EFFECTS OF NITROGEN AND PHOSPHORUS ON GROWTH, DEVELOPMENT, YIELD, AND OIL CONTENT OF SAFFLOWER (CARTHAMUS TINCTORIUS L.)

MASTER OF SCIENCE (MSC) IN CROP SCIENCE (HORTICULTURE)

BY

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JULY 2017

UNIVERSITY OF BOTSWANA

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EFFECTS OF NITROGEN AND PHOSPHORUS ON GROWTH, DEVELOPMENT, YIELD, AND OIL CONTENT OF SAFFLOWER (*Carthamus tinctorius* L.)

A research Dissertation submitted to the University of Botswana in partial fulfillment of the requirements for the Degree of Master of Science in Crop Science (Horticulture)

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STATEMENT OF ORIGINALITY

I, the undersigned hereby declare that work contained in this dissertation was carried out by the author at the Botswana University of Agriculture and Natural Resources. It is original work except where due reference is made and neither has been nor will be submitted for the award of a Degree by any other University.

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ACKNOWLEDGEMENT

Firstly, I would like to thank the Almighty God, for in Him all this was made possible. Thank you to my supervisor Prof. V. E. Emongor for his endless patience, continuous support and willingness to dedicate valuable time to the success of my research. Thanks to my co-supervisor Prof. F. Pule-Meulenberg for her constructive critique.

I am grateful to the following Heads of Department; Crop Science, Animal Science and the Director of Agricultural Research, for the opportunity to carry out experiments in their laboratories. Thanks also go to Mr Mpofu, Mr Mathaio, George, Thabo, Kethabile and Shima for their invaluable assistance rendered when conducting experiments.

Lastly, but far from least I would like to acknowledge my parents, Dr. L. M Mazhani, Mrs. Chililo Mazhani, Mr. Kelitile Gabana, Mrs. Gladys Kelitile, my wife, Dineo Mazhani, my sister Carol, my children Bokani, Wesley, Imani and my entire extended family. Thank you for your endless support, this is for you.

DEDICATION

This research is dedicated to my family.

ABSTRACT

Safflower is a multipurpose crop grown for the orange-red dye that is obtained from its petals, medicinal properties, feed value and especially for its high quality oil (Dajue and Mündel, 1996; Dwivedi, 2005; Sirel and Aytac, 2016). A field experiment was carried out to evaluate the effects of nitrogen (N) and phosphorus (P) on the growth, development, seed yield and oil content of safflower. The experimental design was a split-plot design in randomized complete blocks with three replications. The treatments were 0, 25, 50, 75, and 100 kg N/ha (main-plots) and P at 0, 25, 50, and 75 kg P/ha (sub-plots). Nitrogen and P fertilizer application interacted significantly ($P < 0.05$) to increase safflower capitula number/plant, seed protein and oil contents, and leaf N and P contents. While N application independently significantly ($P < 0.0001$) increased safflower plant height, 100-seed weight and seed yield. Phosphorus fertilizer application significantly ($P < 0.0001$) increased safflower seed yield. Based on vegetative growth, yield, yield components and oil content of safflower, it was concluded that under sandy loam soils of Botswana, the optimal N and P fertilizer application rate to optimize safflower seed yield and oil content was 75 kg N/ha and 50 kg P/ha. Application of N and P beyond 75 and 50 kg/ha, respectively, resulted in decrease in safflower yield, yield components and oil content. The author recommended that for wider application of these fertilizer rates in Botswana, N and P trials be undertaken in various parts of the country under different soil types, irrigation regimes and rain-fed agriculture to confirm the current findings.

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LIST OF ABBREVIATIONS

ATP- Adenosine triphosphate

ANOVA- Analysis of variance

ADF- Acid detergent fibre

BUAN- Botswana University of Agriculture and Natural Resources

CEC- Cation exchange capacity

Cu- Copper

DNA- Deoxy ribonucleic acid

FAD- Flavin adenine dinucleotide

FAO- Food and Agricultural Organisation

Fe- Iron

GDP- Gross domestic product

GLM- General linear models

Ha- Hectare

HDL- High density lipoprotein

LAI- Leaf area index

LAN- Limestone ammonium nitrate

LDL- Low density lipoprotein

LSD- Least significant difference

MoA- Ministry of Agriculture

Mn- Manganese

N- Nitrogen

NADPH- Nicotinamide adenine dinucleotide phosphate

NDF- Nitrogen detergent fibre

OC- Organic carbon

P- Phosphorus

RNA- Ribonucleic acid

SSP- Single superphosphate

WUE- Water use efficiency

CHAPTER 1

1.0 INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is a member of the family Compositae or Asteraceae (Weiss, 2000), cultivated mainly for its seed, which is used as edible oil, birdseed or for its flowers, used as dye sources, and medicinal purposes (Ekin, 2005; Dordas and Sioulas, 2008; Istanbulluoglu, 2009, Emongor, 2010). Safflower is a temperate zone plant grown in arid and semi-arid regions of the world (McPherson *et al.*, 2004). Safflower is native to the Middle East and Iran is thought to be one of the centres of origin (Ashri, 1975; Weiss, 2000; McPherson *et al.*, 2004; Zareie *et al.*, 2013; Khalili *et al.*, 2014). Compared to other oilseed crops worldwide and its many uses, safflower remained a minor and neglected crop (Ekin, 2005; Emongor, 2010). The research and development on different aspects of safflower, despite its adaptability to varied growing conditions with very high yield potential and diversified uses of different plant parts, have not received due attention. This probably is the main reason for its status as a minor crop around the world in terms of area and production, compared to the other oilseed crops.

Interest in cultivation of safflower has increased because of increased demand for vegetable oil, biodiesel and edible oil (Mailer *et al.*, 2008). There is a huge shortfall in oilseed production in countries having a sizable area with scanty rainfall, to which safflower is most suited, the preference of consumers for healthy oil with less amounts of saturated fats, for which safflower is well known, and the medicinal uses of flowers in China and extraction of edible dyes from flowers have become more widely known (Singh and Nimbkar, 2006; Emongor, 2010). Interest in cultivating safflower as source of edible oil has further been stimulated since the identification of safflower oil as a rich source of polyunsaturated essential fatty acid linoleic acid (70-87%) and monounsaturated fatty acid oleic acid (11-80%) (Murthy and Anjani, 2008; Aghamohammadreza *et al.*, 2013). Linoleic acid has been shown to offer nutritional and therapeutic benefits such as prevention of coronary heart disease, arteriosclerosis, high blood pressure and hyper lipaemia

(Wang and Li. 1985; Cosge *et al.*, 2007). 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). Now safflower is grown commercially in China, India, Ethiopia, Kenya, Argentina, Australia, Mexico, Canada, Spain, Italy, Turkey, Iraq, Iran, Syria, Kazakhstan, Uzbekistan, Israel, Morroco, Pakistan and Russia (Dajue and Mündel, 1996; Emongor, 2010). It is cultivated on 800,000 ha in the world, with a yield of between 650,000-921,000 tons (Rowland; 1993; Gyulai, 1996; Camas *et al.*, 2005; Rajranshi, 2005; Singh and Nimbkar; 2006; FAO, 2011). The largest producer of safflower is India, mainly in the states of Maharashra and Karnataka (Nimbkar, 2002). India grows the crop on 402,000 ha, producing about 206,000 tons of seed annually (Camas *et al.*, 2007; Rajranshi, 2005).

1.1 Uses of safflower

Safflower is a multipurpose crop grown for the orange-red dye that is obtained from its petals, medicinal properties, feed value and especially for its high quality oil (Dajue and Mündel, 1996; Dwivedi, 2005; Sirel and Aytac, 2016). Traditionally, safflower was grown for its seeds, for colouring foods, as medicines and for making red and yellow dyes, especially before cheaper aniline dyes became available (Weiss, 1971; Emongor, 2010).

