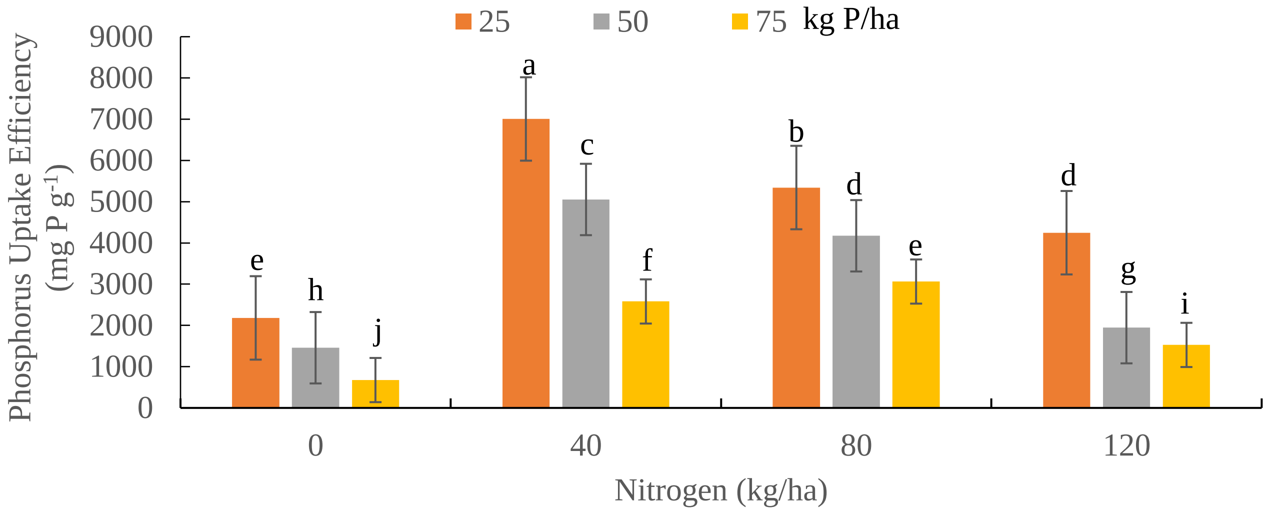


Figure 4.14. Interaction effect of nitrogen and phosphorus on the phosphorus uptake efficiency of safflower in summer, bars are standard error. Means with the same letter(s) are not significantly different.

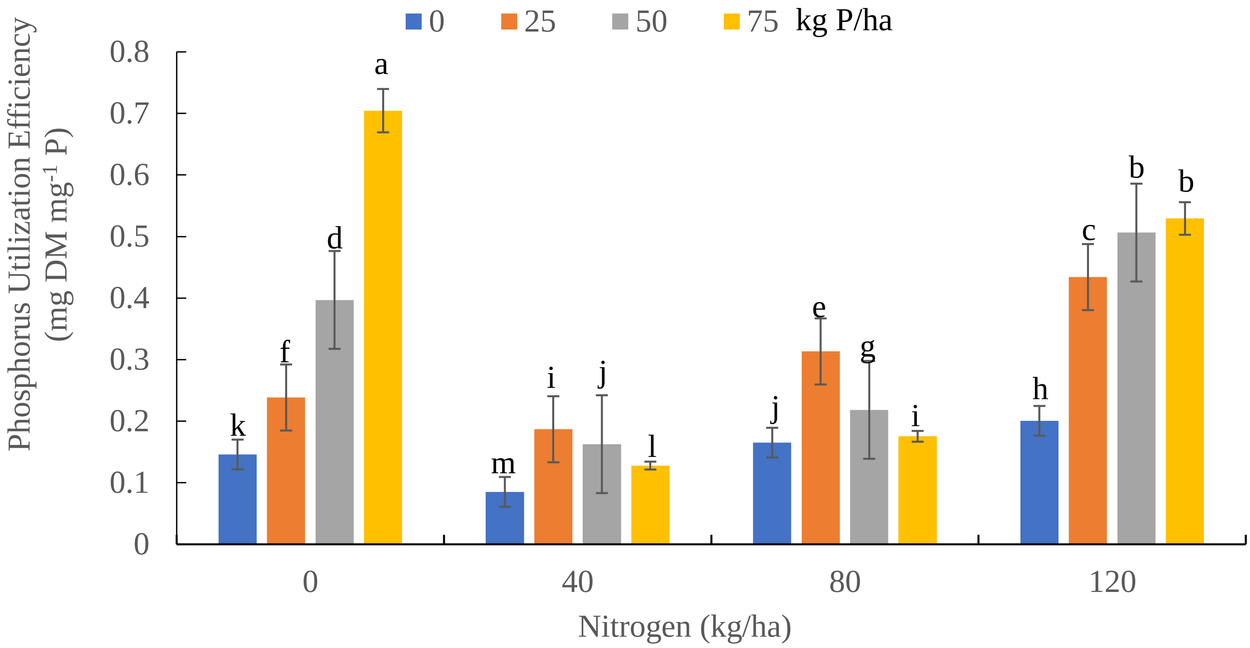


Figure 4.15. Interaction effect of nitrogen and phosphorus on the phosphorus utilization efficiency of safflower in summer, bars are standard error. Means with the same letter(s) are not significantly different.

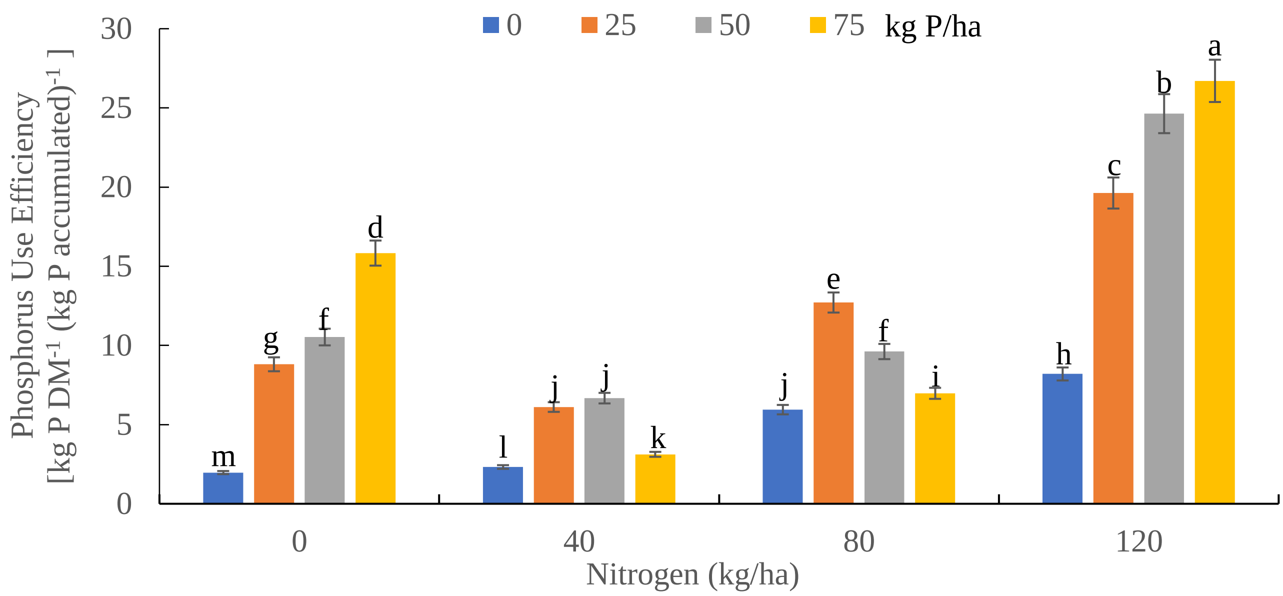


Figure 4.16. Interaction effect of nitrogen and phosphorus on the phosphorus use efficiency in summer, bars are standard error. Means with the same letter(s) are not significantly different.

Winter

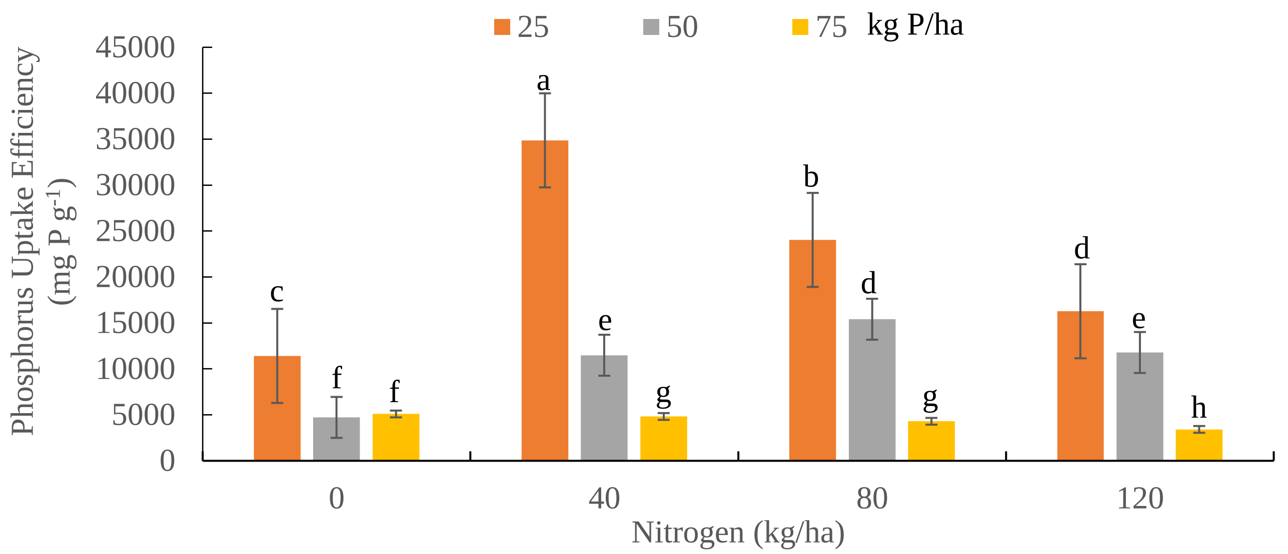


Figure 4.17. Interaction effect of nitrogen and phosphorus on the phosphorus uptake efficiency of safflower in winter, bars are standard error. Means with the same letter(s) are not significantly different.

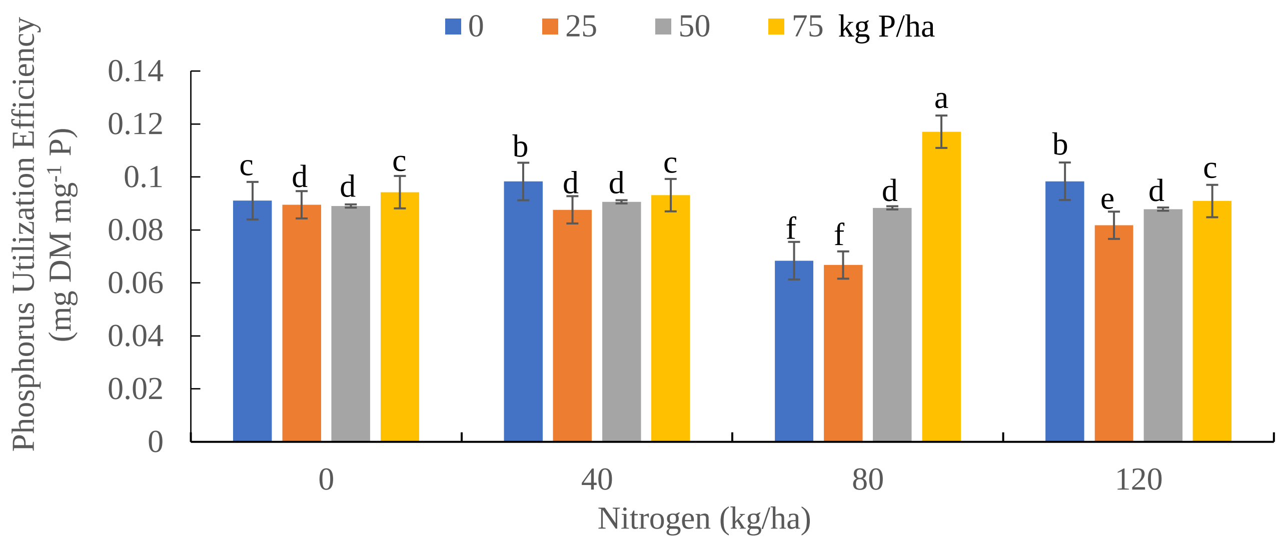


Figure 4.18. Interactin effect of nitrogen and phosphorus on the phosphorus utilization efficiency of safflower in winter, bars are standard error. Means with the same letter(s) are not significantly different.

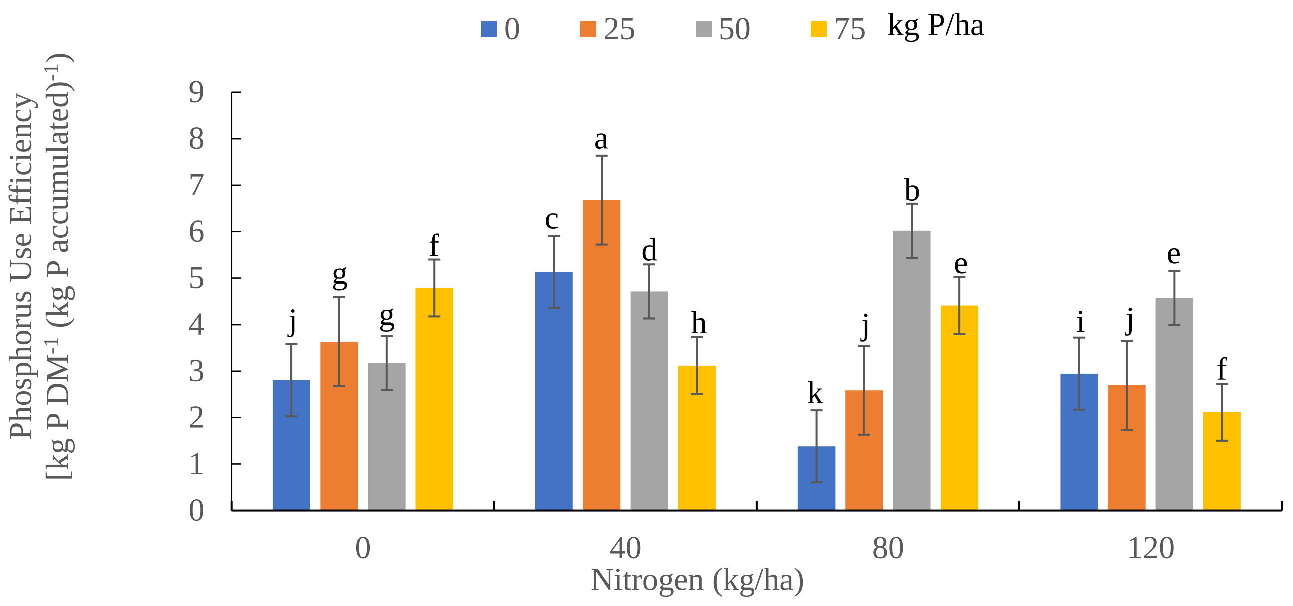


Figure 4.19. Interaction effect of nitrogen and phosphorus on the phosphorus use efficiency of safflower in winter, bars are standard error. Means with the same letter(s) are not significantly different.

4.5 Yield components

The effect of N and P on the dependent variables that define the yield components of safflower in both summer and winter are shown in Table 4.7. The table shows the independent and interaction significance of both N and P on yield components of safflower in summer and winter.

Table 4.7. Summarised ANOVA for dependent variables: number of capitulum per plant, capitulum diameter, seed number per capitulum, 1000-seed weight, seed yield, oil content, leaf nitrogen, and leaf phosphorus.

Dependant variables		Summer season		Winter season	
	Effects	**F-value**	**P-value**	**F-value**	**P-value**
Number of capitula per plant	N	30.48	0.0005	0.24	0.8628
	P	5.77	0.0041	1.71	0.1907
	N * P	1.12	0.3840	1.13	0.3832
Capitula diameter	N	6.82	0.0232	0.58	0.6468
	P	97.28	<0.0001	0.95	0.4309
	N * P	25.29	<0.0001	0.39	0.9294
Seed number per capitulum	N	2.54	0.1525	83.22	<0.0001
	P	51.17	<0.0001	12.75	<0.0001
	N * P	13.10	<0.0001	12.26	<0.0001
1000-seed weight	N	3.90	0.0735	5.06	0.0440
	P	3.58	0.0285	0.05	0.9856
	N * P	7.34	<0.0001	0.51	0.8509
Seed yield	N	2119.93	<0.0001	38.36	0.0003
	P	309.24	<0.0001	18.94	<0.0001
	N * P	279.74	<0.0001	7.64	<0.0001
Oil content	N	2.96	0.1194	19.12	0.0018
	P	1.65	0.2043	2.10	0.1274
	N * P	0.64	0.7482	7.89	<0.0001
Leaf N	N	28.61	0.0006	43.33	0.0002
	P	77.48	<0.0001	117.12	<0.0001
	N * P	16.41	<0.0001	167.41	<0.0001
Leaf P	N	39.67	0.0002	12.58	0.0054
	P	54.76	<0.0001	5.18	0.0067
	N * P	4.64	0.0012	4.48	0.0016

4.5.1 Capitula number per plant

There were no significant ($P > 0.05$) interactions of N and P on the capitula number per plant in both summer and winter safflower planting (Table 4.7). In summer, N and P independently significantly ($P < 0.05$) influenced capitula number per plant (Table 4.7). Application of 40, 80, and 120 kg N/ha significantly ($P < 0.05$) increased capitula number per plant of safflower planted in summer (Figure 4.20a). The response of safflower plants to increasing N application rate was quadratic with respect to capitula number per plant (Figure 4.20a). Safflower plants applied with 40 kg N/ha significantly ($P < 0.05$) produced the highest capitula number per plant (36.7) compared to plants applied with 0, 80, or 120 kg N/ha (Figure 4.20a). Application of N above 40 kg/ha significantly ($P < 0.05$) decreased the capitula number per plant in summer (Figure 4.20a).

Phosphorus application in summer significantly ($P < 0.05$) increased safflower capitula number per plant (Figure 4.20b). The response of safflower plants to increasing P application rate was quadratic in summer (Figure 4.20b). Application of 50 kg P/ha produced a higher capitula number per plant (34.3) than any other P application rate (Figure 4.20b). Application of P above 50 kg/ha to safflower plants in summer decreased capitula number per plant (Figure 4.20b).

In winter, no treatment under study had a significant ($P > 0.05$) effect on safflower capitula number per plant (Table 4.7, Figure 4.20c & d). Nitrogen application to safflower plants in winter had a non-significant ($P > 0.05$) increase in capitula number per plant compared to control plants (Figure 4.20c). Similarly in winter, the application of P fertilizer to safflower plants had no significant ($P > 0.05$) increase in capitula number per plant compared to control plants (Figure 4.20d). Plants applied with 50 kg P/ha produced the highest capitula number per plant (22.1) though non-significant compared to control plants or plants applied with either 25 or 75 kg P/ha (Figure 4.20d). Figure 4.20e shows the capitula of safflower during flowering in summer.

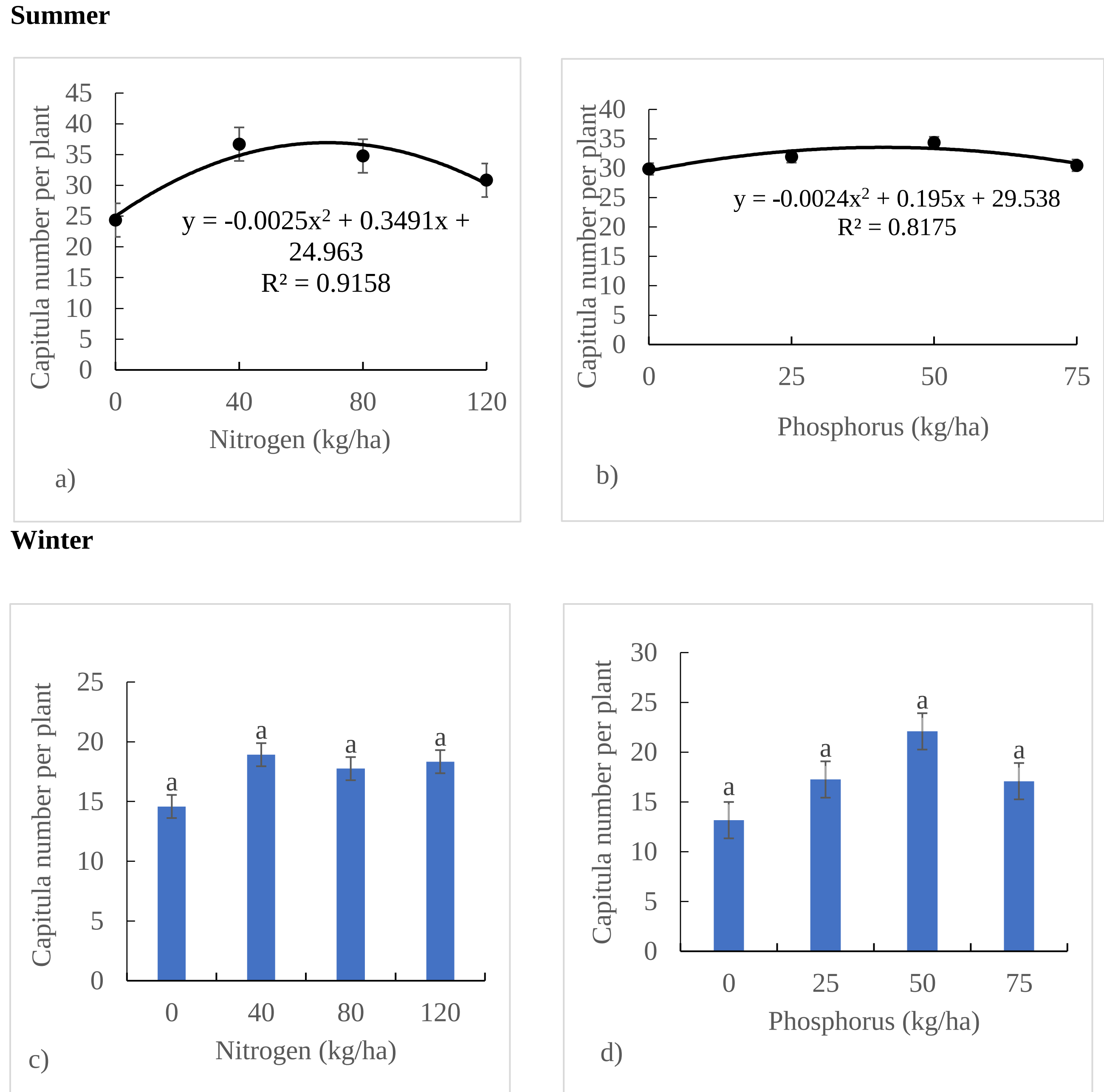


Figure 4.20. Effect of N and P on safflower capitula number: a) N effect on capitula number in summer, b) P effect on capitula number in summer, c) N effect on capitula number in winter, and d) P effect on capitula number in winter; bars are standard error. Means with the same letter(s) are not significantly different; mean separation by LSD at P = 0.05.

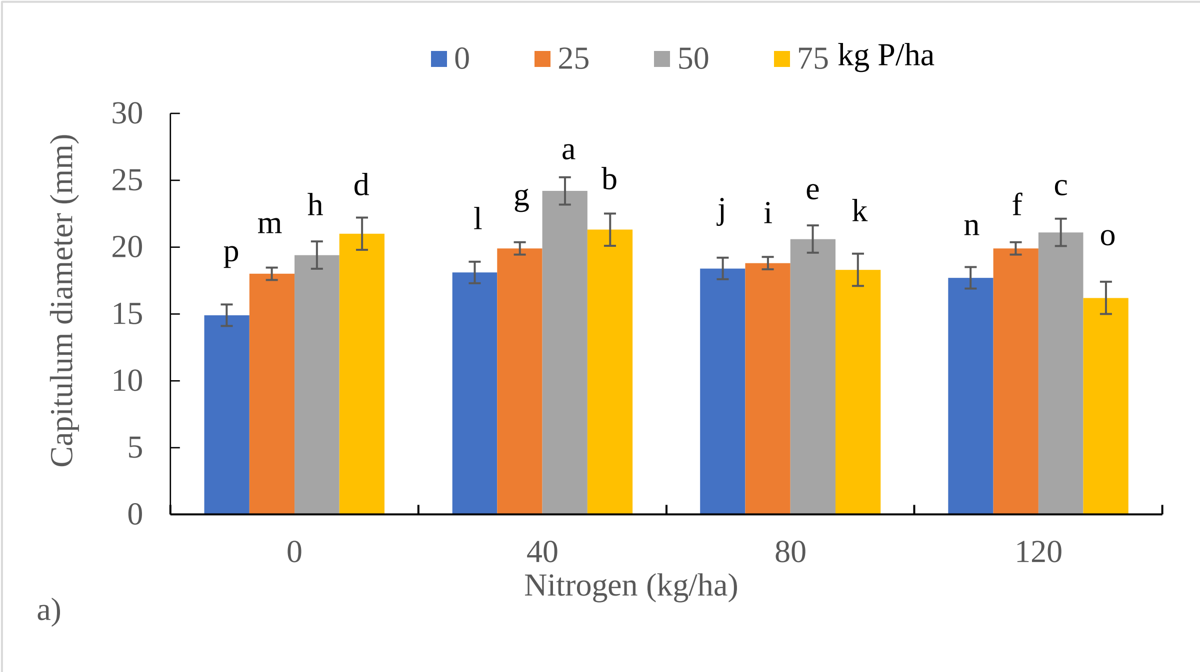
Figure 4.20e. A picture showing the safflower capitula during the flowering stage in summer

4.5.2 Capitula diameter

Nitrogen and P interacted significantly ($P < 0.0001$) to influence the capitulum diameter of safflower plants in summer (Table 4.7). The interaction combinations of N and P were all significantly ($P < 0.05$) different from each other (Figure 4.21a). Safflower plants applied with 40 N + 50 P kg/ha produced the largest diameter of 24.2 mm than any other N and P interaction treatment in summer (Figure 4.21a). On the contrary, plants applied with zero N and P produced plants with the smallest capitula diameter of 14.9 mm compared to any other N and P interaction treatment in summer (Figure 4.21a). The second largest capitula diameter of 21.3 mm was produced by safflower plants applied with 40 N + 75 P kg/ha (Figure 4.21a).

During the winter no treatments under investigation had a significant (P > 0.05) on safflower capitula diameter (Table 4.7). Application of 40 kg N/ha to safflower plants in winter had a non-significant increase in capitula diameter compared to control plants and plants applied with either 80 or 120 kg N/ha (Figure 4.21b). Application of N above 40 kg/ha resulted in a non-significant (P > 0.05) decrease in capitula diameter (Figure 4.21b). Phosphorus application to safflower plants at either 25 or 50 kg/ha produced a non-significant (P > 0.05) increase in capitula diameter compared to control plants (Figure 4.21c). Application of 75 kg P/ha to safflower plants in winter induced a non-significant (P > 0.05) decrease in capitula diameter in winter (Figure 4.21c).

Summer



Winter

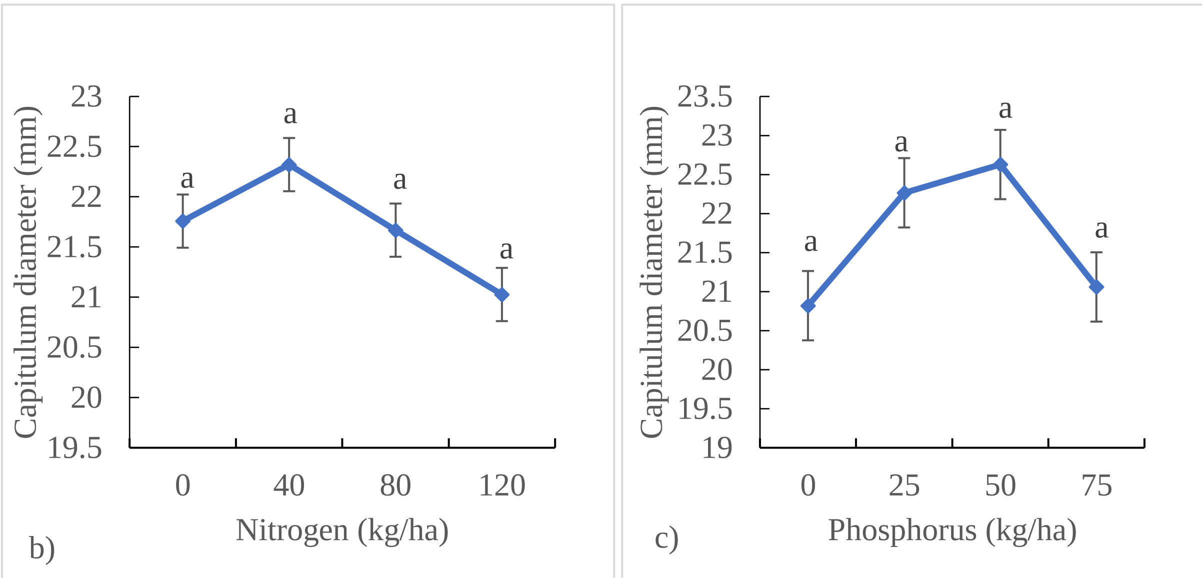


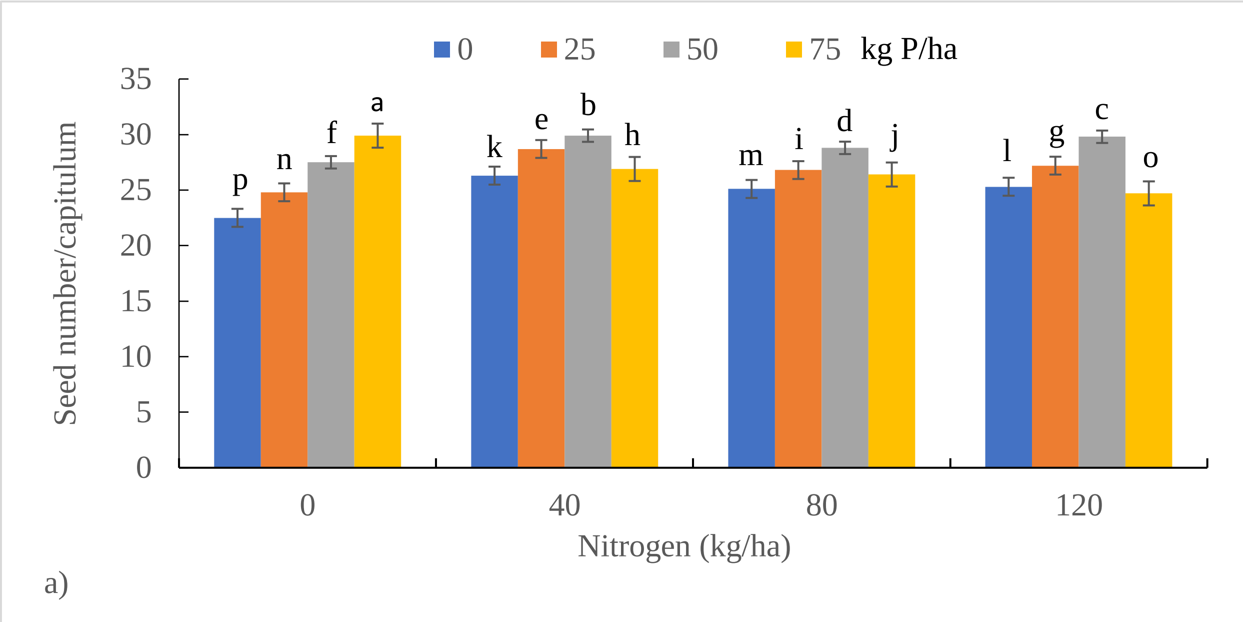
Figure 4.21. Effect of nitrogen and phosphorus on safflower capitulum diameter: a) nitrogen and phosphorus interaction effect on capitulum diameter in summer, b) nitrogen effect on capitulum diameter in winter, c) phosphorus effect on capitulum diameter in winter, bars are standard error. Means with the same letter(s) are not significantly different; mean separation by LSD at P = 0.05.

4.5.3 Seed number per capitulum

There was a significant ($P < 0.0001$) interaction between N and P as they influenced the safflower seed number per capitulum in both summer and winter grown safflower (Table 4.7) In summer, application of 0 N + 75 P, 40 N + 50 P and 120 N + 50 P kg/ha to safflower plants significantly ($P < 0.05$) produced the highest seed number per capitulum (30) compared to any other N and P interaction combination (Figure 4.22a). However, these interactions did not significantly ($P > 0.05$) differ from each other with respect to seed number per capitulum in summer (Figure 4.22a). Safflower plants applied with no N and P produced significantly ($P < 0.05$) the lowest seed number per capitulum (23) compared to any other treatment in summer (Figure 4.22a).

In the winter, application of 0 N + 75 P kg/ha to safflower plants produced capitula with significantly ($P < 0.05$) the highest seed number (77) compared to any other N and P interaction combination (Figure 4.22b). On the contrary in winter, safflower plants applied with 80 N + 0 P kg/ha produced plants with significantly ($P < 0.05$) the lowest seed number per capitulum compared to any other N and P interaction combination (Figure 4.22b). Also in winter, safflower plants applied with 0 N + 0 P and 120 N + 25 P kg/ha significantly ($P > 0 .05$) produced the same seed number (51) per capitulum which was significantly ($P < 0.05$) higher than that of other N and P interactions with exception of 0 N + 75 P kg/ha (Figure 4.22b).

Summer



Winter

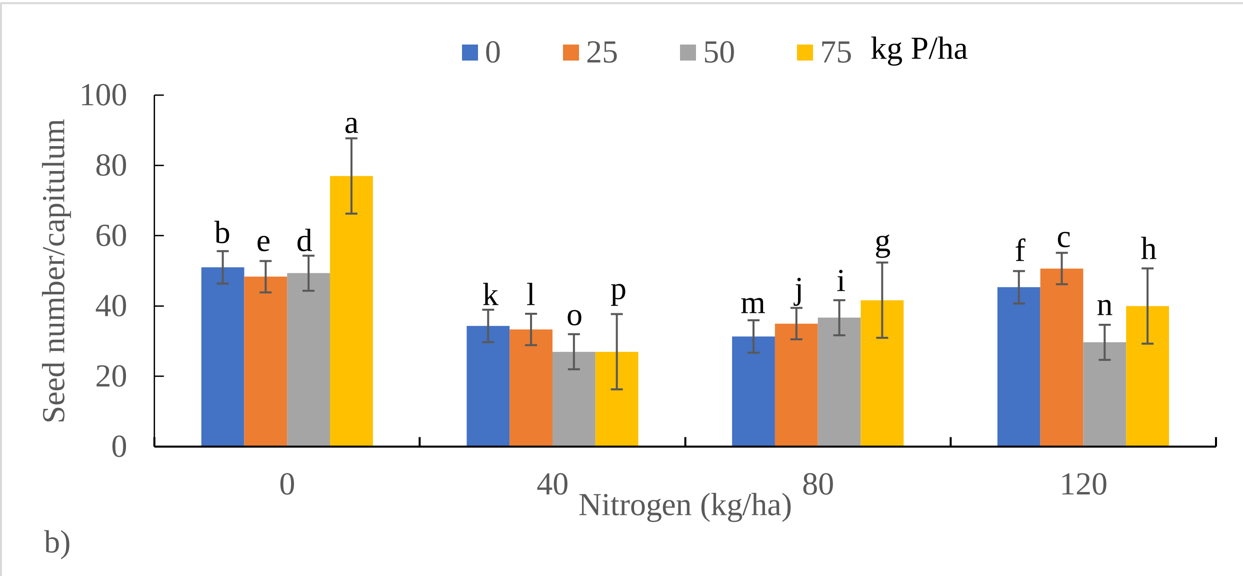


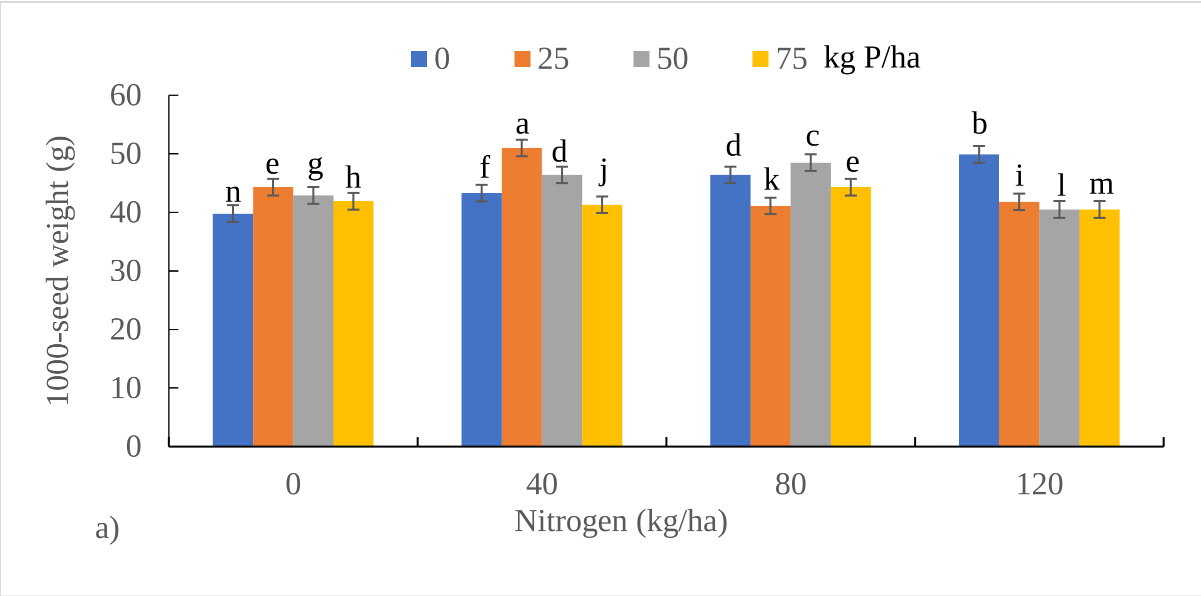
Figure 4.22. Effect of nitrogen and phosphorus on seed number per capitulum: a) nitrogen and phosphorus interaction effect on seed number per capitulum in summer, b) nitrogen and phosphorus interaction effect on seed number per capitulum in winter, bars are standard error. Means with the same letter(s) are not significantly different; mean separation by LSD at P = 0.05.

4.5.4 Thousand seed weight

There was a significant ($P < 0.0001$) interaction between N and P, with respect to 1000-seed weights in summer (Table 4.7). Safflower plants applied with 40 N + 25 P kg/ha in summer significantly ($P < 0.05$) produced the heaviest 1000-seed weight (51 g) compared to all other N and P interactions (Figure 4.23a). On the contrary in summer, safflower plants applied with no N and P significantly ($P < 0.05$) produced the lightest 1000-seed weight (39.8 g) compared to all N and P interaction treatments (Figure 4.23a). Safflower plants applied with 40 N + 50 P and 80 N + 0 P, 40 N + 75 P and 80 N + 25 P, and 120 N + 50 P and 120 N + 75 P kg/ha produced 1000-seed weights of 46.4, 41.3, and 40.5 g, respectively, which were significantly ($P > 0.05$) different from each other within the comparison (Figure 4.23a).

In winter, there was no significant ($P > 0.05$) N and P interaction or independent P effects on the 1000-seed weight of safflower plants, therefore main effects are presented (Table 4.7). Application of N fertilizer to safflower plants significantly ($P < 0.05$) increased 1000-seed weight (Figure 4.23b). The response of safflower plants to increasing N fertilizer application rate was quadratic (Figure 4.23b). The maximum 1000-seed weight of 168 g was significantly ($P < 0.05$) produced by plants applied with 80 kg N/ha (Figure 4.23b). Application of N above 80 kg/ha to safflower plants significantly ($P < 0.05$) caused a decrease in 1000-seed weight (Figure 4.23b). While application of P fertilizer to safflower in winter resulted in a non-significant ($P > 0.05$) increase in the 1000-weight seed weight of safflower plants (Figure 4.23c).