1.1.1 Food uses

Safflower oil is extracted from safflower seed, and it is often considered a healthier option than olive and canola oils (Bergman, 1997; Corleto *et al.*, 1997) as well as sunflower oil (Dajue and Mündel, 1996) because it has lower percentage of saturated fatty acids. Safflower oil consists of two types of unsaturated fatty acids oleic acid (monounsaturated fatty acids) and linoleic acid (polyunsaturated fatty acids). Oleic acid is a beneficial agent in the prevention of coronary artery disease (Dajue and Mündel, 1996) and linoleic acid has been reported to reduce blood cholesterol levels. Arslan *et al.* (2003) reported that safflower oil with greater amount of linoleic acid contained tocopherols, known to have antioxidant effects and high vitamin E content. For this reason, safflower oil is used in the diets of patients with cardiovascular diseases, and bears great importance for its anti-cholesterol effect (Pongracz *et al.*, 1995; Arslan *et al.*, 2003). Safflower in India for a long time has been grown for the orange-red dye extracted from its brilliant florets, high-quality edible oil rich in polyunsaturated fatty acids, which helps in reducing the cholesterol level in blood (Singh and Nimbkar, 2006). Safflower oil is nutritionally similar to olive oil, as it contains high levels of linoleic or oleic acid. The monounsaturated fatty acid like oleic acid is also known to reduce low-density lipoprotein (LDL; bad cholesterol) without affecting high-density lipoprotein (HDL; good cholesterol) in blood (Smith, 1996). Safflower oil is highly stable, and its consistency remains the same at low temperatures, thereby making it suitable for application in frozen/chilled foods (Weiss, 1971). Due to its heat stability, safflower oil is used as a cooking oil. It is also used in the preparation of mayonnaise, salad oil and margarine (Nimbkar, 2002). Safflower oil is also better suited to hydrogenation for margarine production than are soy or canola oils (Kleingarten, 1993).

Safflower leaves are eaten as vegetables (Weiss, 1983) and they are rich in carotene, riboflavin, vitamins A and C, iron, phosphorus and calcium (Nimbkar and Singh, 2006). Its petals are used for extraction of carthamidin and carthamine which are non-toxic food colourants, which are used in colouring and flavouring foods such as cakes, sweets, biscuits, butter, ice cream, rice, soup, sauces and breads (Zohary and Hopf, 2000; Emongor, 2010). With the advent of cheaper synthetic dyes like aniline, use of safflower flowers as a source of edible colour gradually decreased to zero during the 20th century. However, recently interest in safflower flowers as a source of colour for use in food is gaining importance owing to a recent ban on the use of synthetic colours in food in the European countries and elsewhere (Dajue and Mündel, 1996; Singh and Nimbkar, 2006).

China produces approximately 1800 to 2600 MT of flowers annually to use them for extraction of dyes and in medicinal preparations (Zhaomu and Lijie, 2001). Flowers of non-spiny cultivar NARI-6 and non-spiny hybrid NARI-NH-1 in India, have been reported to be rich in protein (10.40 and 12.86%), total sugars (7.36 and 11.81%), calcium (558 and 708 mg/100 g), iron (55.1 and 42.5 mg/100 g), magnesium (207 and 142 mg/100 g), and potassium (3992 and 3264 mg/100 g), respectively. All essential amino acids except tryptophan are reported to be present in safflower flowers (Singh, 2005). With the commercialization of flowers as herbal health tea, extraction of dyes from them, and their use for medicinal purposes, the monetary returns to farmers from both seed and flowers are expected to grow to the extent of 141% of the monetary returns presently earned from the harvesting of seed alone in India (Sawant *et al.*, 2000). A pleasant-tasting tea made with safflower flowers as its main-ingredient has been developed in China (Li and Yuanzhou, 1993), India (Singh, 2005), and Botswana (Emongor, 2010).

1.1.2 Livestock feed

Not only is safflower good for human consumption, it can be grazed by animals and stored as hay or silage (Bar-Tal *et al.*, 2008). Its forage is palatable with feed value and yields similar or better than oats and alfalfa (Smith, 1996; Wichman, 1996). It makes an acceptable livestock forage if cut at or just after bloom (Berglund *et al.*, 2007). Grazed safflower has been shown to support satisfactory growth rates in Australian steers (French *et al.*, 1988) and improved fertility in Canadian ewes (Stanford *et al.*, 2001). Safflower oil cake is a valuable animal feed (Weiss, 2000). Safflower meal contains about 24% protein and is considerably high in fibre. Values of 21.8% protein, 67.4% nitrogen detergent fibre (NDF), 38.5% acid detergent fibre (ADF), 15-20% acid detergent lignin and 3.3% ash have recently been reported (Chidoh, 2012), which are suitable for livestock feed or supplement. It can be taken as a protein supplement in livestock and poultry feeds (Berglund *et al.*, 2007). Safflower seed is also used as birdseed especially for members of the parrot family and pigeons as well as for other small animals such as gerbils. hamsters and chinchillas (Dajue and Mündel, 1996). The birdseed industry prefers to use the white hull or normal hull type of safflower even though striped and partially hull types usually are higher in oil and protein content. The birdseed market does not have a preference for fatty acid type (Dajue and Mündel, 1996).

1.1.3 Textile industry

Dried flower petals are used to extract natural dyes. Natural dyes from plants are getting more important nowadays because they are natural and fashion trends. The colourful matter in safflower is carthamin which is benzoquinone-based (Garcia, 2009). It has a dye of flavonoid type. Hydrophilic fibres like cotton, wool and others can be dyed with safflower dye because it is a direct dye (Badiger *et al.*, 2009). The water soluble yellow dye (carthamidin) and the insoluble red dye (carthamine) are used in the carpert-weaving industry in Eastern Europe and the Indian subcontinent (Weiss, 1983).

1.1.4 Cut flower industry

In Western Europe, Japan, Latin America and Kenya, spineless varieties of safflower are grown as cut flowers for the domestic and the export market (Kizil *et al.*, 2008; Emongor, 2010). Its importance is increasing due to the higher demand for dried flowers in the last two decades of the twentieth century (Ekin, 2005). Stems are normally harvested when colour is visible on most of the flowers and they are one quarter to one half open, as buds do not open well after harvest and freshly cut flowers usually last up to 10 days (Dole *et al.*, 2005). Ekin (2005) reported that in 2000, 35.2 million flowering stems of safflower were supplied to the Dutch flower auction and the total value of sales that year reached about €5.3 million, so that safflower was ranked 39th amongst all cut flowers in terms of commercial importance.

1.1.5 Medicinal and clinical uses

Safflower is also used for medicinal purposes. In traditional Chinese medicine, safflower petals are regarded as a stimulant for blood circulation, phlegm reduction, healing fractures, contusions and strains (Wang and Li, 1985). Safflower petals are reported to be useful in curing several chronic diseases such as hypertension, coronary heart ailments, rheumatism, male and female fertility problems (Wang and Li, 1985; Zhou, 1986; Yu, 1987; Qin, 1990; Qu, 1990; Zhou, 1992; More *et al.*, 2005; Rajranshi, 2005) and the seed for the treatment of urinary calculi (Wang and Li (1985). According to Zhou (1986), treatment of infertility with safflower resulted in pregnancy in 56 of infertile women out of 77 women who had been infertile for 1.5-10 years. In dermatology, it is reported that safflower has many beneficial effects including clearing of vitiligo (Pu *et al.*, 1992; Tan, 1989). An alcoholic preparation of safflower and other herbs was completely effective against acne (Liu, 1985). Other medicinal uses of safflower either as topical dust or injections, are said to include a cure for ear infections (Pan, 1986; Wang and Li, 1985), and eye drops for reducing myopia, especially in children (Tao, 1990; Wang and Li, 1985). Safflower oil is non-allergenic, and therefore suitable in injectable medications (Smith, 1996).

Another important and interesting use of safflower seed has recently emerged by means of its genetic modification to produce high-value proteins as pharmaceuticals and industrial enzymes. SemBioSys- a Calgary-based (Canada) company-transforms safflower tissue genetically in order to get the proteins of interest to accumulate in the seed of the mature transgenic plant (Mündel *et al.*, 2004). The process of transformation of safflower tissues follows the patented Stratosome™ Biologics system, which facilitates the genetic attachment of target proteins of interest to oleosin, the primary protein coating the oil-containing vesicles (oil bodies) of the seed. Such attachment permits the target protein to be purified along with the oil body fraction, which upon centrifugation floats to the surface of ground seeds/water slurry (van Rooijen *et al.*, 1992). The purification process of the Stratosome system makes it more efficient than the other transgenic systems. The attachment of proteins to the oil bodies of safflower in the Stratosome Biologics system is expected to stabilize intracellular accumulation of foreign proteins, and also provide a useful attachment matrix and deliver benefits for end use applications. The transgenic safflower plants are used to produce human insulin because the global demand for the hormone has greatly grown (Anon., 2006). According to SymBioSys its safflower- produced insulin can reduce capital costs compared to existing insulin manufacturing (Anon., 2006).

1.1.6 Other uses of safflower

High linoleic acid safflower oil has an important use in the paint industry. Safflower oil is preferred for the paint and varnish industry owing to its specific properties of absence of linolenic acid, presence of high linoleic acid, low colour values, non-yellowing, low free fatty acids, low unsaponifiables, and no wax, which make the quality in paints, alkyd resins, and coatings beyond comparison (Smith, 1996; Berglund *et al.*, 2007). The high linoleic safflower oil is used in the manufacture of high quality coloured paints (Dajue and Mündel, 1996). However, with the development of cheaper petroleum products and a shift to water-based paints, the use of safflower oil in the paint and varnish industry has been reduced drastically in recent times (Singh and Nimbkar, 2006). Safflower oil is also now used as a diesel fuel substitute (Oelke *et al.*, 1992; Ogut and Oguz 2006). However, for the latter use, like most vegetable oils, it is currently too expensive. Safflower is also used in cosmetics products such as hair cream, shampoo, face cream, perfume, and body lotion (Shouchun *et al.*, 1993).