Summer



Winter

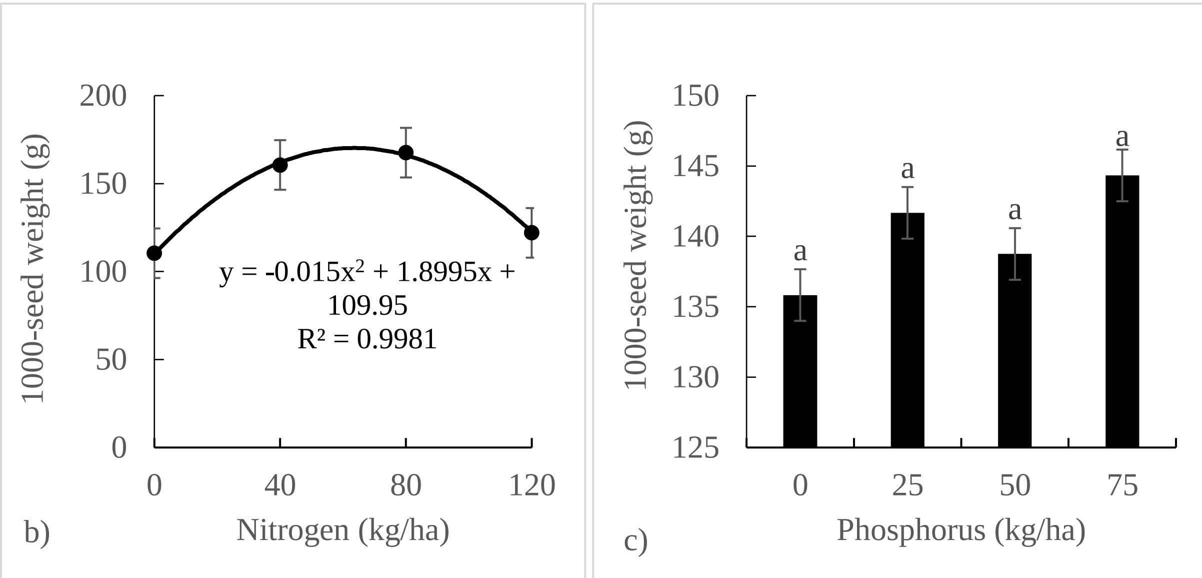


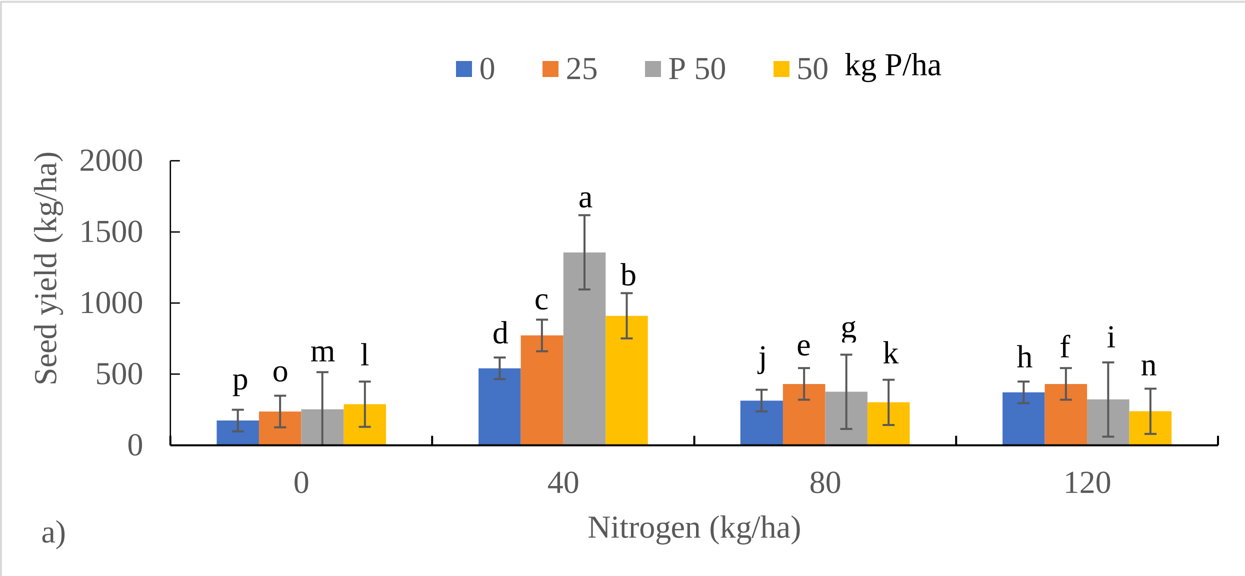
Figure 4.23. Effect of nitrogen and phosphorus on 1000-seed weight: a) nitrogen and phosphorus interaction effect on 1000-seed weight in summer, b) nitrogen effect on 1000-seed weight in winter, c) phosphorus effect on 1000-seed weight in winter, bars are standard error. Means with the same letter(s) are not significantly different; mean separation by LSD at P = 0.05.

4.5.5 Seed yield

There were significant ($P < 0.0001$) interactions of N and P on the seed yield of safflower both in summer and winter (Table 4.7). Safflower plants not applied with N and P significantly ($P < 0.05$) produced the lowest yield of 174 kg/ha compared to the other N and P interactions in summer (Figure 4.24a). On the contrary, safflower plants applied with 40 N + 50 P kg/ha significantly ($P < 0.05$) produced the highest seed yield of 1356 kg/ha in summer (Figure 4.24a). The second-best N and P interaction was an application of 40 N + 75 P kg/ha to safflower plants that produced a seed yield of 910 kg/ha which was significantly ($P < 0.05$) higher than all other N and P interactions with exception of safflower plants applied with 40 N + 50 P kg/ha in summer (Figure 4.24a). Safflower plants applied with 80 N + 0 P, 80 N + 75 P, and 120 N + 50 P did not significantly ($P > 0.05$) differ in their seed yield (Figure 4.24a). Similarly, in summer, safflower plants applied with 0 N + 50P, O N + 75 P, and 120 N + 75 P had statistically similar seed yields (Figure 4.24a). Heavy rainfall which occurred at the flowering and grain filling stages of safflower affected pollination and maturation of seeds leading to blind capitula and poor grain filling as shown in Figure 4.24c in summer.

In winter, application of safflower plants with 80 N + 75 P kg/ha significantly ($P < 0.05$) produced the highest seed yield of 3528 kg/ha compared to any other N and P interaction (Figure 4.24b). While application of 120 N + 50 P kg/ha to safflower plants significantly ($P < 0.05$) produced the lowest seed yield of 1028 kg/ha compared to N and Interactions in winter (Figure 4.24b). Safflower plants applied with 80 N + 0 P and 120 N + 0 P kg/ha did not significantly ($P > 0.05$) differ in their seed yield in winter (Figure 4.24b). Similarly, safflower plants applied with 40 N + 0 P, 40 N + 25 P, 80 N + 25 P, and 120 N + 75 P kg/ha did not significantly ($P > 0.05$) differ in their seed in winter (Figure 4.24b). Also, safflower plants applied with 0 N + 25 P and 80 N + 50 P did not significantly ($P > 0.05$) differ in their seed yield (Figure 4.24b).

Summer



Winter

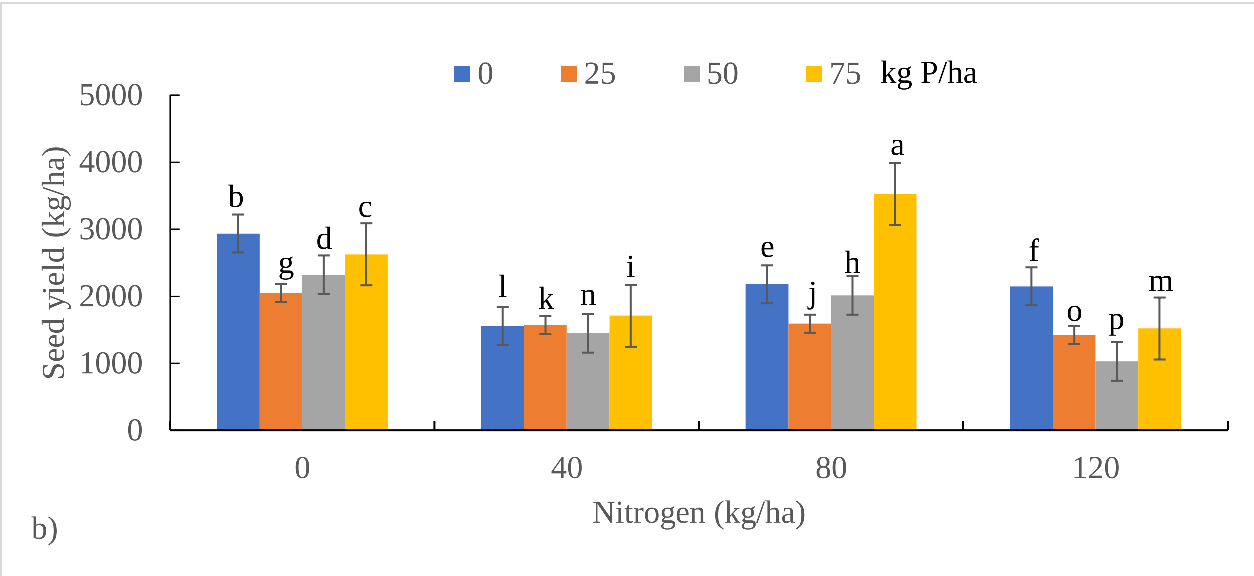


Figure 4.24. Effect of nitrogen and phosphorus on seed yield: a) nitrogen and phosphorus interaction effect on seed yield in summer, b) nitrogen and phosphorus interaction effect on seed yield in winter, bars are standard error. Means with the same letter(s) are not significantly different.

Figure 4.24c. A picture of the safflower plants after heavy rains before harvest in summer

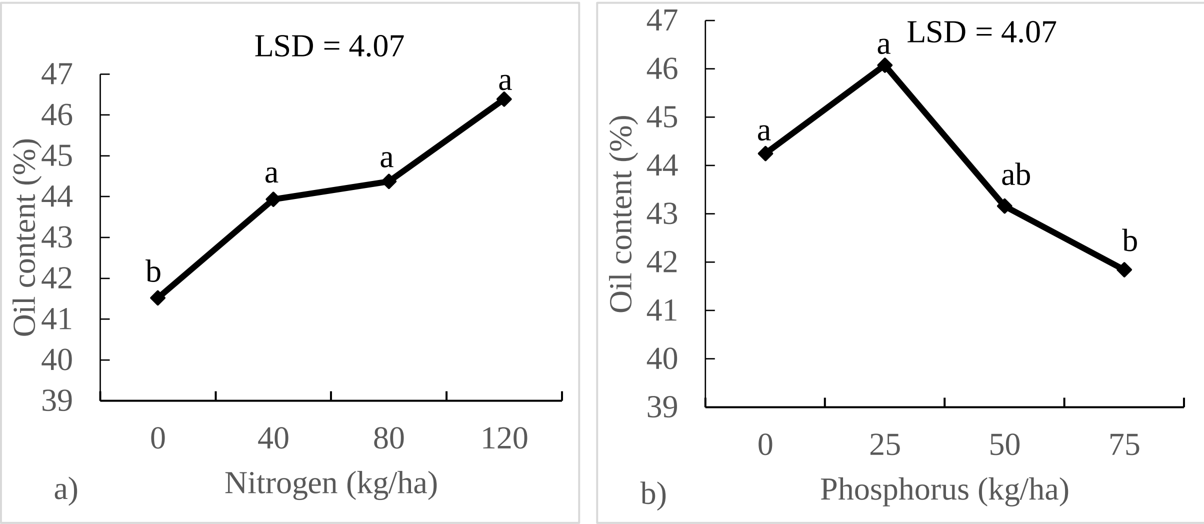
4.5.6 Oil content

Nitrogen and P interaction had no significant ($P > 0.05$) effect on the oil content of safflower seeds in summer, therefore main effects are presented (Table 4.7). Nitrogen and P independently had a significant ($P < 0.05$) effect on safflower seed oil content (Figure 4.25a & b). Application of 40, 80, and 120 kg N/ha significantly ($P < 0.05$) increased safflower seed oil content compared to control plants in summer (Figure 4.25a). However, the seed oil content of safflower plants applied with 40, 80, or 120 kg N/ha did not significantly ($P > 0.05$) differ from each other in summer (Figure 4.25a). Maximum seed oil content was obtained in safflower plants applied with 120 kg N/ha in summer. Phosphorus application at 25 kg/ha resulted in non-significant ($P > 0.05$) increase in safflower seed oil content (46.08%) in summer (Figure

4.25b). However, the application of 75 kg P/ha significantly ($P < 0.05$) reduced safflower seed oil content (41.84%) compared to oil seed content (44.25%) to control plants in winter (Figure 4.25b).

In winter, N and P interaction significantly ($P < 0.0001$) influenced safflower seed oil content (Table 4.7). Safflower plants applied with 0 N + 25 P kg/ha significantly ($P < 0.05$) produced the highest seed oil content of 71.8% compared to all other N and P interactions (Figure 4.25c). The second-highest seed oil content of 69.9% was produced by safflower plants applied with 0 N + 50 P kg N/ha in winter (Figure 4.25c). The lowest safflower seed oil content of 52.5% was significantly ($P < 0.05$) produced by plants applied with 40 N + 25 P kg/ha compared to other N and P interactions in winter (Figure 4.25c).

Summer



Winter

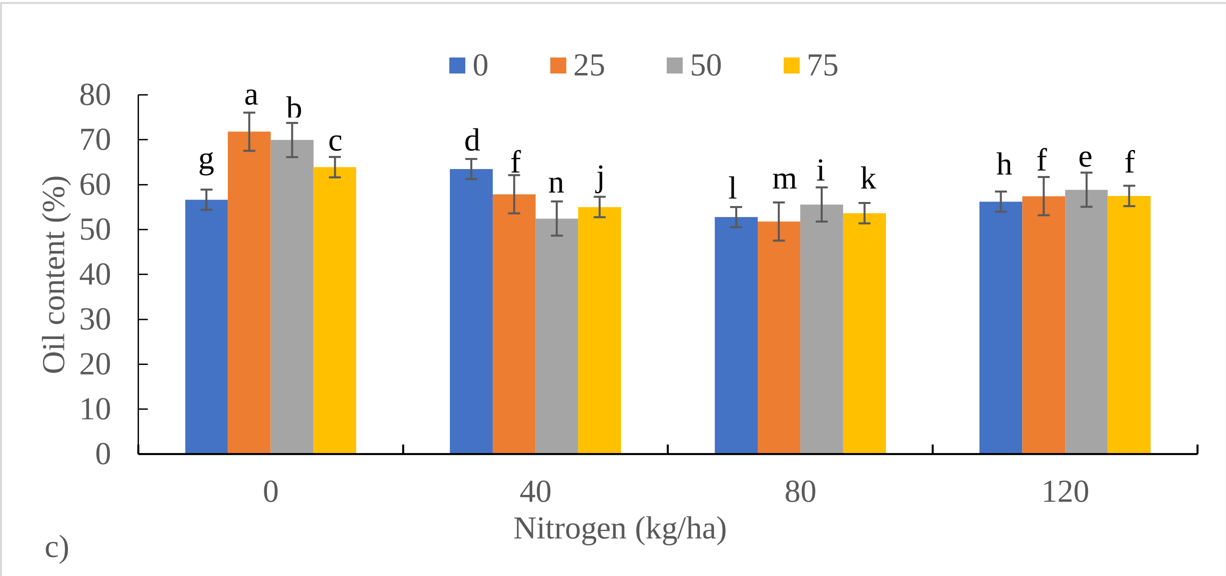


Figure 4.25. Effect of N and P on seed oil content: a) N effect on seed oil content in summer, b) P effect on seed oil content in summer, c) interaction effect of N and P on seed oil content; bars are standard error. Means with the same letter(s) are not significantly different.

4.6 Leaf N and P contents

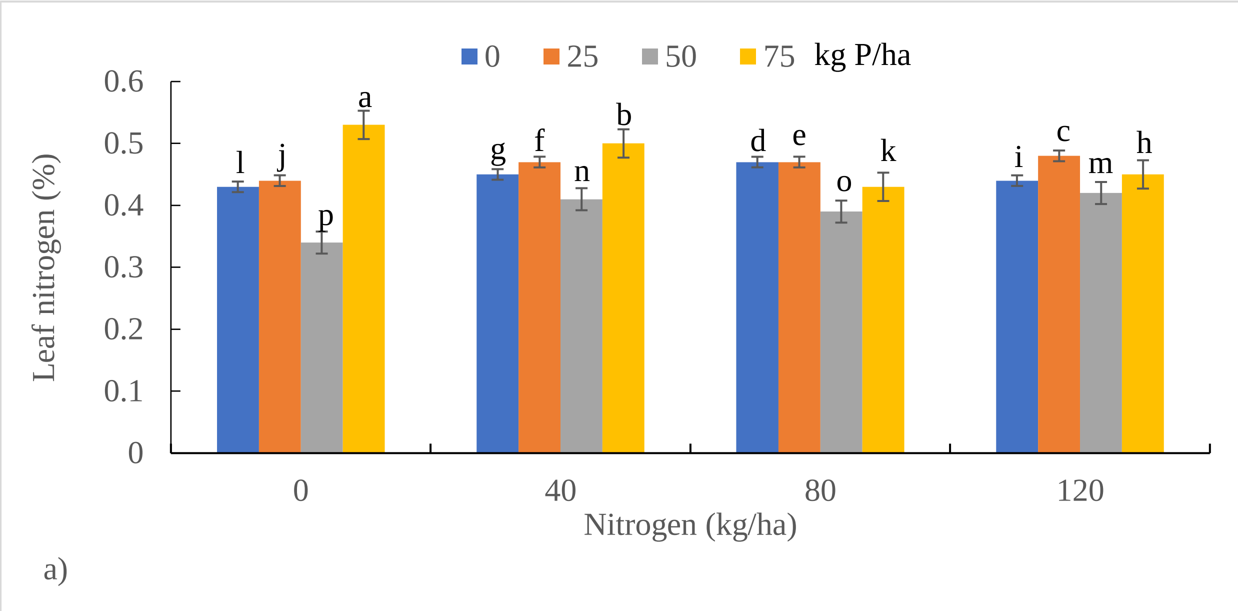
The application of N and P significantly ($P < 0.0001$) interacted to influence leaf N and P contents of safflower in summer (Table 4.7). Safflower plants applied with 0 N + 75 P kg/ha significantly ($P < 0.05$) increased leaf N content (0.53%) in summer than any other N and P interaction (Figure 4.26a). While safflower plants applied with 0 N + 50 P kg/ha produced plants with significantly ($P < 0.05$) the lowest leaf N content of 0.34% in summer (Figure 4.26a). In general, an increase in P application promoted leaf N content in summer (Figure 4.26a). Safflower plants applied with 40 N +25 P, 80 N + 0 N, and 80 N + 25 P kg/ha in summer produced plants that did not significantly ($P > 0.05$) differ in their leaf N contents (Figure 4.26a). Similarly, in summer, safflower plants applied with 40 N + 0 P and 120 N + 25 P kg/ha did not significantly differ in their leaf N contents (Figure 4.26a).

Application of 40 N + 0 N kg/ha to safflower plants in summer produced plants with significantly ($P < 0.05$) the highest leaf content of 120 mg P/ka than any other Nand P interactions. On the contrary in summer, application of 0 N + 25 P kg/ha to safflower plants produced plants with significantly ($P < 0.05$) the lowest leaf P content of 42 mg/kg (Figure 4.26b). The second-best interaction in summer that promoted partitioning of P to the leaves was the application of 40 N + 75 P kg/ha which resulted in leaf P content of 79 mg/kg (Figure 4.26b). However, plants applied with 40 N +50 P and 80 N + 0 P kg/ha did not significantly ($P > 0.05$) differ in their leaf P contents (Figure 4.26b).

In winter, N and P significantly ($P < 0.0001$) interacted to influence leaf N and P of safflower plants (Table 4.7). Application of 80 N + 0 P kg/ha to safflower plants produced plants with significantly ($P < 0.05$) the highest leaf N content of 0.48 % in winter (Figure 4.27a). While safflower plants applied with 80 N + 75 P kg/ha produced plants with significantly ($P < 0.05$) the lowest leaf N content of 0.26% in winter (Figure 4.27a). Safflower plants applied with 0 N + 75 P and 120 N + 25 P kg/ha did not significantly ($P > 0.05$) differ in their leaf N contents but was significantly (P < 0.05) the third highest leaf content of 0.43% in winter (Figure 4.27a). Similarly in winter safflower plants applied with 0 N + 0 P, 0 N + 25 P, 40 N + 25 P, 40 N + 50 P, and 120 N + 50 P kg/ha did significantly (P > 0.05) differ in their leaf N contents in winter (Figure 4.27a).

In winter, safflower plants applied with 80 N + 0 P and 80 N + 25 P kg/ha produced plants with similar leaf P content of about 152 mg/kg but was significantly (P < 0.05) higher than the leaf P content of any other N and P interaction (Figure 4.27b). Plants applied with 40 N + 25 P, 80 N + 50 P, and 120 N + 50 P kg/ha did not significantly (P > 0.05) differ in their leaf P contents, but their leaf P contents was significantly (P < 0.05) the third highest compared to other interactions with exception of plants applied with 80 N + 0 P, 80 N + 25 P, and 120 N + 25 P kg/ha in winter (Figure 4.27b). Plants applied with 0 N + 25 P and 0 N + 75 P kg/ha in winter did not significantly (P > 0.05) differ in their leaf P contents (Figure 4.27b). Similarly in winter, safflower plants applied with 0 N + 75 P and 40 N + 50 P kg/ha did not significantly (P > 0.05) differ in their leaf P contents (Figure 4.27b).

Summer



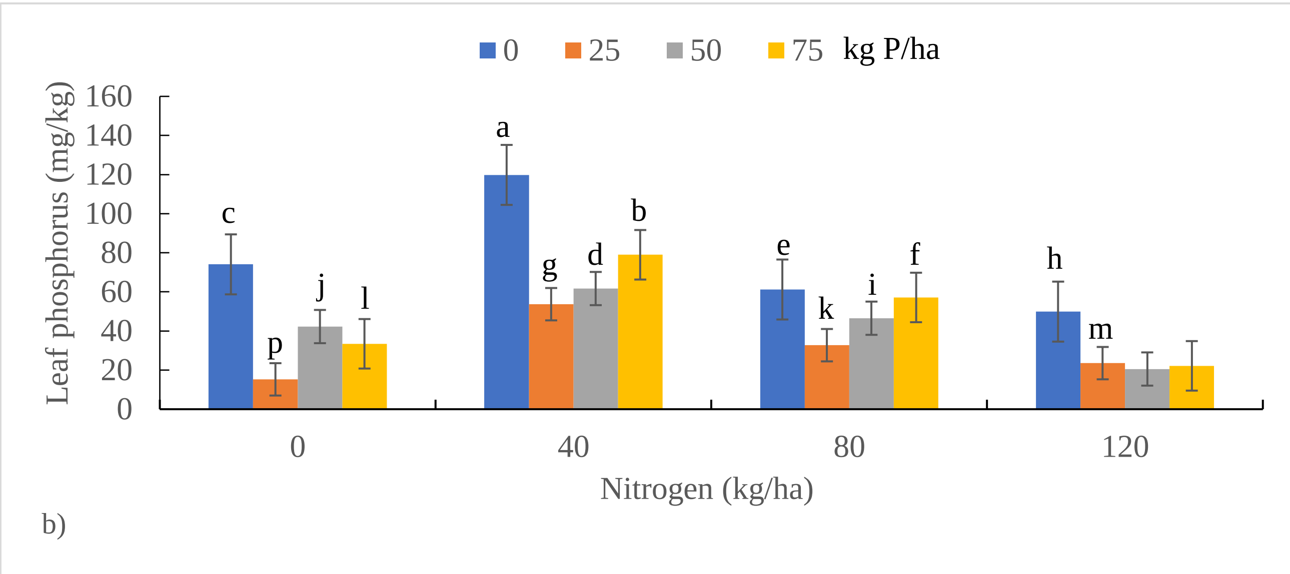
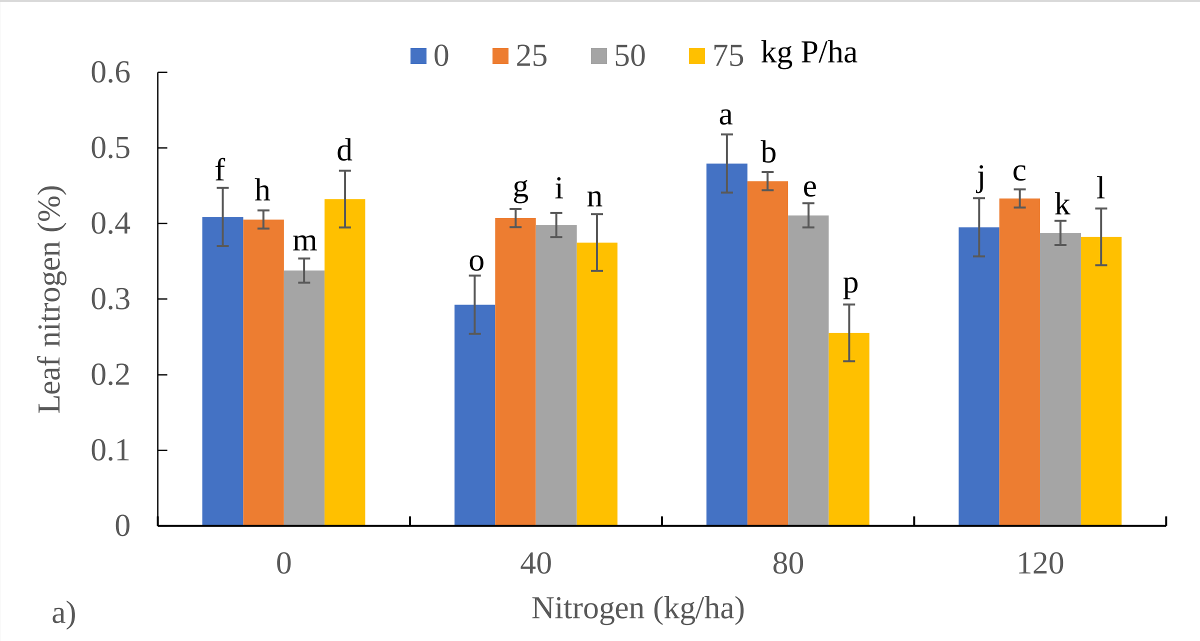


Figure 4.26. Effect of nitrogen and phosphorus on the leaf nitrogen and leaf phosphorus: a) nitrogen and phosphorus interaction effect on leaf nitrogen summer, b) nitrogen and phosphorus interaction effect on leaf phosphorus in summer, bars are standard error. Means with the same letter(s) are not significantly different.

Winter



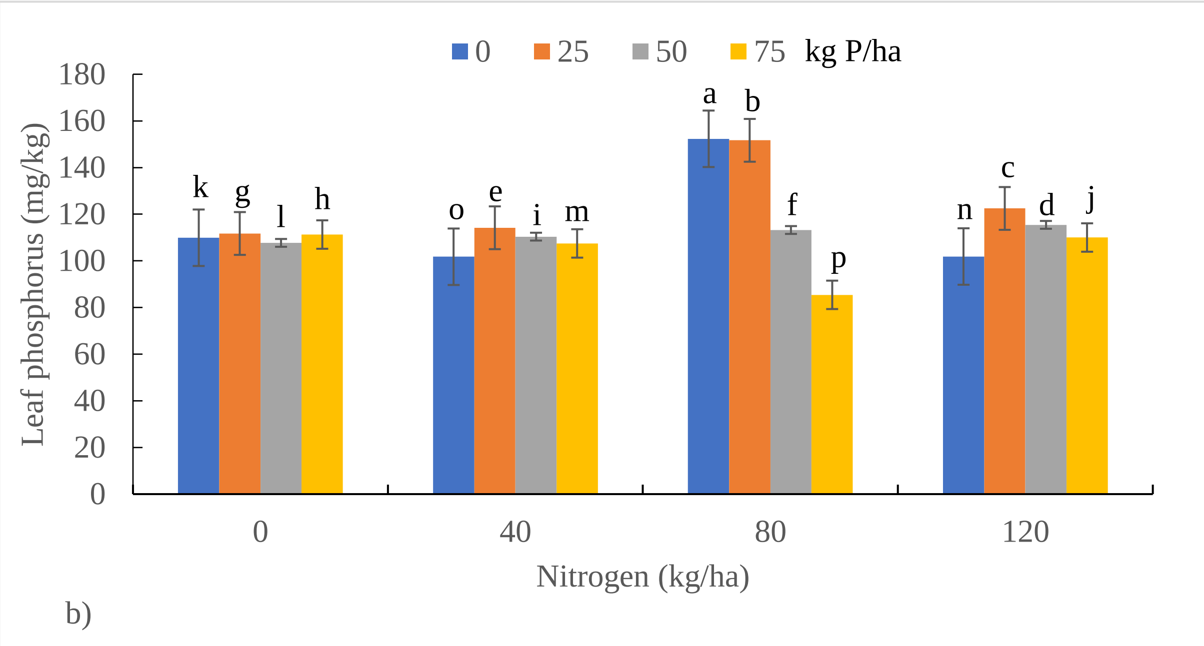


Figure 4.27. Effect of nitrogen and phosphorus on the leaf nitrogen and leaf phosphorus: a) nitrogen and phosphorus interaction effect on leaf nitrogen winter, b) nitrogen and phosphorus interaction effect on leaf phosphorus in winter, bars are standard error. Means with the same letter(s) are not significantly different.

CHAPTER 5

DISCUSSION

5.1 Soil analysis

Soil analysis data of the study site at the start of the experiment showed that soil textural class was sandy clay loam and sandy loam in summer and winter, respectively. The difference in the soil textural class was due to planting in winter in a different plot of the same field having different soils. In the sandy clay loam soil, the soil pH was acidic, and soil organic carbon content and total N were low, but total P and EC were high with a medium CEC (McKenzie et al., 2004). In the sandy loam soil, the soil pH was acidic, and the soil organic carbon, total N, EC, and CEC were low, but total P was high. The CEC of soils varies depending on the clay percentage, type of clay, soil pH, and amount of organic matter. Pure sand has a very low CEC, less than 2 cmol/kg, and the CEC of sand and silt size fractions of most soils is negligible (Moore, 1998; McKenzie et al., 2004). Clay soils such as kaolinite and montmorillonite have CECs of about 10 and 49 cmol/kg, respectively. Other clays such as illite and smectite have CECs ranging from 25 to 100 cmol/kg (Moore, 1998; Mengel and Kirkby, 2001; McKenzie et al., 2004; Marschner, 2005). Organic matter has a very high CEC ranging from 250 to 400 cmol/kg (Moore 1998). Soils with high CEC indicate that more clay and organic matter are present in them and have generally high-water holding capacity than soils low in CEC (Moore, 1998; McKenzie et al., 2004).

The Application of N as calcium ammonium nitrate (CAN) and single superphosphate (SSP) fertilizers significantly ($P < 0.05$) increased total soil N and P, pH, OC, EC, and CEC compared to control plots where CAN and SSP were not applied. The increase in soil pH induced by CAN and SSP application was attributed to calcium in the fertilizers which is a basic (alkaline) element (Mengel & Kirkby, 2001; Marschner, 2005; Havlin et al., 2005). Calcium nitrate is a combination of $CaCO_3$ and NH_4NO_3 and contains 19% Ca, and SSP contains 18-20% Ca (Marschner, 2005; Havlin et al., 2005). The increase in total soil N and P, EC, and CEC due to the application of CAN and SSP fertilizers was attributed to the nutrients which were added to the soil with the application of the fertilizers.

5.2 Effects of N and P application on vegetative growth

The application of N and P fertilizers significantly increased the vegetative growth of variables (first branching height, plant height, number of primary branches, stem diameter, leaf area, dry matter, and leaf chlorophyll content) of safflower compared to control plants in which no fertilizer was applied. Maximum first branching height, leaf area, and leaf chlorophyll content plus plant dry matter were produced by safflower plants applied with 40 N + 50 P and 120 N + 75 P kg/ha, respectively. While plant height, primary branch number, and stem diameter were significantly ($P < 0.05$) increased by independent applications of N and P. The increase in first branching height, plant height, leaf area, dry matter, and leaf chlorophyll content induced by N application was attributed to its role in promoting cell division and elongation which promoted internode elongation (Raven et al., 1999; Mengel & Kirkby, 2001; Marschner, 2005; Eckert, 2011). Nitrogen is an integral part of proteins which are the building blocks of plant structure, protoplasm, purines, pyrimidines, porphyrins, enzymes, coenzymes, chlorophyll, and vitamins (Salisbury & Ross, 1996; Raven et al., 1999; Mengel & Kirkby, 2001; Emongor, 2002; Hu & Schmidhalter, 2005; Marschner, 2005; Weinberg et al., 2007. It also helps in the biosynthesis and maintenance of phytohormone concentrations (auxins, cytokinins, and gibberellins) which may have resulted in enhanced first branching height, plant height, leaf area, leaf chlorophyll content, and dry matter of safflower plants (Emongor, 2002; Mazhani, 2017; Sreekanth et al., 2021). Nitrogen is a major component of the chlorophyll molecule, amino acids; energy-transfer compounds such as ATP, vitamins as well as other components needed for cell growth, development as well as yield hence explaining the increase in leaf area and chlorophyll content and dry matter of safflower plants applied with N (Salisbury & Ross, 1996; Mengel & Kirkby, 2001; Taiz & Zeiger, 2002; Hu & Schmidhalter, 2005; Marschner, 2005; Leghari et al., 2016). Phosphorus plays a major role in several physiological processes such as photosynthesis, respiration, energy storage and transfer, cell division and enlargement, and development of meristematic tissues which helps to increase growth attributes of plants such as dry matter, chlorophyll accumulation, and leaf growth (Salisbury & Ross, 1996; Raven et al., 1999; Mengel and Kirkby, 2001; Taiz & Zeiger, 2002; Marschner, 2005) explaining the increase in vegetative growth of safflower plants applied with P.

The increase in dry matter of safflower plants due to an increase in N and P application was also attributed to increased safflower plant height, leaf area, and chlorophyll content which promoted photosynthesis and accumulation of photosynthates. The increase in safflower dry matter due to N fertilizer application was also attributed to the role of N in dry matter production because the dry matter is directly related to N supply. That is, the lower the N supply, the lower the dry matter, especially in leaves, and this affects the production and distribution of photo-assimilates to the reproductive organs (Koutroubas et al., 2004; Fageria & Baligar, 2005; Dordas et al., 2008; Dordas & Sioulas, 2009). Sreekanth et al. (2021) reported that application of 50 N + 60 P kg/ha to safflower plants in Prayagraj (India), resulted in maximum plant height and dry matter of 102.9 cm and 41.2 g/plant, respectively. Mazhani (2017) in Botswana reported that application of N and P at 50 kg/ha to safflower plants independently (no interaction) increased vegetative growth. Santos et al. (2018) in Brazil, reported that the application of 350 and 199 kg N/ha to safflower plants under irrigated and rainfed conditions produced maximum plant height of 120 and 113 cm, respectively. Megda & Monteiro (2010), Golzafar et al. (2011), and Mohamed et al. (2012) reported that N fertilizer application increased safflower plant dry matter and attributed this to the role of N in improving photosynthetic activity, which enhanced solar energy absorption and crop development. Increased N application was also reported to increase leaf area, which increased light absorption and thus dry matter production, accounting for the increase in safflower vegetative development (Weinberg et al., 2007). An increase in vegetative growth of safflower due to N and P application has been reported in the literature (Vishwanath et al., 2006; Aghamohammadreza et al., 2013; Singh & Singh, 2013; Nathan et al., 2017, 2018; Öz, 2017; Sofy et al., 2020; Eryiğit et al., 2021). Siddiqui & Oad (2006) in Pakistan, observed that the application of 180 kg N/ha to safflower plants led to the production of the tallest plants of 165 cm. However, low N application rates (0, 40, 80 kg/ha) on safflower plants had no effect on vegetative growth in marginal areas of southwest Germany (Elfadl et al., 2009).

In winter, the response of safflower plants to applied N and P fertilizers with respect to first branching height, plant height, and primary branch number per plant was poor due to low temperatures of -6.3-4.2°C (July and August 2021) which occurred at the elongation stage of vegetative growth. The factors that affect the susceptibility and severity to chilling injury by a plant or plant tissue include the origin of the crop, the genetic makeup of the crop, stage of development or maturity, metabolic status of the tissue, and several environmental factors such as temperature, duration of exposure to the low temperature, light, relative humidity, and atmospheric composition (Patterson & Reid, 1990; Lim et al., 2009; Emongor, 2015; Chaudhary et al., 2017; Wassan et al., 2021). The current results are in agreement with those reported in literature (Emongor et al., 2015 and Emongor and Oagile, 2017).Temperatures below -4°C at the elongation and branching stages of the safflower plant have been reported to cause damage to the growing point and could lead to its death (Wachsmann et al., 2010). Although plants can recover from chilling injury to some extent, as shown in fig 4g,by producing new shoots from below the damaged areas, the growth rate and subsequent yield can be negatively affected (Wachsmann et al., 2010). The safflower plants recovered from chilling injury when air temperatures warmed up in September.

5.3 Effect of N and P application on yield components

The yield components of safflower are capitula number/plant, seed number per capitulum, capitulum diameter (size), and 1000-seed weight (Chaundry, 1990; Gonzalez et al., 1994; Bagheri et al., 2001; Camas & Esendal, 2006; Kedikanetswe, 2012; Emongor et al., 2015; Moatshe et al., 2016; Emongor & Oagile, 2017; Emongor et al., 2017b; Moatshe, 2019). In the current study, application of N and P fertilizers independently or interactively depending on season increased the yield components of safflower.

Application of N and P at 40 and 50, 40 N + 50 P, and 40 N + 25 P kg/ha independently or interactively significantly ($P < 0.05$) increased capitula number/plant, capitula diameter (size), and seed number/capitulum, and 1000-seed weight of safflower respectively, compared to control plants in the current study. This was partly attributed to the increase in primary branch number/plant, leaf area, chlorophyll content, and plant dry matter induced by N and P fertilizer application. Positive correlations between vegetative growth variables with yield components of safflower have been reported which may be driven by the source-sink relationship (Sirel & Aytac, 2016; Emongor et al., 2017b; Moatshe, 2019; 2020; Chehade et al., 2021; Koç, 2021). Sirel & Aytac (2016), Moatshe (2019) and Chehade et al. (2021) reported positive correlations between vegetative variables (plant height, primary branch number/plant, plant dry matter) and yield components (capitula number/plant, capitula size, seed number/capitulum, and 1000-seed weight) in the range of $r = 0.5$-0.85, 0.76-0.96 and 0.57-0.89, respectively. The increase in capitula number/plant, capitula diameter, seed diameter, and 1000-seed weight induced by both N and P fertilizer application could be attributed to the role of N and P in the physiological stimulation of meristematic regions of actively growing plant parts (Salisbury & Ross, 1996) such as young leaves and flower buds, followed by translocation of assimilates to plant reproductive parts (Malhotra et al., 2018). In safflower, the translocation and storage of pre-anthesis (vegetative phase of growth) assimilates into the seed is a crucial physiological process during the development of reproductive structures such as capitula and seeds (Koutroubas et al., 2004; Koutroubas & Papakosta, 2010). Sreekanth et al. (2021) reported that application of 50 N + 60 P kg/ha produced a maximum number of capitula/plant (24.5/plant) and the number of seeds/capitulum (29.53) of safflower plants. The increase in capitula number/plant and seed number/capitulum was attributed to better growth of safflower plants due to the availability of N and P which promoted photosynthesis leading to more photo-assimilates produced and translocated to the formation and development of capitula and seed (Sreekanth et al., 2021). Haliloglu & Beyyavas (2019) in Turkey reported that application of 50, 100, and 150 kg N/ha to safflower plants significantly increased capitula number/plant, the number of seeds/capitulum, and 1000-seed weight with an application of 100 and 150 kg N/ha giving the highest capitula number/plant, number of seeds/capitulum and 1000-seed weight, respectively. Whereas in Brazil, Santos et al. (2018) reported that application of 50, 100, 150, 200, 250, 300, 350, and 400 kg N/ha to safflower significantly increased capitula number/plant but N had no significant effect on 100-seed weight. Application of N above 400 kg/ha significantly decreased yield components (capitulum number/plant and 100-seed weight) due to N toxicity (Santos et al., 2018). While Sampaio et al. (2016) in Brazil reported that application of 0 N + 0 P, 8 N + 10 P, 16 N + 20 P, 24 N + 30 P, and 32 N + 40 P kg/ha to safflower plants had no effect on capitula number/plant and 1000-seed weight. The lack of response of safflower plants to fertilizer applied by Sampaio et al. (2016) could be due to the low nutrient application rate compared to the results of Santos et al. (2018) still in Brazil. Mazhani (2017) reported a significant increase in capitula number/plant with an application of 75 N + 50 P kg/ha in Botswana. However, N application at 75 kg/ha increased safflower capitulum diameter and 100-seed weight but had no significant effect on seed number/capitulum (Mazhani, 2017).

From literature, there is significant variation in the recommended amount of N and P to be applied for optimum safflower production which could be attributed to differences in soil types, climatic conditions, genotypes, and agronomic practices (irrigated or rainfed) (Siddiqui & Oad, 2006; Abbadi & Gerendas, 2008; Mirzakhani et al., 2009; Golzarfar et al., 2012; Abd El-Mohsen & Mahmound, 2013; Emongor & Oagile, 2017; Santos et al., 2018).