Safflower is considered to be ideal for cosmetics and is used in 'Macassar' hair oil and Bombay 'Sweet Oil' (Weiss, 1971). Carthamin is used to create cosmetics for *Geisha* and *Kabuki* artists in Japan, where the colour is called *Beni* (Nishimura *et al.*, 2008).

1.2 Plant Nutrition

Plants need to be supplied adequately with nutrients during their entire growth period. Crop requirements for different nutrients vary with various stages of plant development and have different physiological requirements. The concentration of plant nutrients in the soil must be maintained at satisfactory level for plant growth (Mengel and Kirkby, 2001). As arable land throughout the world is decreasing, increasing yield within the available land is possible through among other things better crop management practices such as optimizing fertilizer application rates in order to increase yield. Plants including safflower require mineral elements supplied by the soil for their growth and development. In natural ecosystems, the minerals absorbed by growing organisms return to the soil after organic matter decomposition, and soil fertility is more or less maintained through nutrient cycling. In cultivated ecosystems, however, all harvested biomass withdrawn from the field contains nutrients that no longer return to the soil. Hence, maintenance of soil fertility and plant yield production depends on the counter balancing fertilizer inputs (Bot *et al.*, 1997). Dahnke *et al.* (1992) reported that the most efficient fertilizer rate will depend on the residual soil nutrient level as determined by a soil test and the yield goal. Yield is influenced by local climate, soil type, management in terms of timeliness of field operations, plant population, variety, fertility and weed control (Barker and Pilbeam, 2007).

1.3 Importance of nitrogen on the growth and development of plants

Nitrogen (N) is one of the mineral nutrients that are required in larger quantities than any other element for the completion of the plant's life cycle (Mengel and Kirkby, 2001; Marschner, 2005). Nitrogen affects the growth, development, yield and quality of crops (Eckert, 2010). Nitrogen is a major component of many essential plant organic compounds such as chlorophyll and coenzymes (Green *et al.*, 1995; Raven *et al.*, 1999; Heidari and Mohammad, 2012), nucleic acids, 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.*, 1995; Mengel and Kirkby, 2001; Marschner, 2005). A good supply of N stimulates root growth and development, the uptake of other nutrients, encourages vegetative growth, giving leaves a deep green colour (Marschner, 2005; Heidari and Mohammad, 2012). Because plants require very large quantities of N, an extensive rooting system is essential to allow unrestricted uptake. Plants with restricted roots by compaction may show signs of deficiency even when adequate amount of the N is in the soil (Marschner, 2005). Plants respond quickly to increased availability of nitrogen (Mengel and Kirkby, 2001; Marschner, 2005).

1.4 Importance of phosphorus on the growth and development of plants

Phosphorus (P) is an essential part of phospholipids which are important in cellular membrane structure (Marschner, 2005; Sanchez, 2007). It is also an essential component of the organic compound often called the energy currency of the living cell: adenosine triphosphate (ATP) (Mengel and Kirkby, 2001; Marschner, 2005; Havlin *et al.*, 2013). Synthesized through respiration and photosynthesis, ATP contains a high energy phosphate group that drives most energy-requiring biochemical processes (Marschner, 2005). For example, the uptake of nutrients and their transport within the plant, as well as their assimilation into different biomolecules, are energy using plant processes that require ATP. Adequate P nutrition enhances many aspects of plant physiology, including the fundamental processes of photosynthesis, nitrogen fixation, flowering, fruiting (including seed production), and maturation (Raven *et al.*, 1999; Marschner, 2005; Adebooye *et al.*, 2010). Phosphorus promotes the growth and development of lateral roots and rootlets (Brady and Weil, 1996; Mengel and Kirkby, 2001; Marschner, 2005; Adebooye *et al.*, 2010). Phosphates form the rails of the nucleic acids, DNA and RNA (Terrence, 2009).

1.5 Justification

In mid 1960's agriculture was the heart of Botswana's economy contributing 40% of the country's gross domestic product (GDP). By 2006/7, this had fallen to 1.6% (MoA, 2010). Much of this may be attributed to the vastly increased importance of the mining industry over the same period. At the moment, diamonds are the most beneficial resources for development in Botswana. The discovery of other resources apart from minerals can impact on diversification of the economy.

Plants are natural resources, which can be used commercially to diversify the economy, but only a few have been explored for their commercial uses. Safflower is one such crop that can be explored with regard to finding viable alternatives to arable agriculture especially for a number of regions in Botswana. Safflower is a multipurpose crop grown for edible oil, as a vegetable and cut flower, flavouring and colouring foods, herbal tea, livestock feed, medicines, making cosmetics, dyes, and paints (Dajue and Mündel, 1996; Ekin, 2005; Emongor, 2010). It has exhibited potential medical, pharmaceutical and cosmetic importance all over the world (Berglund *et al.*, 2007; Emongor, 2010; Mündel and Bergman, 2010; Mohamed *et al.*, 2012). It is a drought tolerant crop (Ozturk *et al.*, 2008; Majidi *et al.*, 2011), able to extract water at soil moisture contents that are unavailable to the majority of crops (Weiss, 2000). It has a strong tap-root that can grow to a depth of 2-3 m under dry climates so it can be able to explore deep soil volumes to recover water (Emongor, 2010). It's salt tolerance is a valuable asset as the area affected by some degree of salinity increases world-wide (Weiss, 2000). It is tolerant to saline conditions, drought and a wide range of temperatures from -7 to 40°C, provided during the elongation and flowering stages of growth and development there is no frost. During the rosette stage, safflower plant can withstand a temperature of -7°C (Mündel *et al.*, 1992; Emongor, 2010). Both biotic and abiotic stresses are the important factors reducing crop production: however, drought stresss is the most important factor limiting crop production in agricultural systems in arid and semi-arid regions (Mollasadeghi *et al.*, 2011). Water stress effects on growth and yield are genotype dependent (Bannayan *et al.*, 2008).

Botswana's location in the sub-tropical high pressure belt of southern hemisphere in the interior of Southern Africa and away from oceanic influence makes it experience low rainfall and high temperatures in summer. There is high inter-annual variability of rainfall and drought is a recurring element of Botswana's climate (Emongor, 2009). Drought adversely affects the already fragile food and agricultural situation in the country and seriously impairs the rural economy and socio-cultural structures. Due to the erratic, unreliable, and poorly distributed rainfall, plus high temperatures, water becomes the most limiting factor to agricultural production in Botswana (Emongor, 2009). In Botswana, the annual precipitation and evapotranspiration ranges between 200-650 mm and 1800-3000 mm, respectively, depending on season (Emongor *et al.*, 2008). Therefore, growing a saline, drought and winter tolerant, and high value crop such as safflower will improve food security, reduce reliance on food imports especially cooking oils and improve income levels of farmers in Botswana, hence the importance of this study.

Plants require mineral elements supplied by the soil for their growth and development. Nitrogen and phosphorus are two essential nutrients which are required for growth, development and increased seed yield and fatty acid composition of safflower. Nitrogen compounds are important chemical compounds such as protein, nucleic acids, chlorophyll and enzymes structure (Herdrich, 2001). Nitrogen has an important role in the tissues structure of plants (Herdrich, 2001). Low phosphorus availability and loss of nitrogen due to leaching in sandy soils are some of the limiting factors to crop production in Botswana (Beynon. 1991; Pardo *et al.*. 2000; Pule-Meulenberg and Batisani, 2003). According to Smil (1999), Socolow (1999) and Graham and Vance (2000), N is a critical limiting element for growth of most plants due to its unavailability. Production of high quality protein rich food is extremely dependent upon availability of sufficient nitrogen.

There is contradictory information in literature about the amount of nitrogen and phosphorus needed for maximum yield production in safflower. Optimum N amounts in the range of 60-120 kg/ha, and 30-100 kg P_2O_5/ha have been reported for safflower (Armendari, 1984; Ahmed *et al.*, 1985; Siddiqui and Oad, 2006; Dordas, 2009; Haghighati, 2010). The importance of N fertilization for safflower in the field (Zaman and das, 1990), and its physiological response (Dordas and Sioulas, 2008) has been evaluated in only a limited manner. These studies showed that increased N levels up to 200 kg/ha, the form of ammonium sulphate, increased leaf N and chlorophyll contents leading to increase in photosynthetic rate and stomatal conductance. The increased photosynthetic rate and stomatal conductance resulted in enhanced water use efficiency and net increase in seed yield. Information on proper mineral nutrition for optimum safflower production is necessary to design a management system which allows maximum expression of genetic potential. Presently, there is no information on the effects of N and P on the growth, development, oil yield and fatty acid composition of safflower in Botswana. Therefore, the objective of this study is to determine the N and P application rates for high safflower yields in Botswana.