Nitrogen and P are essential elements needed for photosynthesis and plant reproductive growth such as flower formation and grain (seed) filling (Mengel & Kirkby; 2001; Marschner, 2005; Malhotra et al., 2018). Phosphorus is reported to be portioned to the plant's fruiting zones, where significant energy requirements are necessary for successful safflower capitula formation (Marschner, 2005; Malhotra et al., 2018). Phosphorus is transported from the source (leaves) to the sink (heads) via translocation (Marschner, 2005; Malhotra et al., 2018). The larger increased leaf area and chlorophyll content induced by N and P application in the current study provided a greater leaf surface area, rate of photosynthesis, and photosynthetic efficiency resulting in the production of more photo-assimilates and dry matter accumulation leading to high capitula number/plant, seed number/capitulum and 1000-seed weight. Nitrogen is reported in literature to be an important nutrient for crop production because of its promoting effects on dry matter production via its influence on leaf area development, maintenance, and photosynthetic efficiency (Kirby & Mengel, 2001, Marschner, 2005; Attia et al., 2011). Several reports in literature indicate that N is one of the most affective factors in increased vegetative growth, yield, yield components, and grain filling of safflower (Ahmed et al., 1985; Steer & Harrriggen, 1986; Sary et al., 1987; Ezz El-Din, 1989; Koutroubas et al., 2004; Massignam et al. 2009; Koutroubas & Papakosta, 2010; Attia et al., 2011; Santos et al., 2018; Sreekanth et al., 2021). According to Borrás et al. (2004), a shortage of assimilate supply results in a significant decrease in grain filling time and grain weight.

However, in winter the effects of N and P on safflower yield components in the current study were negatively affected by the extremely low temperatures of -6.3-4.2°C which occurred on two different days in July and August 2021. Abiotic stresses such as drought and low temperature reduce water uptake of plants, water use efficiency, leaf area, leaf chlorophyll content, photosynthesis, plant biomass, and seed yield (Hajihashemi et al., 2018; Wassan et al., 2021; Marang et al., 2022; Zhang et al., 2022).

5.4 Effects of N and P application on seed yield and oil content

In the current study, the application of N and P fertilizers to safflower plants significantly ($P < 0.05$) interacted to increase seed yield. Application of 40 N + 50 P kg/ha significantly produced the highest safflower seed yield of 1356 and 3528 kg/ha in summer and winter, respectively. Nitrogen and P increased safflower seed yield because these macronutrients increased vegetative growth (plant height, first branching height, primary branch number/plant, leaf area and chlorophyll content, and dry matter) and yield components (capitula number/plant, capitula diameter, seed number/capitulum, and 1000-seed weight) of safflower. Nitrogen and P are macronutrients that are required in larger quantities for the completion of the plant's life cycle including safflower (Mengel & Kirkby, 2001; Marschner, 2005). Nitrogen and P affect the growth, development, yield, and quality of crops (Marschner, 2005; Sanchez, 2007; Eckert, 2010). Nitrogen and P are major components of many essential plant organic compounds such as chlorophyll and coenzymes (DNA and RNA) (Green et al., 1995; Raven et a*l*., 1999; Heidari & Mohammad, 2012), nucleic acids (DNA and RNA), amino acids, alkaloids, and some plant hormones, and energy transfer compounds such as ATP (adenosine triphosphate) which allows cells to conserve and use the energy released in metabolism (Green et al., 1990; Mengel and Kirkby, 2001; Havlin et al., 2005; Marschner, 2005; Terrence; 2009). Phosphorus (P) is an essential part of phospholipids which are important in cellular membrane structure (Marschner, 2005; Sanchez, 2007). Phosphorus is an essential component of adenosine triphosphate (ATP)

(Mengel and Kirkby, 2001; Marschner, 2005; Havlin et al., 2005), synthesized through respiration and photosynthesis, it contains a high energy phosphate group that drives most energy-requiring biochemical processes (Marschner, 2005). For example, nutrient uptake and transport within the plant, as well as their assimilation into various biomolecules, are energy-intensive plant processes that necessitate the use of ATP. Adequate P nutrition enhances many aspects of plant physiology, including the fundamental processes of photosynthesis, nitrogen fixation, flowering, fruiting (including grain filling and seed production), and maturation (Raven *et al*., 1999; Marschner, 2005; Akanbi et al., 2010). Phosphorus promotes the growth and development of lateral roots and rootlets (Brady &Weil, 1996; Mengel & Kirkby, 2001; Marschner, 2005; Akanbi et al., 2010). A good supply of N and P stimulates root growth and development, the uptake of other nutrients, and encourages vegetative growth, giving leaves a deep green colour (Marschner, 2005; Heidari & Mohammad, 2012). Because plants require very large quantities of N, an extensive rooting system is essential to allow unrestricted uptake. The above physiological roles of N and P in plants explain why the yield of safflower was increased by N and P fertilizer application.

Sreekanth et al. (2021) reported that the application of 50 N + 60 P kg/ha to safflower plants significantly produced the highest seed and biological yield compared to other treatments. They attributed the high seed yield of safflower induced by N + P application to increased capitulum/plant and increased N and P mining capacity of the safflower plants due to better root development and increased translocation of photo-assimilates from the source to meristems in plots applied with N and P fertilizer, which resulted in high seed yield (Sreekanth et al., 2021). The increase in safflower seed yield due to N + P application was attributed to the role of N and P in promoting cell division and elongation, vigorous root system development for effective absorption of applied nutrients, and chlorophyll synthesis. The increase in biological yield due to the application of 50 N + 60 P kg/ha was explained by the increase in plant height, and the number of branches/plants from the application of N and P fertilizers. Safflower plants applied with 90, 120, and 150 kg N/ha produced seed yields of 1364, 1587, and 1762 kg/ha, respectively, but control plants produced a seed yield of 1074 kg (Al-Zubaidy & Al-Mohammad, 2021). While Santos et al. (2018) in Brazil reported that N fertilizer application at 50, 100, 150, 200, 250, 300, 350, and 400 kg/ha significantly increased safflower seed yields under irrigated and rainfed conditions. However, optimum safflower seed yield of 4216 and 2048 kg/ha was obtained by application of 200 kg N/ha under irrigated and rainfed conditions, respectively (Santos et al., 2018). Golzarfar et al. (2012) reported comparable results, when they found that safflower plants applied with 22 and 44 kg P kg/ha produced 2,392 and 2,642 kg/ha of seed, respectively, compared to control plants that produced 1936 kg/ha. Application of 150 kg N/ha to safflower plants produced a maximum seed yield of 3228 kg/ha (Golzarfar et al., 2012). An increase in safflower seed yield due to N and P fertilizer application has been reported in literature (Dordas & Sioulas, 2008; Singh et al., 2013; Sandhya Rani et al., 2014; Mazhani, 2017; Nathan et al., 2017, Haliloglu & Beyyavas, 2019; Sofy et al., 2020).

The difference in safflower seed yield between summer and winter was attributed to differences in days to physiological maturity. In summer and winter, the safflower crop took 103 and 169 days after emergence, respectively to physiological maturity. The longer growth period in winter resulted in more vegetative growth, dry matter accumulation, and yield components of safflower than in summer (Emongor et al., 2013; Emongor et al., 2017; Moatshe, 2019; Moatshe et al., 2020). The longer growth period in winter than summer was attributed to low air temperatures, especially at night. Similar results have been reported in literature (Emongor et al., 2013; Hassan et al., 2015; Tahmasebpou et al., 2016; Emongor et al., 2017; Moatshe, 2019; Moatshe et al., 2020). Emongor et al. (2013) in Botswana reported that the safflower genotype 'Kiama' took 106 and 142 days after sowing to physiological maturity in summer and winter, respectively. While Moatshe (2019) also in Botswana reported that safflower took 100-116 and 135-147 days after sowing depending on genotype and plant density.

The results of the current study on seed yield in both summer and winter were low compared to the average yields of 2000-3600 and 4000-5600 kg/ha in summer and winter, respectively (Emongor et al., 2013; Oarabile, 2017; Moatshe et al., 2016; Emongor et al., 2017; Moatshe, 2019). The low yields in summer were explained by heavy rainfall which occurred during flowering and physiological maturity. Waterlogging adversely affects safflower flowering and capitulum set which is a dryland plant (Kolte, 1985; Dajue & Mündel, 1996; Emongor, 2010). Pollination and seed set are negatively affected by prolonged rainfall during seed set, resulting in a considerable loss in seed yield (Mündel et al., 1992; Dajue & Mündel, 1996). Prior to harvesting, heavy rains opened the capitula in the current study causing most of the seeds to be damaged or destroyed, hence leading to decreased seed yield. During the winter study, the seed yield was reduced due to frost which occurred at the end of the elongation phase of growth, however plants recovered from the low-temperature stress. Low-temperature stress below -2°C at the flowering stage significantly reduces safflower seed yield (Emongor, 2010; Emongor & Oagile, 2017; Grains Research and Development Corporation [GRDC], 2017; Bergman & Kandel, 2019).

The most essential economic trait of safflower is its oil content because for commercial production, knowing the seed oil content is crucial and it determines the success of safflower production (Dajue & Mündel, 1996; Bassil & Kaffka, 2002; Singh & Nimbkar, 2006; Emongor et al., 2017). For commercial production of safflower oil for food, pharmaceutical, and other industrial purposes the minimum seed oil content should be 28% (Dajue & Mündel, 1996; Bassil & Kaffka, 2002; Singh & Nimbkar, 2006; Emongor, 2010; Singh & Singh, 2013; Emongor et al., 2017). In the current study application of 40 and 25 kg/ha of N and P, significantly increased safflower oil seed content in summer. Safflower plants applied with 40 and 25 kg/ha of N and P, produced seed oil contents of 43.9 and 46.1%, respectively in summer. While in winter the highest seed oil content of 71.8% was produced by safflower plants applied with 0 N + 25 P kg/ha. The increase in seed oil content of safflower with the application of N and P fertilizers was attributed to the increase in vegetative growth, dry matter accumulation, yield components, and uptake of N and P of safflower as induced by N and P application in the current study. Nitrogen and P caused a favourable effect on plant growth and development of safflower due to proper partitioning of the photosynthates from source to sink resulting in high seed yield and oil content. The oil content increased successively with an increase in P application because it is a constituent of phospholipid and is essential for the biosynthesis of oil (Salisbury & Ross, 1996; Marschner, 2005; Bates et al., 2013; Kong et al., 2020). Plants biosynthesize and store fatty acids mostly as triacylglycerol (TAG) in their seeds to support seedling development as a carbon and energy resource (Kong et al., 2020). The biosynthesis of TAG requires P since it's a constituent of the molecule and plays an important role in carbohydrate metabolism (Salisbury & Ross, 1996; Marschner, 2005; Bates et al., 2013; Kong et al., 2020). TAG (often familiar to many people as vegetable oils) is a highly energy-rich natural resource, as it has higher energy compared to carbohydrates and proteins (Kong et al., 2020). Sofy et al. (2020) in Iraq reported that safflower plants applied with 50 and 100 kg P_2O_5/ha significantly produced seed with a high oil content of 30.0 and 32.6%, respectively, but control plants produced seed oil content of 28.7%. Mazhani (2017) in Botswana, reported that application of N and P significantly increased safflower seed oil content, with a maximum seed oil content of 49.1% obtained by application of 100 N + 50 P kg/ha while control plants produced seed oil content of 21.9%. Singh & Singh (2013) reported that safflower plants applied with N at 40-80 kg/ha and P at 40-80 kg/ha increased safflower seed oil content compared to control plants. Safflower plants applied with N (40-80 kg/ha) and P (40-80 kg/ha) produced seed oil content of 33.3-33.5 and 33.8-34.0%, respectively, but control plants produced seed oil content of 31.9-32.4% (Singh & Singh, 2013). While Arani et al. (2011) reported that safflower plants applied with 50, 75, and 100 kg P/ha produced seed with significantly higher oil content by 2.1, 3.8, and 4.6%, respectively, than seed oil content from control plants. El-Nakhlawy (1991) reported that application of 46 N + 46 P, 92 N + 46 P, and138 N + 46 P kg/ha decreased the oil content by 2.8%, 3.3% and 4.9%, respectively, compared to the control plants. Golzarfar et al., (2011) reported that application of 75 N + 50 P, 75 N + 100 P, 150 N + 50 P, and 150 N + 100 P kg/ha to safflower plants had no significant effect on the seed oil content.

5.5 Effect of N and P application on the N and P leaf content

Nitrogen and P are important components of the basic cell structure of plants. Plant photosynthesis, growth, reproduction, and eco-physiological activities are heavily influenced by leaf N and P concentrations and their stoichiometric relationship. Leaf N and P concentrations, as significant functional features, play critical roles in revealing plant nutrient-use strategies and their evolution in terrestrial environments. They also have an impact on physiological and ecological processes in leaves as well as productivity at the ecosystem level (He et al., 2014). In the current study, the safflower leaf N content was higher than the leaf P content. This suggests that P was more limited than N or it could also mean that P was used in other plant processes. The higher leaf N may be due to limited plant N fixation (Aerts & Chapin, 1999) and limited N losses as dissolved organic N (Perakis & Hedin, 2002) or as gaseous N (McCalley & Sparks, 2009), whereas the lower P may be due to soil fixation due to the experimental site's low soil pH. Lower tissue nutrition content may also be related to high nutrient utilization efficiency. Phosphorus is notorious for being the most limiting nutrient in the soils of Botswana. Nitrogen and P application, as suggested by Emongor et al. (2012), enhances nutrient uptake and partitioning within the plant, hence the increased leaf N and P contents. This could be an explanation for the increased leaf N and P contents of the safflower leaves during the winter experiment.

5.6 Nutrient use efficiency

5.6.1 Effect of N and P application on nitrogen use efficiency

The total N (NO_3^- and NH_4^+ ions) absorbed in relation to the total N available in the soil is defined as the uptake efficiency (Moll et al., 1982; Gerloff & Gabelman, 1983; Baligar et al., 1990). Safflower plants were more efficient in N uptake with low N input but in the presence of P. Increasing N application above 40 kg/ha in the presence of P significantly ($P < 0.05$) decreased NUpE in summer and winter. Safflower plants were efficient in the uptake of N at the interaction of 40 N + 75 P kg/ha and 40 N + 25 P kg/ha which resulted in maximum N uptake of 12.43 and 10.2 mg in summer and winter, respectively. The efficient uptake of N by safflower plants at a low N (40 kg/ha) application rate in the current study, implies that the plants were able to adapt to reduced nutritional status by increasing their physiological ability to acquire the limiting nutrients. Plants thriving in nutrient-limited soils have evolved structural traits and nutrient-absorbing mechanisms that enable them to grow (Morgan & Connolly, 2013). A change in root structure, which may increase the overall surface area of the root to improve nutrient acquisition or increase the extension of the root system to access new nutrient sources, is one of the most common responses to nutrient-limited soils (Morgan & Connolly, 2013). Singh & Singh (2013) reported that N uptake by safflower plants was highest at 80 kg N/ha. The results of the current study confirm the findings of Abbadi (2007) and Koutroubas et al. (2020). Abbadi (2009) reported that safflower plants were more efficient in NUpE at lower N supplies compared to sunflowers. Koutroubas et al. (2020) reported that increasing N (69, 138, 276 kg/ha) application above 69 kg/ha in sunflower significantly decreased NUpE. The differences between the maximum NUpE obtained at different N rates in the study of Singh & Singh (2013) and the current study may be due to other external factors such as soil type and pH, agronomic practices, and climatic conditions (Tao et al., 2018). Soil pH affects nutrient availability for example in acidic soils, the levels of Al, Mn, Fe, and H^+ are usually phyto-toxic resulting in a deficiency of N, P, K, and Ca that support good plant growth (Baligar & Fageria, 1997; Mengel & Kirkby, 2001; Marschner, 2005). The reduced NUpE at higher N rates may be because of excess salt (salinity) accumulation affecting N uptake and reduced permeability of the roots, subsequently decreasing nutrient uptake (Frota & Tucker, 1978; Mengel & Kirkby, 2001; Silveira et al., 2001; Marschner, 2005; Debouba et al., 2006). Salinity is a major factor responsible for low N availability (Debouba et al. 2006), because nitrate reductase activity in leaves is reliant on nitrate flux from roots, and is significantly affected by osmotic shock caused by NaCl (Silveira et al. 2001; Mokhele et al., 2012). Phosphorus application in the current study improved the NUpE of safflower plants. Nitrogen and P interactions are known for their synergistic interactions (Chapin & Shaver, 1989; Mengel & Kirkby, 2001; Aulakh & Malhi, 2005; Marschner).

The most significant obstacle in modern agriculture is maintaining and improving crop yields while lowering production expenses. One method would be to improve nutrient utilization efficiency. Nitrogen utilization efficiency (NUtE) is the effective remobilization and translocation of N from vegetative parts of the plant to developing tissues representing strong sinks for N during the seed-filling period which is predominantly present as protein (Masclaux-Daubresse et al., 2010). Because inorganic fertilizers are costly, a crop that will make greater use of fertilizer at a lower application rate will be ideal for a farmer. In the current study safflower plants applied with 120 N + 75 P and 40 N + 50 P kg/ha had significantly the highest NUtE of 116.1 and 187.3 kg/ha in summer and winter, respectively. In summer, increasing N application increased NUtE of safflower plants, though it was low compared to winter grown safflower. The high NUtE in both summer and winter due to N and P application is partly attributed to the high dry matter accumulation by the safflower plants. Nitrogen is a constituent of amino acids, which are required for the synthesis of proteins and other related compounds, and it plays many roles in almost all plant metabolic processes hence explaining the high NUtE of safflower plants applied with N fertilizers (Tucker, 1999; Marschner, 2005; Mokhele et al., 2012). The results of the current study in summer disagree with those of Abbadi (2007), who found that safflower was more efficient in utilizing N at low N rates than at optimal rates. However, in winter, the results of the current study agreed with those of Abbadi (2007). The differences in the NUtE may be attributed to the different soil textures that the trials were carried out in summer (sandy clay loam) and winter (sandy loam). Soils with clay are known to hold nutrients whilst those predominant in the sand are prone to nutrient loss through leaching (Mengel & Kirkby, 2001; Marschner, 2005). This partly explains why at low N input in winter, safflower plants were able to perform better than low N in summer in the current study. High NUtE in summer due to high N input was due to the ability of the soil to hold N. This implies that soil texture plays an important role when it comes to the nutrient efficiency of a plant. The presence of P increased safflower's ability to utilize N which again, symbolizes the importance of both N and P application to the safflower plants hence proving the synergistic interactions of N and P.

The two basic components of NUE are NUpE and NUtE. In theory, NUE can be improved by enhancing either NUpE, NUtE, or both (Ranjan et al., 2019). Food production through minimum use of inorganic fertilizer to reduce their footprints in the environment is integral in today's world, hence improving the NUE of cropping systems is needed (Ranjan & Yadav, 2019). In the current study, the NUE of the safflower plant decreased as the rate of N application increased in both summer and winter. Application of 40 N +50 P kg/ha to safflower plants in summer and winter resulted in significantly the highest NUE of 1.02 and 1.91 kg, respectively. The decrease in NUE with an increase in N above 40 kg/ha was attributed to elevated N. A negative correlation between the amount of applied N and NUE has been well established in several crops (Barbieri et al., 2008; Fageria, 2014; Koutroubas et al., 2014; 2020). The results of the current study agree with those of Shahrokhnia & Sepaskhah (2016) who found high NUE of safflower at low N rates of 46 kg/ha. Soleymanifard et al. (2022) also obtained a high NUE of safflower when N was applied at 50 % compared to 100%. Nitrogen use efficiency was high in lower nitrogen rates in wheat and decreased with increased N application (Sepaskhah & Hosseini, 2008; Faraj, 2011; Mehrabi & Sepaskhah, 2018).

The application of P resulted in the improvement of the NUE compared to when N was applied alone in the current study. The N and P synergistic interactions are not only important for improved yield but also for enhanced nutrient use efficiency. The high NUE at low N input implies that the safflower plant does not require a large N input to produce a substantial quantity and quality of seed yield and oil content. The results of the current study suggest that safflower is an economic plant that can benefit farmers as it will lessen their economic burden due to the low N requirement to produce optimum seed yield and oil content and reduce the N footprint in the environment.

5.6.2 Effect of N and P application on phosphorus use efficiency

Phosphorus is an essential element required by the plant for increased productivity. The application of P fertilizer is important and effective concerning yield formation in safflower (Mündel et al., 1997; Abbadi & Gerendás, 2011; 2015). In the current study, the application of N and P fertilizers increased PUpE. In both summer and winter application of 40 N + 25 P kg/ha significantly increased PUpE by 7.0 and 34.9 mg, respectively. Application of P above 25 kg/ha decreased PUpE in the current study. Safflower plants were able to absorb more P at low rates of P. The ability of the safflower plants to efficiently absorb the given P fertilizer at the lowest P rates in the current study could be attributed to the plant's adaptability to inadequate soil nutrients. The safflower plants may also have been able to establish long and lateral roots, as P is known for its role in root development, which improved the surface area for nutrient absorption and thus improved the uptake efficiency. The decreased uptake efficiency in both summer and winter at high P rates by the safflower plant could be attributed to the unavailability of P due to acidic soils as P is deficient in acidic soils (Mengel & Kirby, 2001; Marschner, 2005). The addition of N fertilizer substantially improved P uptake, but as N rates increased and P increased, a decrease in PUpE was observed. The increased uptake efficiency of P at low P fertilizer rates suggests that it can reduce the economic burden of inputs on a farmer whilst improving yield. Abbadi (2007) and Abbadi & Gerendás (2015) reported that PUpE (Moll et al., 1982) in safflower was much less at low P supplies. Safflower had much less P concentration compared to sunflower at low P supplies but outyielded that of sunflower at their best levels because safflower accumulated less dry matter than sunflower (Abbadi and Abbadi & Gerendás, 2015).