1.6 Objective of the Study

The objective of this study is to evaluate the effects of nitrogen and phosphorus on the growth, development, seed and oil content of safflower.

1.7 Hypothesis

H_0: Nitrogen and phosphorus have no effect on the growth, development, seed and oil content of safflower.

H_1: Nitrogen and phosphorus have an effect on the growth, development, seed and oil content of safflower.

CHAPTER 2

2.0 LITERATURE REVIEW

Safflower requires adequate supply of nutrients for its full production potential even under limiting moisture conditions (Rao, 1985; Kubsad *et al.*, 2001). The yield levels obtained in research farms are an indication that the productivity can be increased to a great extent if proper technology is used. It is, however, known that farmers are unable to adopt the entire package as per recommendation due to certain difficulties, sometimes beyond their control.

2.1 Vegetative growth

2.1.1 Nitrogen

Tunçtürk and Yildirim (2004) reported that application of N at 40, 80, and 120 kg/ha significantly ($P < 0.05$) increased safflower plant height compared to control plants, with 120 kg N/ha producing the tallest plants. They further reported that N in the form of ammonium nitrate significantly ($P < 0.05$) increased safflower plant than ammonium sulphate or urea. Siddiqui and Oad (2006) reported that application of N to safflower at 30, 60, 80, 120, 150, and 180kg/ha increased plant height and branch number per plant than control plants, but these variables peaked at 120 kg N/ha. Application of N above 120 kg/ha had no significant ($P > 0.05$) effect on safflower plant height and branch number (Siddiqui and Oad, 2006). However, increasing application of N from 30 to 180 kg/ha, significantly ($P < 0.05$) delayed safflower crop maturity by 3.34-24.34 days compared to plants with no N application (Siddiqui and Oad, 2006). Strašil and Vorlíček (2002) in the Czech Republic reported that N application at 40 or 80 kg/ha had no effect on safflower plant. Similar results were reported by Malek and Ferri (2014) and Haghighati (2010) who reported that application of 30, 60 or 90 kg N/ha had no significant ($P > 0.05$) effect on plant height of safflower. While, Taleshi *et al.* (2012) reported that application of

N at 84, 120 and 154 kg/ha increased primary and secondary branch number by 31 and 44%, respectively compared to control plants. Haghighati (2010) reported that N application at 30, 60 and 90 kg/ha increased safflower plant biomass by 13.4, 15.3, and 22.9%, respectively, compared to control plants. In another study, increasing N application from 0 to 300 kg/ha improved ensiling quality of safflower by improving the aerobic stability, lactic acid production and smaller fermentation losses (Weinberg *et al.*, 2007). Increased supply of N enhanced total dry matter production and accumulation in various plant parts, explaining the increase in safflower vegetative growth (Weinberg *et al.*, 2007; Mündel *et al.*, 1997). Nitrogen is an integral part of amino and nucleic acids, proteins, nucleotides, chlorophyll and all enzymes (Weinberg *et al.*, 2007; Mundel *et al.*, 1997). Increased supplies of N, leads to increased leaf area index and thereby increased light absorption, enhancing total dry matter production and accumulation in various plant parts, explaining the increase in safflower vegetative growth (Weinberg *et al.*, 2007; Mündel *et al.*, 1997).

Application of N at 0, 25, 50, 75, 100, 125, 150, and 175 kg/ha had significant ($P < 0.05$) effect on safflower physiology and productivity (Mohamed *et al.*, 2012). Increasing N to 100 kg/ha significantly ($P < 0.05$) increased the assimilation and transpiration rates, stomatal conductance and leaf area index (LAI) by 42, 32, 52 and 42%, respectively, compared to control plants (Mohamed *et al.*, 2012). The above ground dry weight and water use efficiency (WUE) increased by an average of 42 and 41% on N applied safflower plants compared to control plants (Mohamed *et al.*, 2012). Chlorophyll a, b and total chlorophyll contents at anthesis were significantly ($P < 0.05$) influenced by N application of 25, 50, 75, 100, 125, and 150 kg/ha. However, application of N above 150 kg/ha had no effect ($P > 0.05$) on chlorophylls a and b, and total chlorophyll contents (Mohamed *et al.*, 2012). Dordas and Sioulas (2008) reported that safflower plants treated with N had increased net assimilation rate by 51% compared to control plants. Nitrogen fertilization at 100 and 200 kg/ha increased safflower biomass at anthesis by an average of 24% and at maturity by an average of 25% compared with control plants (Dordas and Sioulas, 2009). Nitrogen application also increased dry matter partitioning in leaves and stems of safflower (Dordas and Sioulas, 2009).

2.1.2 Phosphorus

Phosphorus is the most limiting nutrient for crop growth and yield in many regions of the world (Rodriguez *et al.*, 1999) including Botswana (Emongor and Mabe, 2012), and application of P fertilizer represents an important measure to correct nutrient deficiencies and to replace nutrients having removed in the products harvested (Dambroth and El-Bassm. 1990). Abbadi and Gerendás (2011) reported that application of P to safflower at 0.04, 0.08, 0.024 and 0.72 g/pot in 2004 and 0.25, 0.5, 1 and 2 g/pot in 2005, significantly ($P < 0.05$) increased plant growth and morphology (stem diameter, leaf dry matter and stem dry matter). Maximum total dry matter of safflower was obtained with application of 1 g P/pot (Abbadi and Gerendás, 2011). Application of P at 50, 75 or 100 kg P_2O_5/ha to safflower plants increased plant biomass by 2.8 to 9.4% compared to control plants, depending on the irrigation regime and stage of safflower plant growth and development when irrigation regime was applied (Arani *et al.*, 2011). The highest plant biomass was obtained with application of 100 kg P_2O_5/ha, irrespective of irrigation regime and stage of plant growth and development when irrigation regime was applied (Arani *et al.*, 2011). Golzarfar *et al.* (2011) found similar results when they reported that application of P to safflower at 50 or 100 kg P_2O_5/ha significantly ($P < 0.01$) increased plant height, stem diameter, number of branches/plant and plant biomass compared to control plants. The highest plant height, stem diameter, number of branches/plant and plant biomass was obtained with application of 100 kg P_2O_5/ha (Golzarfar *et al.*, 2011). Singh and Singh (2013) reported that P application at 40 or 80 kg P_2O_5/ha significantly ($P < 0.05$) increased safflower plant biomass and

P uptake by 24.6-31.2% and 17.9-35.3%, respectively, compared to control plants. Application of 40 and 80 kg P_2O_5/ha to safflower plants also increased the uptake of N and sulphur (S) by 17.0-24.1% and 11.5-23.8%, respectively, compared to control plants (Singh and Singh, 2013). However, Haghighati (2010) reported that application of P at 30 or 60 kg/ha had no significant ($P > 0.05$) effect on safflower plant height and biomass.

2.1.3 Nitrogen and phosphorus interaction

There is a wide range of nonspecific as well as specific interactions between mineral nutrients in plants (Robson and Pitman, 1983) which affect the critical contents. The critical deficiency content of N increases as the P content increases and vice versa (Marschner, 2005). Interactions between two mineral nutrients are important when the contents of both are near the deficiency range (Mengel and Kirkby, 2001; Marschner, 2003). Increased P or N alone brings about lower vegetative growth than when N and P are added together. Mirzakhani *et al.* (2009) working with Azobacter and Mycorrhiza bacteria, with N and P reported that there was a significant ($P < 0.05$) interaction of N and P application on root dry weight. Application of N and P at 50 N + 25 P, 100 N + 50 P and 150 N+ 75 P kg/ha, increased root dry matter by 28.1, 48.3 and 67.4% compared to control plants, respectively (Mirzakhani *et al.*, 2009). Haghighati (2010) reported that there was significant ($P < 0.05$) interaction effect of variety with N and P fertilizer application. A combined application of 60 N + 30 P or 60 N + 60 P kg/ha significantly ($P < 0.05$) increased safflower plant height, but had no effect on plant biomass (Haghighati, 2010).

2.2 Yield components

The yield components of safflower are days to 50% flowering, capitula number/plant, capitulum size, number of achenes (seed) per capitula, and achene weight (100-seed weight) (Gonzalez *et al.*; 1994; Kedikanestwe, 2012; Emongor *et al.*, 2013). Even though yield components are under genetic control, they do respond with various degrees of flexibility to cultural practices (Gonzalez *et al.*, 1994; Emongor *et al.*, 2013).

2.2.1 Nitrogen

Sufficient N supply increases leaf expansion resulting in increased interception and efficient utilization of solar radiation leading to greater accumulation of dry matter in the leaves and shoots, increased yield components and yield of crops (Ahmed *et al.*, 2007). Bonfim-Silva *et al.* (2015) reported in a pot experiment, that application of N at 60, 120, 180, 240 and 300 mg/dm^3 to safflower plants increased the capitula number/plant (61.79%) compared to control plants and the response was quadratic to increasing N application rate. Safflower plants applied with 180 mg N/dm^3 produced highest capitula number of 18/plant. Abd El-Mohsen and Mahmoud (2013) reported that application of 0, 40, 80 and 120 kg N/ha to safflower plants significantly ($P < 0.05$) increased 1000-seed weight, capitula number/plant and days to 50% flowering and the response was quadratic to increasing N application rate. Application of 80 kg N/ha gave the highest 1000-seed weight and capitula number/plant. Application of N beyond 80 kg/ha resulted in a decrease in all the yield components traits (Abd El-Mohsen and Mahmoud, 2013). Elfadl *et al.* (2009) reported that variance components for agronomic traits, yield components and yield were significantly ($P < 0.05$) influenced by the environment. Nitrogen application at 0, 40 and 80 kg/ha only had a significant ($P < 0.05$) increase on 1000-seed weight compared to control, but N application had no effect number of seeds/plant, harvest index, number of capitula/plant and number of seeds/capitulum (Elfadl *et al.*, 2009). Soleimani (2010) reported that the application of N at 50, 75, 100, 125, and 150 kg/ha significantly (P <

0.05) increased 1000-seed weight and number of seed/capitulum, and the response was quadratic to increasing N application rate. Application of N increased 1000-seed weight and number of seeds/capitulum by 4.7% and 5.5%, respectively, compared to the same variables on control plants (Soleimani, 2010). Application of 100 kg N/ha to safflower plants produced the optimum yield components (Soleimani, 2010). Soleimani (2010) also reported that split application of N at sowing, early stem elongation and early flowering stages significantly ($P < 0.01$) increased 1000-seed weight than split application of N at sowing and late stem elongation. Siddiqui and Oad (2006) in Pakistan, reported that N application at 0, 30, 60, 80, 120, 150 and 180 kg/ha significantly ($P < 0.05$) increased the capitula number/plant and 1000-seed weight compared to control plants. Increasing N application from 30 to 180 kg/ha significantly ($P < 0.05$) increased capitula number/pland and 1000-seed weight by 31.4-167% and 13.9-61.1%, respectively, compared to control plants.

Tunçtürk and Yildirim (2004) reported that application of safflower plants with 40, 80, and 120 kg N/ha significantly ($P < 0.05$) increased the capitula number/plant. Increasing N application from 40 to 120 kg/ha increased the capitula number/plant by 6.1-23% compared to control plants and the response plateaued at 80 kg N/ha (Tunçtürk and Yildirim, 2004). They further reported that the form of N-fertilizer had a significant ($P < 0.05$) effect on capitula number/plant. Application of N in the form of ammonium nitrate significantly ($P < 0.05$) improved the yield components, yield and agronomic traits of safflower compared to when N was applied either as urea or ammonium sulphate, however, ammonium sulphate was better than urea (Tunçtürk and Yildirim, 2004). Application of N in the form of urea to safflower plants at 75 and 150 kg/ha significantly ($P < 0.05$) increased capitula number/plant, capitula number/primary branch, capitula number/secondary branch, seed number/capitulum, seed number/primary capitulum, seed number/secondary capitulum and 1000-seed weight compared to control plants (Golzarfar *et al.*, 2012). Application of 75 or 150 kg N/ha significantly ($P < 0.05$) increased the safflower yield components in the range of 27.9 to 125% compared to control plants, depending on the yield component trait (Golzarfar *et al.*, 2012). Mohamed *et al.* (2012) in the United Kingdom, reported that application of N at 0, 25, 50, 75, 100, 125, 150, 175 kg/ha increased capitula number/plant and seed number/plant in the range of 16.6-41.7% and 16.6-308.3%, respectively, compared to the same variables from control plants.

2.2.2 Phosphorus

Increasing P application to safflower plants increased yield components of safflower (Arani *et al.*, 2011; Golzarfar *et al.*, 2012; Singh and Singh, 2013). Singh and Singh (2013) reported that application of 0, 40 and 80 kg P_2O_5/ha significantly ($P < 0.05$) increased capitula number/plant, seed weight/capitula, 1000-seed weight and harvest index. Application of P at 0, 40 and 80 kg P_2O_5/ha significantly ($P < 0.05$) increased capitula number/plant, seed weight/capitula, 1000-seed weight and harvest index in the range of 22.4-49.4, 15.5-35.2, 14.0-38.5 and 2.0-4.7%, respectively (Singh and Singh, 2013). Application of P to safflower plants at 50 and 100 kg/ha significantly ($P < 0.05$) increased capitula number/plant, capitula number/primary branch, capitula number/secondary branch, seed number/capitulum, seed number/primary capitulum, seed number/secondary capitulum and 1000-seed weight compared to control plants (Golzarfar *et al.*, 2012). Application of 50 or 100 kg P/ha significantly ($P < 0.05$) increased the safflower yield components in the range of 7.9 to 25.4% compared to control plants, depending on the yield component trait (Golzarfar *et al.*, 2012). Phosphorus application at 0, 50, 75, and 100 kg P_2O_5/ha significantly ($P < 0.01$) increased a 1000-seed weight and harvest index of safflower (Arani *et al.*, 2011). Increasing P application from 50, 75, and 100 P_2O_5 kg/ha increased a 1000-seed weight and harvest index from 20.1 to 45.5 g and 7.63 to 26.74, respectively, depending on the P applied and irrigation regime (Arani *et al.*, 2011). Application of P at 0.25, 0.5, 1.0, and 2.0 g/pot significantly ($P < 0.05$) increased capitula dry matter and capitula number/pot per pot from 15.4 to 26.5 g and 8.7 to 16.0, respectively (Abbadi and Gerendás, 2011).

2.2.3 Interaction of nitrogen and phosphorus

Increased P or N alone resulted in lower yield components than when N and P are added together (El-Nakhawy, 1991; Golzarfar *et al.*, 2012). El-Nakhawy (1991) reported that application of 46 N + 46 P, 92 N + 46 P, 138 N + 46 P kg/ha increased 100-seed weight by 8, 18.7, and 19.2 % , respectively, compared to control plants where no fertilizer was applied. They further reported that application of 46 N + 46 P, 92 N + 46 P, 138 N + 46 P kg/ha increased capitula number/plant and seed weight/capitulum 10.6, 43.2, 35.7%, and 17.6. 54.4, 32.4%, respectively, control plants. 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, 150 N + 100 P_2O_5 significantly ($P < 0.05$) increased capitula number/primary branch, capitula number/plant, capitula number/secondary branch, seed number/secondary capitulum, and 1000-seed weight compared to safflower plants applied with no fertilizer or N or P alone.

2.3 Seed Yield

2.3.1 Nitrogen

Nitrogen and P have been reported to be the two essential nutrients for safflower growth and development, therefore optimization of their application rates can strongly increase seed yield and oil content of safflower (Steer and Harrigan, 1986; Belanger *et al.*, 2000; Antoniadou and Wallach, 2002; Henke *et al.*, 2007). Safflower response to N is reported generally to be greater than other nutrients (Weiss, 2000). Malek and Ferri (2014) in Iran researching on N fertilizer application in two locations and different safflower cultivars, reported that application of 0, 30, 60, and 90 kg N/ha significantly ($P < 0.01$) increased safflower seed yield irrespective of location or cultivar. Safflower plants fertilized with 30, 60, and 90 kg N/ha produced 769.5, 779.6 and 837.1 kg/ha of seed yield compared to control plants that produced 679.3 kg/ha. Singh and Singh (2013) in India, reported that application of N at 40 or 80 kg/ha to safflower plants significantly