After uptake, the plant must be able to use the accumulated nutrients. In the current study, safflower plants applied with 80 N + 75 P and 0 N + 75 P kg/ha had significantly the highest PUtE of 0.12 and 0.7 mg in winter and summer, respectively. The maximum rate of application of P fertilizer in both summer and winter enhanced the safflower plant's PUtE. Safflower was able to produce more dry matter by using more of the accumulated P. The high PUtE of safflower at higher rates of P indicated a high P requirement at optimal growth. The results of the current study agree with those of Abbadi & Gerendas (2015) who found that safflower required higher rates of P for optimal growth compared to sunflower. Plants with low utilization efficiency were unable to produce sufficient dry matter. The addition of N improved the utilization of P of safflower in winter, when N was increased, P was better utilized.

In agricultural cropping systems, phosphorus is one of the most limited nutrients. (Roberts & Johnston, 2015; Guignard et al., 2017; Khan et al., 2018). Phosphorus deficiencies are estimated in nearly 67% of world land designated for crop production (Dhillon et al., 2017). Also, PUE for cereal production in the world is too low, varying between 15 and 30% (Dhillon et al., 2017). Given the importance of P fertilizers in agricultural productivity and their association with population growth, PUE should be enhanced, particularly given the non-renewable nature of P-reserves (Dhillon et al., 2017). In the current study, safflower plants applied with 120 N + 75 P and 40 N + 25 P kg/ha had the highest PUE of 26.7 and 6.68 in summer and winter, respectively. This indicated that for safflower to produce a high yield, more and less P fertilizer was required in summer and winter. The high demand for P for a significant output in summer may be costly for a farmer unlike in winter when costs may be substantially decreased. The addition of N, on the other hand, improved the safflower plant's P use efficiency. This could be because the presence of N aids in the uptake of P and, ultimately, the usage of P, as Emongor et al. (2012) argued. As a result, when P was applied alone, safflower plants were not efficient users of applied P Abbadi & Gerendás (2015) reported that safflower's agronomic P requirement was much higher than that of sunflower at their respective best P supplies, which indicated high P requirement in safflower compared to sunflower at optimal growth. Unlike that of safflower, the requirement of sunflower to produce the same amount of achene decreased with decreasing P supply indicating that sunflower was more efficient in utilizing external P than safflower at suboptimal, best, and high P supplies to express achene yield (Abbadi & Gerendás, 2015).

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The results of the current study showed that nitrogen and phosphorus application increased vegetative growth, yield components, seed yield, and oil content of safflower. Maximum capitula number/plant, capitula diameter, seed number/capitulum, and 1000-seed weight were produced by safflower plants applied with 40 N + 50 P and 40 N + 25 P kg/ha. Application of 40 N + 50 P and 80 N + 75 P kg/ha to safflower plants significantly increased the seed yield in both summer and winter, respectively. Application of 120 kg N/ha and 25 kg P/ha and 0 N + 25 P kg/ha significantly increased safflower seed oil content in summer and in winter, respectively. The results revealed that winter grown safflower produced seed with high oil content (71.8%) than summer grown safflower (43.9% and 46.1%).

6.2 Recommendations

From the results based on the performance of safflower as N and P influenced vegetative, yield components, seed yield, and seed oil content it was concluded that application of 40 N + 50 P kg/ha was recommended to maximize safflower production. However, it was also recommended that this study be repeated in Southern Botswana for confirmation of the current results. Also, fertilizer trials be done in other parts of the country where farmers are currently growing safflower.

REFERENCES

Abbadi, J. (2007). Importance of nutrient supply (N, P, K) for yield formation and nutrient use efficiency of safflower (*Carthamus tinctorius L.*) compared to sunflower (*Helianthus annuus L.)* including an assessment to grow safflower under north German conditions. https://macau.uni-kiel.de/receive/diss_mods_00002618

Abbadi, J., Gerendás, J., & Sattelmacher, B. (2008). Effects of nitrogen supply on growth, yield, and yield components of safflower and sunflower. *Plant and Soil*. https://doi.org/10.1007/s11104-008-9569-5

Abbadi, J., & Gerendás, J. (2009). Nitrogen use efficiency of safflower as compared to sunflower. *Journal of Plant Nutrition*, *32*(6), 929–945. https://doi.org/10.1080/01904160902870705

Abbadi, J., & Gerendás, J. (2011). Effects of phosphorus supply on growth, yield, and yield components of safflower and sunflower. *Journal of Plant Nutrition*, *34*(12), 1769–1787. https://doi.org/10.1080/01904167.2011.600405

Abbadi, J., & Gerendás, J. (2015). Phosphorus use efficiency of Safflower (Carthamus tinctorius L.) and Sunflower (Helianthus annuus L.). *Journal of Plant Nutrition*, *38*(7), 1121–1142. https://doi.org/10.1080/01904167.2014.983115

Abbadi, J. (2017). Phosphorous Use Efficiency of Safflower and Sunflower Grown in Different Soils. 5(4), 212–220. http://dx.doi.org/10.12691/wjar-5-4-3

Abd El-Mohsen, A. A., & Mahmoud, G. (2013). Modeling the influence of nitrogen rate and plant density on seed yield, yield components, and seed quality of safflower. American Journal of Experimental Agriculture, 3(2): 336-360.

http://dx.doi.org/10.9734/AJEA/2013/2886

Akanbi, W. B., Adebooye, C.O., Togun, A.O., Ogunride, J. O., & Adeyeye, S. A. (2007). Growth, herbage and seed yield and quality of *Telfaria occidentalis* quality as affected by cassava peel compost and mineral fertilizer. *World Journal of Agriculture Sciences*, 3(4), 508-516.

http:// doi=10.1.1.415.3591&rep=rep1&type=pdf

Aerts, R., & Chapin, F. S. (1999) The mineral nutrition of wild plants revisited: A reevaluation of processes and patterns. Adv. Ecol. Res. 30 1–67. https://doi.org/10.1016/S0065-2504(08)60016-1

Aghamohammadreza, M., Mirhadi, M. J., Delkhosh, B., & Omidi, A. (2013). Evaluation of native and exotic safflower (*Carthamus tinctorius* L.) genotypes for some important agronomic traits and fatty acid composition. *Annals of Biological Research*, 4(6), 200-204.

Aguirrezabal, L.A.N., Lavaud, Y., Dosio, G.A.A., Izquierdo, N.G., Andrade, F.H., & Gonzalez, L.M. (2003). Intercepted solar radiation during seed filling determines sunflower weight per seed and oil concentration. Crop. Sci, 43:152-161. https://doi.org/10.2135/cropsci2003.1520

Ahmed, Z., Medakkar, S., & Mohammed, S. (1985). Response of safflower to nitrogen and phosphorus. Indian Journal of Agronomy, 30(1), 128-130.

Alivelu, K., Subba-Rao, A., Sanjay, S., Sing K. N., Raju, N. S., & Madhuri, P. (2006). Prediction of optimal nitrogen application rate of rice based on soil test values. *European Journal of Agronomy*, 25, 71–73.

Al-Zubaidy, Z. N. M. A., & Al-Mohammad, M. H. S. (2021). Effect of planting dates and nitrogen fertilizer on growth and yield of safflower. Fourth International Conference for Agricultural and Sustainability Sciences, IOP Publishing, IOP Conference Series: Earth and Environmental Science 910 (2021) 012026. https://doi.org:/10.1088/1755-1315/910/1/012026.

Amini, F., SaeidI, G. A., & Arzani, A. (2008). Relationship among seed yield and its components in genotypes of safflower (Carthamus tinctorius L.). Journal of Science and Technology of Agriculture and Natural Resources. 12: 525-535, Issue 45.

Antoniadou, T., & Wallach, D. (2002). Evaluating optimal fertilizer rates using plant measurements. *Journal of Applied Statistics*, 27, 1083–1099.

Arani, M. S., Shirani-Rad, A. H., Delkosh, B., & Tajalli, S. (2011). Effects of different amounts of phosphorus on the improvement of qualitative and quantitative traits in safflower during moisture stress. International Journal of Science and Advanced Technology, 1 (8): 109 113.

Attia, A. N. E., Badawi, M. A., Seadh, S. E., & El-Hety, S. M. S. (2011). Response of growth and petal yield of safflower to sowing dates, nitrogen fertilizer levels and times of foliar application with natural growth promoters "Melagrow". *Journal of Plant Production, Mansoura University*, 2(12), 1703- 1716.

Aulakh, M. S., & Malhi, S.S. (2005). Interactions of nitrogen with other nutrients and water: Effect on crop yield and quality, nutrient use efficiency, carbon sequestration, and environmental pollution. Advances in Agronomy 86:341–409.

Bagheri, A., Yazdi-Samadi, B., Taeb, M., & Ahmadi, M. R. (2001). Study of correlations and relations between plant yield and quantitative and qualitative traits in safflower. Iranian

Journal of Agricultural Sciences 32(2): 295-307.

Baligar, V. C., Duncan, R.R., & Fageria, N.K. (1990). Soil-plant interactionson nutrient use efficiency in plants. An overview. In: Crops as enhancers of nutrient use,eds. V. C. Baligar and R. R. Duncan, pp. 351–373. San Diego, California: Academic Press.

Baligar, V. C., & Fageria, N.K. (1997). Nutrient use efficiency in acid soils: nutrient management and plant use efficiency pp. 75–93. In: A. C. Monitz, A.M.C. Furlani, N. K. Fageria., C. A. Rosolem, and H. Cantarells. (eds.), Plant-Soil Interactions at Low pH: Sustainable Agriculture and Forestry Production. Brazilian Soil Science Society Compinas, Brazil.

Baligar, V. C., Fageria, N. K., & He, Z. L. (2001). Nutrient use efficiency in plants. *Communications in Soil Science and Plant Analysis*, *32*(7–8), 921–950. https://doi.org/10.1081/CSS-100104098

Baligar, V.C., & Fageria, N.K. (2015). Nutrient Use Efficiency in Plants: An Overview. In: Rakshit A., Singh H.B., Sen A. (eds) Nutrient Use Efficiency: from Basics to Advances. Springer, New Delhi. https://doi.org/10.1007/978-81-322-2169-2_1.

Barbieri, P. A., Echeverría, H. E., Saínz Rozas, H. R., & Andrade, F. H. (2008). Nitrogen use efficiency in maize as affected by nitrogen availability and row spacing. Agronomy Journal, 100(4): 1094-1100.

Bassil, E. S., & Kaffka, S. R. (2002). Response of safflower (Carthamus tinctorius L.) to saline soils and irrigation. I. Consumptive water use. Agricultural Water Management, 54: 67-80.

Bates, P. D., Stymne, S., & Ohlrogge, J. (2013). Biochemical pathways in seed oil synthesis. *Current Opinion inPlant Biology*. http://dx.doi.org/10.1016/j.pbi.2013.02.015.

Belanger, G., Walsh, J. R., Rechards, J. E., Melburn, P. H., & Zeade N. (2000). Comparison of three statistical models describing potato yield response to nitrogen fertilizer. *Agronomy Journal*, 92, 902–908.

Berglund, D., Riveland, R.R., & Bergman, J. (2007). Safflower Production Environment-Natural Resources, Dacota State University, NSDU, 870p.

Bergman, J. & Kandel, H. (2019). *Safflower production*. North Dakota State University Fargo, North Dakota Extension Bulletin A870, 4p.

Bernstein, L., Bosch, P., Canziani, O., Chen, Z., Christ, R., Davidson, O., Hare, W., Huq, S., Karoly, D., Kattsov, V., Kundzewicz, Z., Liu, J., Lohmann, U., Manning, M., Matsuno, T., Menne, B., Metz, B., Mirza, M., Nicholls, N., … Yohe, G. (2007). *Climate change synthesis report in UN and other languages. An Assessment of the Intergovernmental Panel on Climate Change*. http://www.ipcc.ch/site/assets/uploads/2018/06/ar4_syr.pdf/.

Bitarafan, Z., Shirani-Rad, A. H., & Delkhosh, B. (2011). Nitrogen rates and sowing date effect on yield and oil content of spring safflower. *International Journal of Science and Advanced Technology*, 1(6), 25-30.

Bloom, A. J., Frensch, J., & Taylor, A. R. (2006). Influence of inorganic nitrogen and pH on the elongation of maize seminal roots. Annals of Botany, 97(5), 867-873.

Bojović, B., & Stojanović, J. (2006). Some wheat leaf characteristics in dependence of fertilization. Kragujevac J. Sci, 28, 139-146.

Bonfim-Silva, E. M., Paludo, J. T. S., Sousa, J. V. R., Sousa, H. H, & Araujo da Silva, T. J. (2015). Development of safflower subjected to nitrogen rates in cerrado soil. American Journal of Plant Sciences, 6: 2136-2143.

Borrás, L., Slafer, G. A., & Otegui, M. E. (2004). Seed dry weight response to source–sink manipulations in wheat, maize and soybean: a quantitative reappraisal. Field Crops Research, 86(2-3), 131-146.

Botswana Agriculture Sector Policy Brief. (2012). Agriculture and Food Security Policy Brief Reflecting on the Challenges of Attaining a Green Economy for Botswana. National Report, pp. 2.

Brady, N.C., & Weil, R. R. (1996). *The nature and properties of soils.* 11th Edition, Prentice-hall International, Inc, New Jersey.

Burgener, D.L., Harveson, P.R., Hein, R., & Hergert, G. (2004). Growing Safflower in Nebraska. *Asian Journal Plant Science* 3:683- 686.

Camas, N., & Esendal, E. (2007). Estimates of broad-sense heritability for seed yield and yield components of safflower (Carthamus tinctorius L.). Hereditas. 143. 55-7. https://doi.org/10.1111/j.2006.0018-0661.01914.x

Challinor, A. J., Watson, J., Lobell, D. B., Howden, S.M., Smith, D.R., & Chhetri, N. (2014). A meta-analysis of crop yield under climate change and adaptation. *Nature Climate Change*, 4, 287-291.

Chapin, F. S., & Shaver, G. R. (1989). Differences in Growth and Nutrient use Among Arctic Plant Growth Forms. Functional Ecology, 3(1), 73–80. https://doi.org/10.2307/2389677.

Chaudhari, S. K. (1990). Path analysis for seed yield in safflower (*Carthumus tinctorius* L.) in acid soil under mid altitude conditions. International Journal of Tropical Agriculture, 8:129-132.

Chaudhary, P. R., Yu, X., & Jayaprakasha, G. K. (2017). Influence of storage temperature and low-temperature conditioning on the levels of health-promoting compounds in Rio Red grapefruit. *Food Science and Nutrition,* 5, 545-553.

Chehade, L. A., Angelini, L. G., & Tavarini, S. (2021). Genotype and seasonal variation affect yield and oil quality of safflower (*Carthamus tinctorius* L.) under Mediterranean conditions. *Agronomy*, 2022, 12, 122. https//doi.org/10.3390/agronomy12010122.

Chen, L., & Liao, H. (2017). Engineering crop nutrient efficiency for sustainable agriculture. *Journal of Integrative Plant Biology*, *59*(10), 710–735. https://doi.org/10.1111/jipb.12559.

Ciompi, S., Gentili, E., Guidi, L., & Soldatini, G. F. (1996). The effect of nitrogen deficiency on leaf gas exchange and chlorophyll fluorescence parameters in sunflower. *Plant Science* 118, 177-184.

Cosge, B., Gurbuz, B., & Kiralan, M. (2007). Oil content and fatty acid composition of some safflower (*Carthamus tinctorius* L.) varieties sown in spring and winter. *International Journal of Natural and Engineering Sciences*, 1, 11-15.

Cunniff, P., & Association of Official Analytical Chemists. (1995). *Official methods of analysis of AOAC international*. Washington, DC: Association of Official Analytical Chemists.

Cunniff, P., & Association of Official Agricultural Chemists. (1996). *Official methods of analysis of AOAC International*. Gaithersburg, Maryland: AOAC International.

Dajue, L. & Mündel, H. H. (1996). *Safflower* (*Carthamus tinctorius* L.). Promoting the conservation and use of utilized and neglected crops,. Germany and International Plant Genetic Resources Institute, Rome.

Daughtry, C. S., Walthall, C. L., Kim, M. S., De Colstoun, E. B., & McMurtrey Iii, J. E. (2000). Estimating corn leaf chlorophyll concentration from leaf and canopy reflectance. Remote sensing of Environment, 74(2), 229-239.

Dayal, J., Goswami, C. L., & Munjal, R. (2004). Influence of phosphorus application on water relations, biochemical parameters and gum content in cluster bean under water deficit. Biologia Plantarum, 48(3), 445-448.

de Anicésio, E. C. A., Bonfim-Silva, E. M., da Silva, T. J. A., & Koetz, M. (2015). Dry mass, nutrient concentration, and accumulation in safflower (Carthamus tinctorius L.) influenced by nitrogen and potassium fertilization. *Australian Journal of Crop Science*, *9*(6), 552–560.

Debouba, M., Gouia, H., Suzuki, A., & Ghorbel, M. H. (2006). NaCl stress effects on enzymes involved in nitrogen assimilation pathway in tomato ‘‘*Lycopersicon esculentum*’’ seedlings. *Journal of Plant Physiology*,163, 1247-1258.

Dhillon, J., Torres, G., Driver, E., Figueiredo, B., & Raun, W. R. (2017). World Phosphorus Use Efficiency in Cereal Crops. Agron. J. 109(4): 1670-1677. https:doi:10.2134/agronj2016.08.0483.

Dong, S., Cheng, L., Scagel, F. C., & Fuchigami, H. L. (2005) Method of nitrogen application in summer affects plant growth and nitrogen uptake in autumn in young fuji/m.26 apple trees. *Communications in Soil Science and Plant Analysis*, 36, 1465–1477.

Dole, J.M., & Wilkins, H.F. (2005). Floriculture principles and species. 2nd ed. Prentice Hall, Upper Saddle River, NJ.

Dordas, C.A., & Sioulas, C. (2008). Safflower yield, chlorophyll content, photosynthesis, and water use efficiency response to nitrogen fertilization under rainfed conditions. *Industrial*

Crops and Products, 27, 75‑85.

Dordas, C. A., Lithourgidis, A. S., Matsi, T., & Barbayiannis, N. (2008). Application of liquid cattle manure and inorganic fertilizer affect dry matter, nitrogen accumulation, and partitioning in maize. Nutrient Cycling in Agroecosystems, 80(3), 283-296.

Dordas, C. A. & Sioulas, C. (2009). Dry matter and nitrogen accumulation, partitioning, and re-translocation in safflower (*Carthamus tinctorius* L.) as affected by nitrogen fertilization. *Fields Crops Research*, 110, 35-43. https://doi:10.1016/j.fcr.2008.06.011.

Eckert, D. (2011). *Efficient fertilizer use-nitrogen*. New York Publishing Inc. New York, United States of America.

Ekin, Z. (2005). Resurgence of safflower (*Carthamus tinctorius* L.) utilization: A global view. *Journal of Agronomy*, 4, 83-87.

Elfadl, E., Reinbrecht, C., Frick, C., & Claupein, W. (2009). Optimization of nitrogen rate and seed density for safflower (*Carthamus tinctorius* L.) production under low input farming conditions in temperate climate. *Field Crops Research*, 114(1), 2-13.

El-Nakhlawy, F. S. (1991). Response of safflower to different levels of nitrogen, phosphorus, and potassium. *Acta Agronomica Hungarica*, 40 (1-2), 87-92.

Emongor, V. E. (2002). Effect of benzyladenine and gibberellins on growth, yield, and yield components of common bean (Phaseolus vulgaris L.). UNISWA Res. J. Agric. Sci. and Tech. 6(1): 65-72.

Emongor, V. E. (2007). Gibberellic acid (GA3) influence on vegetative growth, nodulation, and yield of cowpea (vigna unguiculator (L.) walp). Journal of Agronomy,

Emongor, V. E. (2009). The major cations and trace elements in Notwane river, Botswana, and its suitability for irrigation. *Botswana Journal of Agriculture and Applied Sciences*, 5(1), 63-70.

Emongor, V. E. (2010). Safflower (*Carthamus tinctorius L.*) the underutilized and neglected crop: A review. *Asian Journal of Plant Sciences*, 9 (6), 299- 306.

Emongor, V. E., & Mabe, O. (2012). Effects of phosphorus on growth, yield, and yield components of chilli pepper (*Capsicum annuum* L.). *Acta Horticulturae*, 936, 327-333.

Emongor, V. E., Oagile, O., & Kedikanetswe, B. (2013). Effects of plant population on growth, development, and oil yield of safflower. Journal of Agricultural Science and Technology B, 3:321-333.

Emongor, V. E., Oagile, O., & Kedikanetswe, B. (2015). Effects of plant population and season on growth and development of safflower (Carthamus tinctorius L.) as an ornamental plant. Acta Horticulture, 1077: 35-45.

Emongor, V. E. (2015). The effects of temperature on storage life of mango. *American Journal of Experimental Agriculture,* 5(3), 252-261.

Emongor, V. E., & Oagile, O. (2017). *Safflower Production*. Impression House Publishers, Industrial West, Gaborone, Botswana, 62p, ISBN 978-99968-0-607-0.

Emongor, V. E., Malikongwa, T., & Tshwenyane, S. O. (2017a). The influence of nitrogen and phosphorus on vegetative growth, yield components and yield of watermelon. *International Journal of Plant & Soil Science*, 15(2), 1-16. www.scienedomain.org.

Emongor, V.E., Oarabile, P., Phuduhudu, D., & Oagile, O. (2017b). Effects of genotype on vegetative growth, yield components and yield, oil content and oil yield of safflower. *Agricultural Research Journal*, 7(12), 381-392.

Eryiğit, T., Yıldırım, B., Kumlay, A. M., & Sancaktaroğlu, S. (2015). The Effects of different row distances and nitrogen fertilizer rates on yield and yield components of safflower (*Carthamus tinctorious L.*) under micro-climate conditions of Iğdır Plain –Turkey. https://doi.org/10.15242/iicbe.c0915025.

Eryiğit, T., Aldemir, R., Kaya, A. R., Tuncturk, M., & Yildirim, B. (2021) The influence of nitrogen doses on yield and yield properties of safflower (*Carthamus tinctorius*) varieties under micro-climate conditions of Igdir plain Turkey, Journal of Plant Nutrition, 44:19, 2840 2848, https://doi.org/10.1080/01904167.2021.1921199.

Evans, J. R. (1989). Photosynthesis and nitrogen relationships in leaves of C_3 plants. *Oecologia*, 78:9-19.

Ezz EL-Din, A. A. (1989). *Response of seeds and plants yield of safflower to nitrogen fertilization and plant density*. Master of Science Thesis, Faculty of Agriculture, Ain Shams University.

Fageria, N. K., & Baligar, V.C. (2005). Enhancing nitrogen use efficiency in crop plants. *Advances in Agronomy*, 88, 97–185.

Fageria, N. K., Baligar, V. C., & Li, Y. C. (2008). The role of nutrient efficient plants in improving crop yields in the twenty first century. *Journal of Plant Nutrition*, *31*(6), 1121–1157. https://doi.org/10.1080/01904160802116068.

Fageria, N. K. (2014). Nitrogen harvest index and its association with crop yields. *Journal of Plant Nutrition*, 37(6), 795–810.

Faraj, B. A. (2011). *Evaluation of nitrogen use effciency (NUE) in wheat*. Doctoral dissertation, University of Adelaide, School of Agriculture, Food and Wine.

Fixen, P., Brenturp, F., Bruulsema, T. W., Garcia, F., Norton, R., & Zingore, S. (2015). Nutrient/ Fertilizer Use Efficiency: Measurment, Current Situation and Trends. *Managing Water and Fertilizer for Sustainable Agricultural Intensification*, *December 2014*, 8–38.

Food and Agriculture Organization. (2014). Food and Agriculture Organization of the United Nations and Government of Botswana Country Programming Framework for Botswana. Availability at http://www.fao.org.

Frota, J. N. E., & Tucker, T.C. (1978). Absorption rates of ammonium and nitrate by rice and kidney beans under salt and water stress. Soil Sci. Soc. Am. J. 42:753–756.

Gerloff, G. C., & W. H. Gabelman. (1983). Genetic basis of inorganic plant nutrition.pp 453–480. In: A Lauchli and R L Bieleski. (eds.), Inorganic Plant Nutrition. Encyclopedia and Plant Physiology New Series, Volume 15B. Springer Verlag, New York, NY.

Gonzalez, J. L., Schneiter, A. A., Riveland, N. R., & Johnson, B. L. (1994). Response of hybrid and open-pollinated safflower to plant population. Agronomy Journal, 86: 1070-1073.

Golzarfar, M., Shirani Rad, A. H., Delkhosh, B., & Bitarafan, Z. (2011). Changes of safflower morphological traits in response to nitrogen rates, phosphorus rates and planting season. Int J Sci Adv Techn 1:84–89

Golzarfar, M., Shirani Rad, A. H., Delkhosh, B., & Bitarafan, Z. (2012). Safflower (*Carthamus tinctorius* L.) response to different nitrogen and phosphorus fertilizer rates in two planting seasons. *Žemdirbystė Agriculture*, 99(2), 159–166.

Grant, C. (2006). *Enhancing nitrogen use efficiency in dryland cropping systems on the Northern Great Plains*. 18th World Congress of Soil Science, Philadelphia, PA, United States of America.

Green, N. P. O., Stout, G. W., Taylor, D. J., & Soper, R. (1990). *Biological Science*. 2nd edition Cambridge University Press. Cambridge. UK.

Grains Research and Development Corporation, (2017). *GrowNote Safflower*. Australia.

Guignard, M. S., Leitch, A. R., Acquisti, C., Eizaguirre, C., Elser, J. J., & Hessen, D. O. (2017). Impacts of nitrogen and phosphorus: from genomes to natural ecosystems and agriculture. Frontiers in Ecological Evolution, 5:70. https://doi: 10.3389/fevo. 2017.00070.

Haghighati, A. (2010). Study on the effects of nitrogen and phosphorus fertilizer on the yield and oil content of safflower lines in drylands. Research Journal of Agronomy, 4(3): 57-62.

Hajihashemi, S., Noedoost, F., Geuns, J. M. C., Djalovic, I., & Siddique, K. H. M. (2018). Effect of cold stress on photosynthetic traits, carbohydrates, morphology, and anatomy in nine cultivars of *Stevia rebaudiana*. Frontiers in Plant Science, 9, 1430. https://doi: 10.3389/fpls.2018.01430.

Haliloglu, H., & Beyyavas, V. (2019). The effects of nitrogen and zinc applications on the yield, yield components and oil ratio of safflower (*Carthamus tinctorius* L.) under semi-arid conditions. *Applied Ecology and Environmental Research*, 17(4): 7591-7604. DOI: http://dx.doi.org/10.15666/aeer/1704_75917604.

Hassan, F. U., Khurshid, M. Y., Ahmed, M., Akmal, M., & Afzal, O. (2015). Growth and development of safflower (*Carthamus tinctorius* L.) under rainfed conditions. *International Journal of Agriculture and Biology*, 17, 105-110.

Havlin, J. L., Beaton, J. D., Tisdale, S. L., & Nelson, W. L. (2005). *Soil fertility and fertilizers:*

*An introduction to nutrient managemen*t. 7th Edition, Pearson Prentice Hall, Upper Saddle River, 515p.

Hasanuzzaman, M., Fujita, M., Oku, H., Nahar, K., & Hawrylak-Nowak, B. (2018). Plant nutrients and abiotic stress tolerance. *Plant Nutrients and Abiotic Stress Tolerance*, *July*, 1–590. https://doi.org/10.1007/978-981-10-9044-8.

He, M., Dijkstra, F. A., Zhang, K., Li, X., Tan, H., Gao, Y., & Li, G. (2014). Leaf nitrogen and phosphorus of temperate desert plants in response to climate and soil nutrient availability. Scientific Reports, 4(1), 1-7.

Heidari, M., & Mohammad, M. M. (2012). Effect of rate and time of nitrogen application on fruit yield and accumulation of nutrient elements in *Momordica charantia*. *Journal of Saudi Society of Agriculture Sciences*, 11(2), 129-133.

Henke, J., Breustedt, G., Sieling, K., & Kage, H. (2007). Impact of uncertainty on the optimum nitrogen fertilization rate and agronomic, ecological, and economic factors in an oilseed rape-based crop rotation. The Journal of Agricultural Science, 5, 455–468.

Hocking, P. J., & Meyer, C.P. (1991). Effects of enrichment and nitrogen stress on growth and partitioning of dry matter and nitrogen in wheat and maize. *Australian Journal of Plant Physiology*, 18, 330-356.

Hu, Y., & Schmidhalter, U. (2005). Drought and salinity: a comparison of their effects on mineral nutrition of plants. *Journal of Plant Nutrition and Soil Science*, *168*(4), 541-549.

Jin, J., Wang, G., Liu, X., Pan, X., & Herbert, S. J. (2005). Phosphorus application affects the soybean root response to water deficit at the initial flowering and full pod stages. Soil Science & Plant Nutrition, 51(7), 953-960.

Johnston, A. E. (2000). Soil and Plant Phosphate. International Fertilizer Industry Association.

Katar, D., Arslan, Y., & Subasi, I. (2012): Effect of different doses of nitrogen on the yield and yield components of safflower (Carthamus tinctorius L.) under Ankara ecological conditions. – Suleyman Demirel University Journal of the Faculty of Agriculture 7(2): 56-64.

Kedikanetswe, B. (2012). Effect of plant population on growth, development and oil yield of safflower (Carthamus tinctorius L.). Master of Science Thesis, Faculty of Agriculture, University of Botswana, 70p.

Khalili, M., Pour-Aboughadareh, Alireza., Mohammad, N., & Esmail, A. (2014). Evaluation of Drought Tolerance in Safflower Genotypes Based on Drought Tolerance Indices. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 42. 214-218. https//:doi.org/10.15835/nbha4219331.

Khan, A., Lu, G., Ayaz, M., Zhang, H., Wang, R., & Lv, F. (2018). Phosphorus efficiency, soil phosphorus dynamics and critical phosphorus level under long-term fertilization for single and double cropping systems. Agric. Ecosyst. Environ. 256, 1–11. https://doi: 10.1016/j.agee.2018.01.006.

Knowles, P.F. (1976). Evolution of crop plants Longman Group Ltd.

Koç, H. (2021). Selection criteria for yield in safflower (*Carthamus tinctorius* L.) genotypes under rainfed conditions. *Grasas Aceites*, 72(3). https://doi.org/10.3989/gya.0449201.

Kolte, S. J. (1985). *Diseases of Annual Edible Oilseed Crops, III*. Sunflower, safflower and niger seed diseases, CRC Press, Boca Raton, Florida, USA.

Kong, Q., Yang, Y., Guo, L., Yuan, L., & Ma, W. (2020). Molecular basis of plant oil biosynthesis: Insights gained from studying the WRINKLED1 transcription factor. *Frontiers in Plant Science*, 11, 24. Doi. 10.3389/fpls.2020.00024.

Koutroubas, S. D., Papakosta, D. K., & Doisinis, A. (2004). Cultivar and seasonal effects on the contribution of pre-anthesis assimilates to safflower yield. *Field Crop Research*, 90, 263-274.

Koutroubas, S. D., & Papakosta, D. K. (2010). Seed filling patterns of safflower: Genotypic and seasonal variations and association with other agronomic traits. *Industrial Crops Production*, 31, 71–76.

Kubsad, V. S., Mallapur, C. P. & Mansur, C. P. (2001). Contribution of production factors on yield of safflower under rainfed conditions. Vth International Safflower Conference, Williston, North Dakota Sidney, Montana, USA, pp. 231-225.

Kumar, S., Ambreen, H., & Murali, T. V. (2015). Assessment of genetic diversity and population structure in a global reference collection of 531 Accessions of Carthamus tinctorius L. (safflower) using AFLP markers. *Plant Molecular Biology Reporter*, 33, 1299-1313.

Lawlor, D. W. (2002). Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. Journal of experimental Botany, 53(370), 773-787.

Leghari, S. J., Laghari, G. M., Laghari, A. H., & Ahmed, T. (2016). Role of Nitrogen for Plant Growth and Development: A review in Environmental Biology Role of Nitrogen for Plant Growth rowth and Development: A Review. September.

Li, H., Li, M., Luo, J., Cao, X., Qu, L., Gai, Y., ... & Luo, Z. B. (2012). N-fertilization has different effects on the growth, carbon and nitrogen physiology, and wood properties of slow-and fast-growing Populus species. Journal of experimental botany, 63(17), 6173-6185.

Li, X., Wang, R., Kang, Y., Liu, J., Zuo, Y., Zeng, X., & Cheng, G. (2016). Effects of safflower yellow on the treatment of severe sepsis and septic shock: A randomized controlled clinical trial. *Evidence-Based Complementary and Alternative Medicine, 2016,* Article ID 3948795, 8p. https://dx.doi.org/10.1155/2016/3948795.

Liao, H., Li, Y., Zhai, X., Zheng, B., Banbury, L., Zhao, X., & Li, R. (2019). Comparison of inhibitory effects of safflower decoction and safflower injection on protein and mRNA expressions of iNOS and IL-1β in LPS-activated RAW264.7 cells. *Journal of Immunology Research*, 2019, Article ID 1018274, 11p. https://doi.org/10.1155/2019/1018274.

Lim, C. S., Kang, S. M., Cho, J. L., & Gross, K. C. (2009). Antioxidizing enzyme activities in chilling sensitive and chilling tolerant pepper fruit as affected by stage of ripeness and storage temperature. *Journal of the American Society for Horticultural Science*, 134, 156-163.

Malek, H. A., & Ferri, F. (2014). Effects of nitrogen and phosphorus fertilizer on safflower yield in dry land conditions. International Journal of Research in Agricultural Sciences, 1(1): 28 – 33.

Malhotra, H., Sharma, S., & Pandey, R. (2018). Phosphorus nutrition: plant growth in response to deficiency and excess. In *Plant nutrients and abiotic stress tolerance* (pp. 171-190). Springer, Singapore.

Malikongwa, T. (2015). *Effects of nitrogen and phosphorus on growth, yield, yield components and fruit quality of watermelon* (*Citrullus lanatus* thumb). Master of Science Thesis, Department of Crop Production, Faculty of Agriculture, Botswana College of Agriculture, University of Botswana.

Marang, M., Emongor, V. E., & Malambane, G. (2022). A review of drought tolerance in safflower. *International Journal of Plant & Soil Science*, 34(10), 140-149. https://doi: 10.9734/IJPSS/2022/v34/1030930.

Marschner, H. (2005). Mineral Nutrition of Higher Plants, 2nd Edition, Academic Press, London, Great Britain, pp. 231-277.

Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L., & Suzuki, A. (2010). Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. Ann. Bot. 105:1141-57.

Massignam, A. M., Chapman, S. C., Hammer, G. L., & Fukai, S. (2009). Physiological determinants of maize and sunflower grain yield as affected by nitrogen supply. Field crops research, 113(3), 256-267.

Mazhani, C.L. (2017). *Effects of nitrogen and phosphorus on growth, development, yield, and oil content of safflower (Carthamus tinctorius L.).* Master of Science Thesis, Botswana University of Agriculture and Natural Resources, Faculty of Agriculture, Botswana.

McCalley, C. K., & Sparks, J. P. (2009). Abiotic gas formation drives nitrogen loss from a desert ecosystem. Science, 326(5954), 837-840.

McKenzie, N. J., Jacquier, D. J., Isbell, R. F., & Brown, K. L. (2004). *Australian Soils and Landscapes: An Illustrated Compendium*. CSIRO Publishing: Collingwood, Victoria.

McPherson, M.A., Yang, R.C., Good, A.G., Nielson, R.L., & Hall, L.M. (2009). Potential for seedmediated gene flow in agroecosystems from transgenic safflower (*Carthamus tinctorius* L.) intended for plant molecular farming. *Transgenic Research*, 18, 281-299.

Megda, M.M., & Monteiro, F.A. (2010). Nitrogen and potassium supply and the morphogenic and productive characteristics of marandu palisadegrass. R Bras Zootec. 39:1666-1675.

Mengel, K., & Kirkby, E. A. (2001). *Principles of Plant Nutrition*. Fifth Edition, Kluwer Academic Publishers.

Mehrabi, F., & Sepaskhah, A. R. (2018). Interaction efects of planting mpethod, irrigation regimes, and nitrogen application rates on yield, water and nitrogen use efciencies of winter wheat (*Triticum aestivum* L.). *International Journal of Plant Production*. https://doi.org/10.1007/s42106-018-0025-z.

Ministry of Agriculture. (2010). Annual Report, Agriculture Production Statistics, Botswana Government Printer, Gaborone, pp. 1-5.

Mirzakhani, M., Ardakani, M. R., Aeene, B. A., Shirani Rad, A. H., & Rejali, F. (2009). Effects of dual inoculation of azotobacter and mycorrhiza with nitrogen and phosphorus fertilizer rates on grain yield and some of the characteristics of spring safflower. *International Journal of Civil and Environmental Engineering*, 1,39-43.

Moatshe, O. G., Emongor, V. E., Balole, T. V., & Tshwenyane, S. O. (2016). Yield and yield components of safflower as influenced by genotype and plant density grown in the semi-arid conditions of Botswana. Scientific Journal of Crop Science, 5(9): 125- 136.

Moatshe, O.G., (2018b). Genotype, Planting Density and Seasonal Effects on Phenological Stages of Safflower (*Carthumus tinctorious* L) in Sebele, Botswana 8, 10.

Moatshe, O. G. (2019). *Genotype and plant density effects on growth, yield, and oil composition of safflower (Carthamus tinctorius L.)*. PhD Thesis, Botswana University of Agriculture and Natural Resources, Crop and Soil Sciences Department.

Moatshe, O. G., Emongor, V. E., Balole, T. V., & Tshwenyane, S. O. (2020). Genotype and plant density effects on oil content and fatty acid composition of safflower. *African Crop Science Journal*, 28, 83-101. https://dx.doi.org/10.4314/acsj.v28il.7S.

Mohamed, S. J., Jellings, A. J., & Fuller, M. P. (2012). Effect of Nitrogen on Safflower Physiology and Productivity. *African Crop Science Journal*, 20(4), 225–237. https://doi.org/10.4314/acsj.v20i4.

Mokhele, B., Zhan, X., Yang, G., & Zhang, X. (2012). Review: Nitrogen assimilation in crop plants and its affecting factors. *Canadian Journal of Plant Science*. https://doi.org/10.4141/cjps2011-135.

Moll, R.H., Kamprath, E.J., & Jackson, W.A. (1982). Analysis and interpretation of factors which contribute to efficiency to nitrogen utilization. Agron. J. 74:562-564.

Moore, G., Dolling, P., Porter, B., & Leonard, L. (1998). Soil Acidity. In G. Moore (Ed.), *Soilguide, a handbook for understanding and managing agricultural soils*. Agriculture Western Australia Bulletin No. 4343.

Morgan, J. B., & Connolly, E. L. (2013) Plant-Soil Interactions: Nutrient Uptake. Nature Education Knowledge 4(8):2.

Mündel, H.H., Morrison, R.J., Blackshaw, R.E., & Roth, B. (eds). (1992). Safflower Production on the Canadian Prairies. Agric. Canada Res. Station, Lethbridge/Alberta Safflower Growers Association with funding by Farming for the Future Project No. 87-0016, Alberta Agric. Research Institute. 35 p

Mündel, H. H., Blackshaw, R. E., Byers, J. R., Huang, H. C., Johnson, D. L., Keon, R., Kubik, J., McKenzie, R., Otto, B., Roth, B., & Stanford, K. (2004). *Safflower Production on the Canadian Prairies*. Agriculture and Agri-Food Canada, Lethbridge Research Centre, Lethbridge, Alberta, Canada.

Mündel, H. H., & Bergman, J. W. (2010). Safflower. Oil Crops, 423-447.

Murthy, I. Y. L. N., & Anjani, K. (2008). *Fatty acid composition in Carthamus species*. Proceedings of the 7th International Safflower Conference, Wagga Wagga, NSW, Australia.

Nathan, R. J. K., Madhavi, L. A., Joseph., B., & Madhavi, A. (2017). Influence of nitrogen and sulphur on growth yield and economics of spineless safflower under irrigated conditions. *International Journal of Current Microbiology and Applied Sciences*, 6(7), 600-606.

Nathan, R. J. K., Madhavi, L. A., Joseph, B., & Madhavi, A. (2018). Influence of nitrogen and sulphur on growth yield and economics of spinless of safflower under irrigated conditions. International Journal of Pure and Applied Bioscience, 6(3):77-81.

NationMaster. (2021). https://www.nationmaster.com/nmx/ranking/safflower-oil-production

Omidi-Tabrizi, A. H. (2000). Correlation between traits and path analysis for grain and oil yield in spring safflower. Sesame and safflower Newsletter, 15:78-83.