(P < 0.05) increased seed yield compared to seed yield from control plants. Safflower plants applied with 40 and 80 kg N/ha produced seed yield of 2270 and 2520 kg/ha, respectively, while control plants produced 1570 kg/ha. Abd El-Mohsen and Mahmoud (2013) in Egypt, reported that N fertilizer application at 0, 40, 80 and 120 kg/ha increased safflower seed yield and the response was quadratic with increase in N application rate. Nitrogen fertilizer application at 40, 80 and 120 kg significantly (P < 0.05) increased safflower seed yield by 38.2, 52.1 and 49.6%, respectively, compared to plants with no N application (Abd El-Mohsen and Mahmoud, 2013). The best seed yield was obtained with 80 kg N/ha, beyond which safflower seed yield significantly (P < 0.05) decreased (Abd El_Mohsen and Mahmoud, 2013). Nitrogen fertilizer application in the United Kingdom (Britain) at 0, 25, 50, 75, 100, 125, 150 and 175 kg/ha, significantly (P < 0.05) increased safflower seed yield (Mohamed *et al.*, 2012) and the response was linear with increase in N application rate. Increasing N fertilizer application rate from 25 to 175 kg/ha, increased safflower seed yield from 4.76 to 323.8% and the best N application rate was 175 kg/ha (Mohamed *et al.*, 2012). While, Golzarfar *et al.* (2012) in Iran, reported that application of N at 0, 75 and 150 kg/ha significantly (P < 0.05) increased seed yield of safflower by 180.3-228.1% compared to safflower plants where N was not applied. Application of N at 0, 75 and 150 kg/ha increased safflower seed yield from 983.83 kg/ha (control) to 2757.83 and 3227.5 kg/ha, respectively. Taleshi *et al* (2012) reported that application rate of 0, 84, 120, and 154 kg N/ha increased safflower seed yield. The response of safflower yield to increasing N application was linear, with application of 154 kg N/ha producing the maximum seed yield of 1507.6 kg/ha. Earlier studies in Iran on N fertilizer application showed that application of 0, 50, 100, and 150 kg N/ha significantly (P < 0.05) increased the safflower seed yield by 36.1-111.1% compared to control plants (Bitarafan *et al.*, 2011). Safflower plants fertilized with 50, 100 and 150 kg N/ha produced seed yield of 2245, 3023 and 3481 kg/ha, respectively, while safflower plants with no N application produced 1649 kg/ha (Bitarafan *et al.*, 2011). Zareie *et al.* (2011) studied the effects of N and iron fertilizers on seed yield and yield components of safflower genotypes and found that N fertilizer application at 0, 50, 100, and 150 kg/ha increased seed yield. Safflower plants fertilized with 50, 100, and 100 kg N/ha produced 5.48, 9.08 and 7.04 tons/ha of seed yield, respectively, and 100 kg N/ha produced the best effect on seed yield and yield components (Zareie *et al.*, 2011). Haghighati (2010) also in Iran, reported that application of N at 0, 30, 60, and 90 kg/ha increased safflower seed yield irrespectively of cultivar and the optimum N application rate was 60 kg/ha beyond which seed yield decreased. Soleimani (2010) reported that in Western Iran, reported that application of N fertilizer at 50, 75, 100, 125, and 150 kg/ ha to safflower plants significantly ($P < 0.05$) increased seed yield. The increase in seed yield was quadratic to increasing N fertilizer application, with 100 kg N/ha giving the best results on yield and yield components of safflower (Soleimani, 2010). Application of N above 100 kg/ha resulted in a decrease in seed yield (Soleimani, 2010). Dordas and Sioulas (2009) in Greece, reported that N fertilizer application at 0, 100 and 200 kg/ha significantly ($P < 0.05$) increased safflower seed yield and the response was quadratic to increasing N fertilizer application. Safflower plants fertilized with 100 and 200 kg N/ha produced 3.99 and 3.98 tons/ha, respectively, while the control plants produced 3.54 tons/ha of seed yield (Dordas and Sioulas, 2009). Siddiqui and Oad (2006) reported that application of N at 0, 30, 60, 80, 120, 150, and 180 kg N/ha in Pakistan, significantly ($P < 0.05$) increased safflower seed yield and the response of seed yield to increasing N application was quadratic. Seed yield peaked at 120 kg N/ha, beyond which seed yield decreased (Siddiqui and Oad, 2006).

2.3.2 Phosphorus

Increasing P application to safflower plants has been reported to increase seed yield (Malek and Ferri, 2014; Singh and Singh, 2013; Golzarfar *et al.*, 2012; Golzarfar *et al.*, 2011; Arani *et al.*, 2011; Haghighati, 2010; Purvimath *et al.*, 1993). Malek and Ferri (2014) reported that application of 0, 30 and 60 kg P/ha to safflower plants produced a non-significant ($P > 0.05$) increase in seed yield. Singh and Singh (2013) reported that application of 40 and 80 kg P_2O_5/ha to safflower plants significantly ($P < 0.05$) increased seed compared to control plants, however, there was no significant ($P > 0.05$) difference in seed yield of plants fertilizer either with 40 or 80 kg P_2O_5/ha. Safflower plants fertilized with 40 and 80 kg P_2O_5/ha produced a seed yield of 2.22 and 2.36 ton/ha, respectively, while the control plants produced a seed yield of 1.77 ton/ha (Singh and Singh, 2013). Golzarfar *et al.* (2012) reported that safflower plants fertilized with 50 and 100 kg P_2O_5 kg/ha produced a seed yield of 2391.5 and 2641.83 kg/ha, respectively, compared to control plants which produced 1935.83 kg/ha. Phosphorus application significantly ($P < 0.05$) increased seed yield by 23.5 and 36.5%, respective to 50 and 100 kg P_2O_5/ha compared to control plants (Golzarfar *et al.*, 2012). Earlier in 2011, Golzarfar *et al.* (2011) had reported similar results in a different site that P application at 0, 50 and 100 kg P_2O_5/ha significantly ($P < 0.05$) increased seed yield of safflower by 23 and 38%, respectively, compared to the control plants. Arani *et al.* (2011) in Iran, reported that P applied at 0, 50, 75, and 100 kg P/ ha significantly ($P < 0.05$) increased the seed yield of safflower by 7, 13 and 15.8%, respectively, compared to control plants. Haghighati (2010) reported that application of P at 0, 30 and 60 kg/ha had no significant ($P > 0.05$) effect on seed yield of safflower. While Purvimath *et al.* (1993) reported that increasing P fertilizer application rate from 75 to 150 kgP/ha increased the seed yield of safflower by 17.6%.

2.3.3 Nitrogen and phosphorus interaction

Golzarfar *et al.* (2011) reported that application of 75 N + 50 P_2O_5, 75 N + 100 P_2O_5, 150 N + 50 P_2O_5, 150 N + 100 P_2O_5 kg/ha significantly (P < 0.05) increased safflower seed yield. They further observed that maximum safflower seed yield was produced by application of 150 N + 100 P_2O_5 kg/ha, constituting an increase of 325% compared to the control plants. Haghighati (2010) reported that application rate of N + P at 30 N + 30 P, 30 N + 60 P, 60 N + 30 P, 60 N + 60 P, 90 N + 30 P Kg/ha increased the seed yield with a maximum being observed at 90 N + 30 P. Mirzakhani *et al.* (2009) observed that with application of 50 N + 25 P, 100 N + 50 P, and 150 N + 75 P kg/ha to safflower significantly (P < 0.05) increased seed yield by 16.4%, 55.4% and 28.3%, respectively, compared to the control plants. Maximum safflower seed yield of 1462 kg/ha was produced by plants fertilized with 100 N + 50 P kg/ha (Mirzakhani *et al.*, 2009). El-Nakhlawy (1991) reported that application rate of 46 N + 46 P, 92 N + 46 P and 138 N + 46 P kg/ha significantly (P < 0.05) increased safflower seed yield, by 25.5%, 70%, 74%, respectively, compared to the control plants. However, no significant N and P interaction effects on safflower seed yield has also been reported (Haghighati, 2010; Malek and Ferri, 2014).

2.4 Oil content

2.4.1 Nitrogen

Safflower plants applied with 40 and 80 kg N/ha significantly (P < 0.05) produced higher seed oil content of 33.3 and 33.5%, respectively, than control plants that produced seed oil content of 32.4% (Singh and Singh, 2103). However, there was no significant (P > 0.05) difference in the oil seed contents of safflower plants fertilized with either 40 or 80 kg N/ha (Singh and Singh, 2013). Application of N at 40, 80 and 120 kg/ha to safflower plants significantly (P < 0.01) increased the seed oil content and oil yield compared to seed oil content and oil yield from control plants (Abd El-Mohsen and Mahmoud, 2013). The response of safflower seed oil content and oil yield was quadratic to increasing N application rate. Maximum seed oil content (33.9%) and oil yield (594.7 kg/ha) was produced with fertilizer application to safflower plants of 80 kg N/ha (Abd El-Mohsen and Mahmoud, 2013). Bitarafan *et al.* (2011) reported that application of N at 50, 100 and 150 kg/ha significantly ($P < 0.05$) increased the seed oil content of safflower compared the seed oil content from control plants. Increasing N application from 50 to 100 kg/ha resulted in a slight increase in seed oil content of safflower, but applying 150 kg N/ha decreased the seed oil content (Bitarafan *et al.*, 2013). Soleimani (2010) reported that application of 50, 75, 100, 125 and 150 kg N/ha to safflower plants increased the seed oil content and oil yield. Application of 100 kg N/ha gave the highest seed oil content and oil yield of 29.9% and 755 kg/ha, respectively (Soleimani, 2010). Elfadl *et al.* (2009) reported that application of 0, 40 and 80 kg N/ha had no significant ($P > 0.05$) effect on safflower seed oil content and oil yield. Dordas and Sioulas (2008) did not find any relationship between rates of N and safflower seed oil content. Tunçtürk and Yildirim (2004) reported that N application at 40 and 80 kg/ha significantly ($P < 0.05$) increased safflower seed oil content compared to seed oil content of control plants. However, application of 120 kg N/ha significantly ($P < 0.05$) decreased the seed oil content compared to control plants or plants fertilized with either 40 or 80 kg N/ha (Tunçtürk and Yildirim, 2004). However, application of 40, 80 and 120 kg N/ha increased safflower seed oil yield compared to control, but there was no significant ($P > 0.05$) difference in the oil seed yield from safflower plants treated with 40, 80 or 120 kg N/ha (Tunçtürk and Yildirim, 2004). El-Nakhlawy (1991) reported that application of nitrogen at 0, 46, 92, and 138 kg/ha decreased safflower seed oil content by 1.0%, 7.8% and 3.9%, respectively, compared to control plants.

2.4.2 Phosphorus

Phosphorus is required for the formation of fats, being a component of the glycerol unit and its needed for conversion of acetate units into fatty acids and its energy expensive, because 2NADPH and 1ATP are needed for each acetyl group present containing a high energy phosphate group that drives most energy requiring biochemical process (Salisbury and Ross, 1992). Singh and Singh (2013) reported that application of 40 and 80 kg P_2O_5/ha to safflower plants significantly ($P < 0.05$) increased the oil seed content compared to control plants. Phosphorus application increased the safflower oil seed content by between 6.0 and 6.6%, respectively. However, there was no significant ($P > 0.05$) difference in the oil seed content of plants fertilized with 40 or 80 kg P_2O_5/ha (Singh and Singh, 2013). Arani *et al.* (2011) reported that P application at 0, 50, 75, and 100 kg/ha significantly ($P < 0.05$) increased safflower seed oil yield. Safflower plants treated with 50, 75 and 100 kg P/ha produced oil yield of 1124, 1262 and 1344 kg/ha, while control plants produced 955.7 kg of oil/ha (Arani *et al.*, 2011). The response of safflower seed oil yield to increasing P application was linear (Arani *et al.*, 2011).

2.4. 3 Interaction of nitrogen and phosphorus

El-Nakhlawy (1991) observed that application of 46 N + 46 P, 92 N + 46 P and 138 N + 46 P kg/ha decreased the oil content by 2.8%, 3.3% and 4.9%, respectively compared to the control plants.

Most literature shows that N, P and the interaction of N and P increased safflower growth, yield components, seed yield, seed oil content and oil yield. However, the optimum amounts of N, P and the interaction of N and P to be applied per hectare to optimize safflower yield potential, oil content and yield varies greatly depending on climatic conditions. agronomic practices. genotypes. growing season (summer or winter) and soil types.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Experimental site

The experiment was conducted at the Botswana University of Agriculture and Natural Resources (BUAN), Notwane Farm, located at 24°33' S; 25°54' E. The site is 964 m above sea level. The soils are shallow, ferruginous tropical soils, mainly consisting of medium to coarse grains and sandy loams with low water holding capacity and subject to crusting after heavy rains (De Wit and Nachtengale, 1996). The soils are deficient in phosphorus, and low in nitrogen and organic matter (Emongor *et al.*, 2004; Emongor *et al.*, 2012). The climate is semi- arid with average annual rainfall of about 538 mm, which mostly falls in summer (late October to April). The experimental site has an average maximum and minimum temperature varying between 33.1–34.7°C and 19.2–19.5°C, respectively (Ramolemana, 1999). However, during the coldest months, from April to September, the average maximum and minimum temperatures range between 26-34°C and 7-16°C, respectively.

3.2 Experimental design

The experiment was a split-plot design in randomized complete blocks with three replications. The treatments were nitrogen (main- plot) applied in the form of calcium ammonium nitrate (28% N) at 0, 25, 50, 75, and 100 kg N/ha and phosphorus (sub-plot) applied as single superphosphate (10.5% P) at 0, 25, 50, and 75 kg P/ha. Soils were sampled at a depth of 0-30 cm for the determination of physico-chemical properties, and total N and P before planting of safflower and at the end of each trial. The main-plots were 23 × 29 m while each sub-plot was 5 × 5 m. Safflower seeds were sown at a depth of 6 mm.

Two trials were conducted. The first trial was done between April to July 2013 and while the second trial was from January to April 2014.

3.3 Soil analysis

A composite soil sample was sampled from the experimental site at a depth of 0-30 cm before the experiment and after the experiment. It was analysed for basic soil properties such as pH, total P, total N, Organic carbon (OC) and Cation exchange capacity (CEC). The soil CEC was determined according to method of Rhoades (1982). Total soil P was determined colorimetrically according to the method of Olsen and Sommers (1982). Total N was determined by the distillation method described by Bremner (1982). Organic carbon was determined using the Walkley Black procedure (Walkley, 1947).

3.4 Crop husbandry

Safflower was planted at a spacing of 20 cm between plants and 45 cm between rows and the variety of safflower used was 'Kiama Composite'. Fertilizer application was done by side banding about 5 cm from the seedlings. Phosphorus (P) was applied two weeks after emergence, while nitrogen (N) was applied in two splits. The first N-split was applied two weeks after emergence and the second split was applied at the start of the elongation phase (end of the rosette stage). Irrigation was done using over- head sprinklers, to a depth of 12mm twice a week. Pest and disease control was done when necessary. Weeding was done by hoeing between the rows regularly.

3.5 Dependent variables determined

Variables determined included, plant height, number of branches per plant, number of capitulum heads per plant, capitulum head diameter, number of seeds per capitulum, 100-seed weight, seed yield, oil content (%), leaf N and P, and seed protein content.

Plant height

The height of plants were measured using a 3- metre measuring tape, from the ground level to the highest point of the plant. Twelve random plants in the centre rows per treatment per replication were measured.

Number of primary branches

Twelve plants per treatment per replication were randomly sampled. The branches were counted on each sampled plant.

Number of capitula

The number of capitula per plant were determined on twelve randomly selected plants per treatment per replication. The capitula number were counted.

Seed number per capitulum

Capitula from twelve plants randomly sampled per treatment per replication were used. The seed number per capitulum were counted.

Capitulum diameter

The capitulum diameter was measured using electronic Vernier calipers from twelve plants randomly selected per treatment per replication.

Hundred-seed weight

The weight of 100 representative seeds per treatment per replication were weighed using a Mettler PM 400 digital balance.

Determination of seed yield

Seed yield was determined from an area of 4 m^2 from the centre of the experimental plots. The harvested capitula were threshed and winnowed to separate seeds from chaff. The seeds were weighed using a digital balance (Mettler PM 400).

Determination of leaf and seed mineral contents

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

Determination of seed oil content and seed protein content

For the extraction of safflower oil, 0.5 g of ground dried safflower seed was weighed and placed into pre-weighed extraction bags, which were sealed and placed in the XT10 Ankom Extraction System (Ankom Technology), with N-hexane for 60 minutes. After extraction, the bags were placed for 20 minutes in an oven to dry and the final weight of the bag and sample was taken. The difference between the final weight and initial weight was the oil content expressed as a percentage of the initial weight of the ground dried safflower seed.

For protein analysis, The protein content in safflower seed was determined by measuring 0.05 g dried and ground safflower seed was wrapped in foil, and placed into an N/Protein analyser (Flash 2000, Organic Elemental Analyzer Thermo Fischer Scientific).

3.6 Statistical analysis

After being checked for normality, measured data on dependent variables was subjected to analysis of variance (ANOVA) using the general linear models (Proc GLM) procedure of the Statistical Analysis System (SAS, 2016) program. Treatment means were separated using the Least Significant Difference (LSD). Appropriate regression models were used to analyze the response of safflower plants to increasing N and P application (Shedecor and Cockran, 1989).

CHAPTER 4

4. 0 RESULTS

4.1 Soil data

The soil physico-chemical properties of the experimental site at the start of the study are shown in Table 1. The results of the soil at the start of the study showed that the soil N, P, CEC, OC were low, while pH was slightly acidic (Table 1). Application of fertilizers, limestone ammonium nitrate (LAN) and single super phosphate (SSP) had no significant ($P \geq 0.05$) influence on the soil pH, N, P, OC, and CEC, at the end of the experiment (Table 2, 3). Application of SSP significantly ($P \leq 0.05$) increased soil P at the end of the experiment (Table 3). However, there were no significant ($P \geq 0.05$) difference of soil P among the plots applied with SSP at 25, 50 or 75 kg P/ha (Table 3).