Omidi, A. H., & Sharifmogadas, M. R. (2010). Evaluation of Iranian safflower cultivars reaction to different sowing dates and plant densities. *World Applcation Science Journal*, 8(8), 953-958.

Omidi, A. H., Khazaei, H., Monneveux, P., & Stoddard, F. (2012). Effect of cultivar and water regime on yield and yield components in safflower (*Carthamus tinctorius* L.). *Turkish Journal of Field Crops*, 17(1), 10-15.

Oarabile, P. (2017). *Effects of safflower (Carthamus tinctorius L.) genotypes on growth,* *development, yield and yield components, and oil content and yield*. Master of Science Thesis, Botswana University of Agriculture and Natural Resources, Faculty of Agriculture, Botswana.

Öz, U. (2017). The effects of different cultivation and ecological conditions on yield and quality of carthamus tinctorius L. (safflower) [Paper presentation]. I. International Congress on Medicinal and Aromatic Plants: " Natural and Life Science", Konya. https://www.researchgate.net/publication/327221093.

Pachauri, R.K., & Meyer, L.A. (2014). Climate Change: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, IPCC, Geneva, Switzerland.

Patterson, B.D., & Reid, M.S. (1990). Genetic and environmental influences on the expression of chilling injury. In C.Y. Wang (Ed.), Chilling Injury of Horticultural Crops (pp.87–112). CRC Press, Boca Raton, FL.

Perakis, S. S., & Hedin, L. O. (2002). Nitrogen loss from unpolluted South American forests mainly via dissolved organic compounds. Nature 415, 416–419.

Place, F., Meybeck, A., Collette, L., de Young, C., Gitz, V., Ehsan Dulloo, E., Hall, S., Muller, E., Nasi, R., Noble, A., Spielman, D., Steduto, P., & Wiebe, K. (2013). Food security and sustainable resource use – what are the resource challenges to food security ? *A Report for the Food Security Futres Conference, April*, 1–5.

Pule-Meulenberg, F., & Batisani, N.J. (2003). A review of Botswana soil fertility research: current status and prospects. UNISWA Journal of Agriculture, 12: 60-67.

Ranjan, R., & Yadav, R. (2019). Targeting nitrogen use efficiency for sustained production of cereal crops. J Plant Nutr. 42(9):1086–1113.https://doi:10.1080/01904167.2019.1589497.

Ranjan, R., Yadav, R., Kumar, A., & Mandal, S. N. (2019). Contributing traits for nitrogen use efficiency in selected wheat genotypes and corollary between screening methodologies, Acta Agriculturae Scandinavica, Section B — Soil & Plant Science, 69:7, 588-595, https://doi: 10.1080/09064710.2019.1623302.

Rastigou, B., Ebadi, A., Vafaie, A., & Moghadam, S. H. (2013). The effects of nitrogen fertilizer on nutrient uptake, physiological traits and yield components of safflower (*Carthamus tinctorius* L.). *International Journal of Agronomy and Plant Products*, 4(3), 355-364.

Raven, P. H., Evert, R. F., & Elchorn, S. (1999). *Biology of Plants*. Worth Publishers, New York, United States of America.

Razaq, M., Zhang, P., Shen, H. L., & Salahuddin. (2017). Influence of nitrogen and phosphorous on the growth and root morphology of Acer mono. *PLoS ONE*, *12*(2). https://doi.org/10.1371/journal.pone.0171321.

Roberts, T. L., & Johnston, A. E. (2015). Phosphorus use efficiency and management in agriculture. *Resources, Conservation and Recycling*, *105*(February), 275–281. https://doi.org/10.1016/j.resconrec.2015.09.013.

Sabbagh, V., Mahalleh, J. K., Roshdi, M., & Hosseini, N. (2012). Effect of nitrogen consuming and deficit irrigation on yield and some characteristic of safflower in relay cropping (northwest of Iran). *Advances in Environmental Biology*, 2674+. https://link.gale.com/apps/doc/A336175988/AONE?u=anon~b2a77c3f&sid=googleScholar&xid=403f94b0

Salisbury, F.B., & Ross, C.W. (1992) Plant Physiology 4th Edition Wadsworth Publishing

Company, Belmont California.

Sampaioa, M. C., Santos, F. R., Bassegio, D., Soares de Vasconselos, E., Silva, M. A., Seccoa, D., & Benetoli da Silva, T. R. (2016). Fertilizer improves seed and oil yield of safflower under tropical conditions. Industrial Crops and Products, 94, 589-595.

Sanchez, C. A. (2007). Phoshorus. In A.V. Barker &D. J. Pilbeam (Eds.), *Handbook of Plant Nutrition* (chapter 3). CRC Press, Taylor and Francis Group, New York.

Sandhya, R. B., Muniratnam, P., Gayathri, N. K., & Padmalatha, Y. (2014). Response of safflower (Carthamus tinctorius L.) varieties to different nitrogen levels in calcareous vertisols. The Journal of Research Acharya NG, Ranga Agricultural University, 42(2), 74-76.

Santos, R. F., Bassegio, D., Sartori, M. M. P., Zannoto, M. D., & Silva, M. (2018). Safflower (*Carthamus tinctorius* L.) yield as affected by nitrogen fertilization and different water regimes. *Acta Agronomica*, 67(2): 264-269.

Sary, G. A., El-Deepah, H. R. A., El-Aaraby, F. I., & Khaled, M. A. I. (1987). Effect of plant density and fertilization of nitrogen and phosphorus on growth characters of safflower. *Annals of Agricultural Science, Moshtohor*, 25(1), 28- 30.

Scheiner, J. D., Gutierrez-Boem, F. H., & Lavado, R. S. (2002). Sunflower nitrogen requirement and 15N fertilizer recovery inWestern Pampas, Argentina. *European Journal of Agronomy* 17: 73‑79.

Schlenker, W., & Roberts, M. J. (2009). *Nonlinear temperature effects indicate severe damages to U.S. crop yields under climate change*. http://www.pnas.org/content/pnas/106/37/15594.

Seadh, S.E., Attia, A.N.E., Badawi, M.A., & El-Hety, S.S. (2012). Response of Seed Yield M and its Components of Safflower to Sowing Dates, Nitrogen Fertilizer Levels and Times of Foliar Application with Milagrow. Journal of Biological Sciences 12(6): 342-348.

Seleka, T. B. (2005). Challenges for Agricultural Diversification in Botswana Under the Proposed SADC-EU Economic Partnership Agreement (EPA). Botswana Institute for Development Policy Analysis (BIDPA).

Sepaskhah, A. R., & Hosseini, S. N. (2008). Efects of alternate furrow irrigation and nitrogen application rates on yield and water-and nitrogen-use efficiency of winter wheat (*Triticum aestivum* L.). *Plant Production Science*, 11(2), 250–259.

Shahrokhnia, M. H., & Sepaskhah, A. R. (2016). Efects of irrigation strategies, planting methods and nitrogen fertilization on yield, water, and nitrogen efficiencies of safflower. *Agricultural Water Management*, 172, 18–30.

Siddiqui, M.H., & Oad, F. C. (2006). Nitrogen requirement of Safflower (*Carthamus tinctorius* L.) for growth and yield traits. *Asian Journal of Plant Sciences*, 5 (3), 563- 565.

Silveira, J. A. G., Melo, A. R. B., Viegas, R. A., & Oliveira, J. T. (2001). A.Salinity-induced effects on nitrogen assimilation related to growth in cowpea plants. *Environment Experimental Botany*, 146, 171-179.

Singh, V., & Nimbkar, N. (2006). Safflower (*Carthamus tinctorius* L.). In R. J. Singh (Ed.), *Genetic resources, chromosome engineering, and crop improvement* (4, 167-194). CRC, New York, USA.

Singh, R. K., & Singh, A. K. (2013). Effect of nitrogen, phosphorus and sulphur fertilization on productivity, nutrient-use efficiency, and economics of safflower (*Carthamus tinctorius* L.) under late-sown condition. *Indian Journal of Agronomy*, 58(4), 583-587.

Sirel, Z., & Aytac, Z. (2016). Relationships between seed yield and some agronomic characteristics of safflower (*Carthamus tinctorius* L.) under semi-arid conditions. *Turkish Journal of Field Crops*, 21(1), 29-35.

Sofy, S. O., Hama, S. J., & Hamma-Umin, B. O. (2020). Influence of phosphorus fertilizer on yield and oil of safflower (*Carthamus tinctorius* L.) varieties under rain fed condition. Applied Ecology and Environmental Research, 18(2), 3409-3418.

Soleimani, R. (2010). Variability of grain and oil yield in spring safflower as affected by nitrogen application. *Journal of Plant Nutrition*, 33, 1744-1750.

Soleymanifard, A., Mojaddam, M., Lack, S., & Alavifazel, M. (2022). Effect of *Azotobacter Chroococcum* and Nitrogen Fertilization on Some Morphophysiological Traits, Grain Yield, and Nitrogen Use Efficiency of Safflower Genotypes in Rainfed Conditions, Communications in Soil Science and Plant Analysis, 53:6, 773-792.

Sreekanth, N., Singh, V., Tiwari, D., & George, S. G. (2021). Effect of nitrogen and phosphorus levels on growth and yield of safflower (*Carthamus tinctorius* L.*). The Pharma Innovation Journal*, 10(10), 2271-2273.

Steer, B.T., & Harriagan, E. K. S. (1986). Rates of nitrogen supply during different development stages affect yield components of safflower (*Carthamus tinctorius* L.). Field Crops Research, 14 (3), 221-231.

Strasil, Z., & Vorlicek, Z. (2002). The effect of nitrogen fertilization, sowing rates and site on yields and yield components of selected varieties of safflower (*Carthamus tinctorius* L.). *Rostlinna Vyroba*, 48 (7), 307-311

Syers, J. K., Johnston, A. E., & Curtin, D. (2008). Efficiency of soil and fertilizer phosphorus use. *FAO Fertilizer and plant nutrition bulletin*, *18*(108).

Tahmasebpou, B., Sofalian, O., Dehghanian, H., & Hoseini, S. S. (2016). Evaluation of drought stress effect in summer safflower genotypes. *Biological Forum-An International Journal*, 8(1), 61-66.

Taiz, L., & Zeiger, E. (2002). *Plant physiology*. 3rd Edition, Sinauer Associates Inc. Sunderland, United States of America.

Tao, H., Morris, T. F., Kyveryga, P., & McGuire, J. (2018). Factors affecting nitrogen availability and variability in cornfields. *Agronomy Journal*, 110(5), 1974-1986.Taiz, L. & Zeiger, E. (2013). Fisiologia vegetal. 5. ed. Porto Alegre, RS: Artmed. 918 p.

Taleshi, K., Shokoh-far, A., Raflee, M., Noormahamadi, G., & Sakinejhad, T. (2012). Safflower yield respond to chemical and biotic fertilizer on water stress condition. World Applied Sciences Journal, 20(11): 1472-1477.

Teixeira Filho, M. C. M., Buzetti, S., Andreotti, M., Arf, O., & Sá, M. E. D. (2011). Application times, sources and doses of nitrogen on wheat cultivars under no till in the Cerrado region. Ciência Rural, 41, 1375-1382.

Terence, C. H. (2009). *Fertilizers and fertility: Phosphorus fertility*. Regents of University of Minesota, USA.

Tucker, M. R. (1999). *Essential plant nutrients: their presence in North Carolina soils and role in plant nutrition*. Agronomic Division, NCDA&CS, USA. http://www.ncagr.gov/agronomi/pdffiles/essnutr.pdf.

Tuncturk, M., & Yildirim, B. (2004). Effects of different forms and doses of nitrogen fertilizers on safflower. Pakistan Journal of Biological Sciences, 7 (8): 1385 ‐ 1389.

Vance, C.P., Uhde-Stone, C., & Allan, D.L. (2003). "Phosphorus acquisition and use: critical adaptations by plants securing a non-renewable resource". New Phytologist, 157, 423-457.

Velasco, L., Perez-Vich, B., & Fernandez-Martinez, J.M. (2005). Identification and genetic characterization of a safflower mutant with modified tocopherol profile. Plant Breeding 124:459-473.

Vishwanath, H., Pujari, B. T., Prakash, S. S., Ramesh, B., & Deshmanya, J. B. (2006). Growth attributes, dry matter production and its partitioning and nutrient uptake studies in spinless Safflower (*Carthamus tinctorius* L.) variety, NARI-6 as influenced by nitrogen and sulphur levels. *Karnataka Journal of Agriculture Science*, 19(4), 913-917.

Wachsmann, N., Jochinke, D., Potter, T., & Norton, R. (2010). Growing safflower in Australia: Part 2 - Agronomic research and suggestions to increase yields and production. 7th International Safflower Conference, Australia.

http://www.australianoilseeds.com/__data/assets/pdf_file/0003/6744/Final_Wachsman paper.pdf.

Walkley, A. (1947). A Critical Examination of a Rapid Method for Determining Organic Carbon in Soils: Effect of Variations in Digestion Conditions and of Inorganic Soil Constituents. Soil Science, 63, 251-264.

Wang, G., & Li, Y. (1985). Clinical application of safflower (*Carthamus tinctorius* L.). *Zhejiang Tradational Chinese Medical Science Journal*, 20, 42-43.

Waraich, E. A., Ahmad, Z., Ahmad, R., Saifullah, & Ashraf, M. Y. (2015). Foliar applied phosphorous enhanced growth, chlorophyll contents, gas exchange attributes and PUE in wheat (Triticum aestivum L.). Journal of Plant Nutrition, 38(12), 1929-1943.

Wassan, S., Xi, C., Jhanjhi, N. Z., & Binte-Imran, L. (2021). Effect of frost on plants, leaves, and forecast of frost events using convolutional neural networks. *International Journal of Distributed Sensor Networks*, *17*(10), 15501477211053777.

Weingberg, Z. G., Bar-Tal, A. Chen, Y., Gamburg, M., Brener, S., Dvash, L., Markovitz, T., & Landau, S. (2007). The effect of irrigation and nitrogen fertilization on the ensiling of safflower (*Carthamus tinctorius* L.). *Animal Feed Science Technology*, 134, 152-161.

Weiss, E. A. (2000). Oilseed Crops. Second Edition, Blackwell Science, Oxford, Chapter 4: 93-129.

Xu, B. C., Niu, F. R., Duan, D. P., Xu, W. Z., & Huang, J. (2012). Root morphological characteristics of Lespedeza davurica (L.) intercropped with Bothriochloa ischaemum (L.) Keng under water stress and P application conditions. Pakistan Journal of Botany, 44(6), 1857-1864.

Yau, S.K., & Ryan, J. (2010). Response of rainfed safflower to nitrogen fertilization under Mediterranean conditions. Industrial Crops and Products, 32, 318-323.

Zhang, Y., Liu, L., Chen, X., & Li., J. (2022). Effects of low-temperature stress during the anther differentiation period on winter wheat photosynthetic performance and spike-setting characteristics. *Plants,* 2022, 11, 389. https://doi.org/10.3390/plants11030389.

Zhao, L., Liu, B., Piao, S., Wang, S., Lobell, D.B., Huang, Y., Huang. M, Yao, Y., Bassu, S., Ciais, P., Durand, J., Elliott, J., Ewert, F., Janssens, I. A., Li, T., Lin, E., Liu, Q., Martre P., Müller, C., Peng, …Asseng, S. (2017). *Temperature increase reduces global yields of major crops in four independent estimates*.

http://www.pnas.org/cgi/doi/10.1073/pnas.1701762114.

APPENDIX

Appendix 1: Rainfall data (mm) for the first trial (2020-2021)

Day	NOV	DEC	JAN	FEB
1	18	3.5	19.9	
2				35
3				26
4				15
5		12		20
6				15
7				
8				
9				
10				
11				
12				
13				
14				
15				

16				
17			2.7	
18				
19				
20				
21				
22		20		5
23				13
24				
25				
26			13	
27			17	27
28				
29				
30				
31				
Total	18	35.5	52.6	156

Appendix 2: Rainfall data (mm) for the second trial (2021)

Day	Apr	Sep	Oct	Nov
1				
2				
3				
4				
5				
6	4.4		13.9	
7			15.8	
8				
9				
10				
11				
12				
13				
14				
15				
16				

17				
18				
19				
20				19
21				
22				
23				
24				
25				
26				
27				
28				
29				
30		4		
Total	4.4	4	29.7	19

Appendix 3. Temperature data (°C) for the second trial (winter 2021)

Month	April		May		June		July		August		September		October		November	
Day	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min
1	34.1	14.6	23.8	8.5	20.6	5.1	26	6.5	23.7	1	31.6	5.2	24.1	17	37.1	17.1
2	32.2	13.8	21.5	14.3	21.4	1	25.7	2.8	26.8	2.3	29.4	13.9	28.7	13.6	34.2	17.2
3	34.1	14.9	22.8	8.9	21.3	5.3	26.9	1.9	23.7	7.1	32.9	13.2	29.3	11.1	36.1	
4	30.2	15.6	24.1	7.8	19.7	6.3	27.9	2	24.7	7.3	31	13.1	28.5	8.4	37.1	18.1
5	32.5	13.1	26.1	7.3	18.5	1.8	25.9	3	25.3	3.5	30.3	18.5	27.2	15.3	37.8	22
6	30.1	15.5	26.7	8.5	19	3.3	18.3	8.1	28	4.7	30.5	17.5	31.7	16.8	37.5	21.4
7	25.9	16.6	26.5	12.3	19.1	6.3	21.6	5.1	28.8	5.1	29	12.3	28.1	17.9	36.6	21.3
8	28.9		26	12.7	19.1	5.8	22.3	3.9	28	11	20.6	16.9	//	//	25.3	20.7
9	31.6	13.2	24.7	6	19.3	9.5	24	4.3	29.4	8.7	26.6	10.9	33.8	17.7	32	14.5
10	31.7	13.3	25.5	6.3	22	7.1	24.1	2.3	25.2	7.7	26	13.6	32.3	13.2	31.5	15.7
11	31.6	14.4	26.7	5.4	23.3	4	23.2	1.8	24.5	12.1	27.1	10	23.4	15		18.5
12	23.3	15.1	28	6.4	25.1	3.2	25.7	4.1	28.7	7.7	27.4	8.9	29.3	10.7		
13	30	14.3	27.1	4.9	26.2	4.6	16.7	7.2	27.7	6.3	29.9	5	33.4	11.2	38.8	
14	32.2	10.6	27.8	4.3	25.3	3.1	16.2	-4.2	17.9	11.9	33.1	10.8	36.3	12.9	38	20.4
15	32.6	15.6	29.1	6.1	24.9	2.4	18.2	1.2	20.2	12.3	35.3	13.5	37.2	15.3	37.5	20.7
16	33.2	15.8	26.2	6.1	24.5	3.7	18.5	3.5	23.8	7.7	34.4	11.1	36.3	17.6	39.1	20.2
17	22	16.6	25.5	6.8	26.1	3	18	1	26	9.8	28.4	11.1	30	15.8		21
18	25	9	25.4	3.4	25.9	3.3	23.1	2	30.1	8.7	22.2	14.7	28.1	14.5	36.7	20.2
19	21.7	7.7	27.5	9.2	20.7	12.5	18.9	3.4	27.5	4.6	27.5	10.9	21.2	14.2		17.5
20	25.6	9.7	25.4	6.3	20.3	9	19.7	-0.7	29.9	5.1	34	15.2	26.1	11.7		
21	25	8.4	27.5	6.3	20.4	4.4	22.9	-0.1	28.4	12.8	31.5	15.1	28.2	14.2		
22	26.4	7.8	25.5	6.3	19.9	2.7	17.4	-3	30.4	15	24	15.3	30.2	16.3		
23	27.9	9	23.8	9.1	23	4.2	16.5	-1.8	26.1	14.9	27.4	9.4	35.2	15.2		
24	29.9	8.5	18.1	2.4	24.5	3.4	17.8	0	27.2	13.6	30.8	12.1	31.6	15.8		

25	31	8.7	23.1	5.7	26.2	3.1	17.6	1.6	27.1	14	33.9	13.2	28.5	16.3		
26	30.2	6	23.3	3.6	26.9	3.1	18.1	-0.8	26.8	12.8	34.6	14.3	29.2	17.6		
27	31.4	6.8	25	3.8	26.3	3.1	19.8	1.7	32.8	15	32.9	16.4	28.7	18.6		
28	30.6	8.5	26	4	28.7	2.3	20.9	-0.6	18.5	12.4	35.9	15.7	20.3	19.3		
29	29.9	13.2	27.2	4.9	28.8	5.2	24.2	1.5	21.2	-6.3	35.7	18.1	30.8	17.3		
30	22.7	15.6	25	2.3	28.5	7.9	23.7	2.3	24.2	5.5	26.4	20.6	32.5	16.7		