Table 1. The physico-chemical properties of the soil before the start of the study

pH	mg N/ kg	mg P/ kg	OC (%)	CEC (cmol /kg)
5.6	1.11	2.2	0.08	2.2

Table 2. Effect of fertilizer limestone ammonium nitrate (LAN) on soil physico-chemical properties.

kg N/ ha	pH	mg N/kg	mg P/kg	OC (%)	CEC (cmol/kg)
0	5.83a	1.10a	2.23a	0.09a	2.17a
25	5.98a	1.32a	2.22a	0.08b	2.40a
50	5.70a	1.45a	2.24a	0.09a	2.07a
75	5.77a	1.42a	2.12a	0.08a	2.29a
100	5.73a	1.32a	2.22a	0.08a	2.27a
Significance	NS	NS	NS	NS	NS
LSD	0.28	0.46	0.83	0.01	0.34

NS non-significant at $P = 0.05$. Means separated using the Least Significant Difference (LSD) at $P = 0.05$; means within column(s) followed by the same letter(s) are not significantly different.

Table 3. Effect of single superphosphate fertilizer on the soil physico-chemical properties.

kg P/ha	pH	mg N/kg	mg P/kg	OC (%)	CEC (cmol/kg)
0	5.82a	1.19a	2.16b	0.08a	2.44a
25	5.77a	1.59a	2.75a	0.09a	2.26a
50	5.86a	1.58a	2.80a	0.08a	2.26a
75	5.75a	1.43a	2.81a	0.08a	2.36a
Significance	NS	NS	*	NS	NS
LSD	0.25	0.41	0.46	0.01	0.30

*, NS, significant at $P = 0.05$ and non-significant, respectively. Means separated using the Least Significant Difference at $P = 0.05$; means within column(s) followed by the same letter(s) are not significantly different.

4.2 Vegetative growth

4.2.1 Plant height

The results showed that the interaction of N and P fertilizer application and P independently had no significant ($P > 0.05$) effect on safflower plant height (Table 4). Application of 25 and 50 kg N/ha to safflower plants significantly ($P < 0.0017$) increased plant height (Figure 1). The response of safflower plants to increasing N application was quadratic (Figure 1). Application of N beyond 50 kg/ha to safflower plants significantly ($P < 0.05$) decreased plant height (Figure 1).

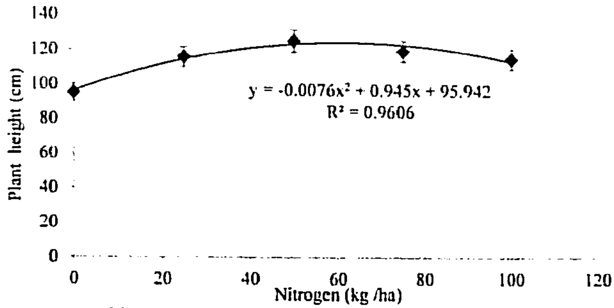

Figure 1. Effect of nitrogen on the plant height in safflower, bars are standard error.

Table 4. Effect of phosphorus on plant height in safflower.

Level of phosphorus added kg P/ ha	Plant height (cm)
0	106.31 a
25	112.22 a
50	114.90 a
75	108.62 a
Significance	NS
LSD	11.62

*, NS, significant at $P = 0.05$ and non-significant, respectively. Means separated using the Least Significant Difference at $P = 0.05$; means within column(s) followed by the same letter(s) are not significantly different.

4.2.2 Primary branch numbers

The application of N and P fertilizers and the interaction of N and P had no significant ($P \geq 0.05$) effect on safflower primary branch development (Figure 1, 2). However, N and P fertilizer application tended to have a non-significant ($P > 0.05$) increase in primary branch numbers (Figure 2, 3).

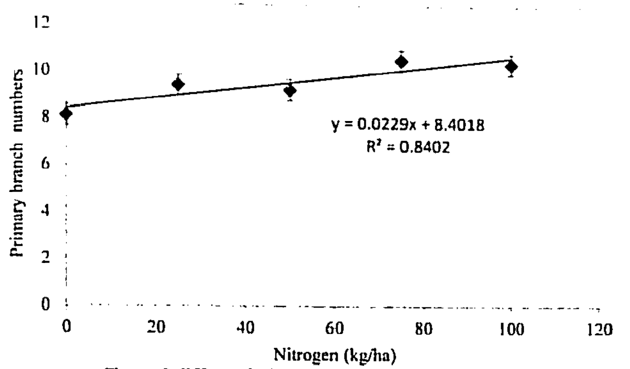

Figure 2. Effect of nitrogen on primary branch numbers of safflower plants, bars are standard error.

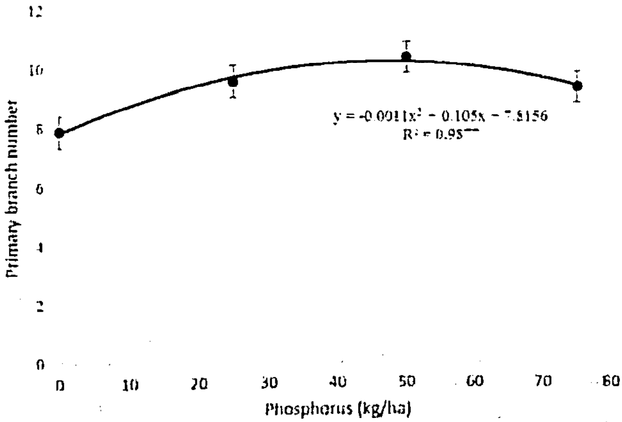

Figure 3. Effect of phosphorus on safflower primary branch number

4.3 Yield components

Application of N, P and the interaction of N and P had no significant (P > 0.05) effect on capitula diameter and seed number/capitulum (Table 5, 6). However, the interaction of N and P significantly (P < 0.001) increased safflower capitula number/plant (Figure 4). Application of 75 kg N/ha plus 50 kg P/ha significantly (P < 0.001) increased capitula number/plant than any other N and P combination or N and P application alone (Figure 4). Application of 100 kg N/ha plus 50 kg P/ha resulted in significant (P < 0.001) decrease in capitula number/plant (Figure 4). Also P and the interaction of N and P had no significant (P > 0.05) effect on 100-seed weight of safflower, but N fertilizer application significantly (P < 0.05) increased 100-seed weight (Figure 5). Increasing N application up to 75 kg/ha significantly (P < 0.05) increased 100-seed weight compared to control (Figure 5). Application of N above 75 kg/ha resulted in a significant (P < 0.05) decrease in 100-seed weight (Figure 5).

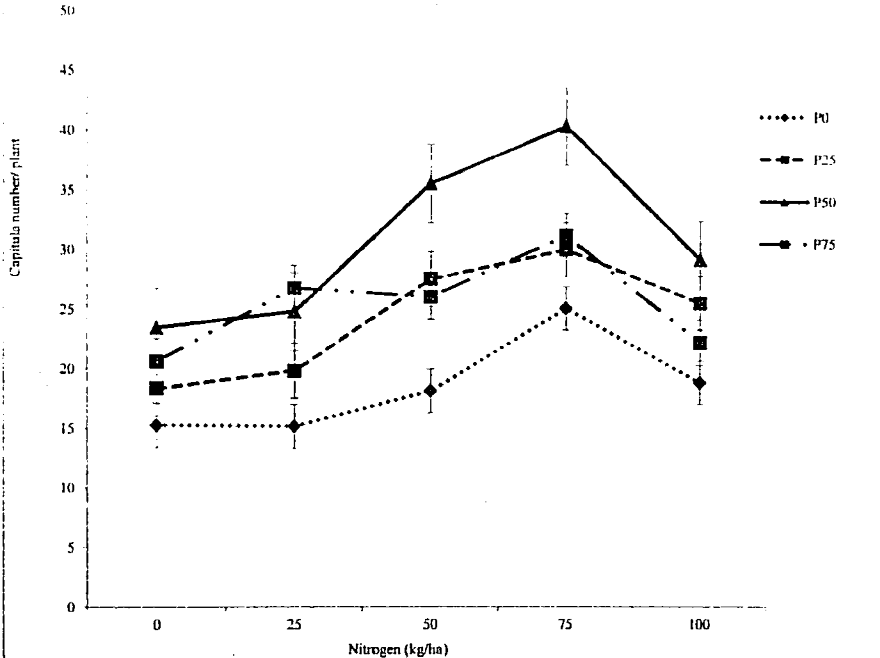

Figure 4. Effect of N and P interaction on capitula number, bars are standard error

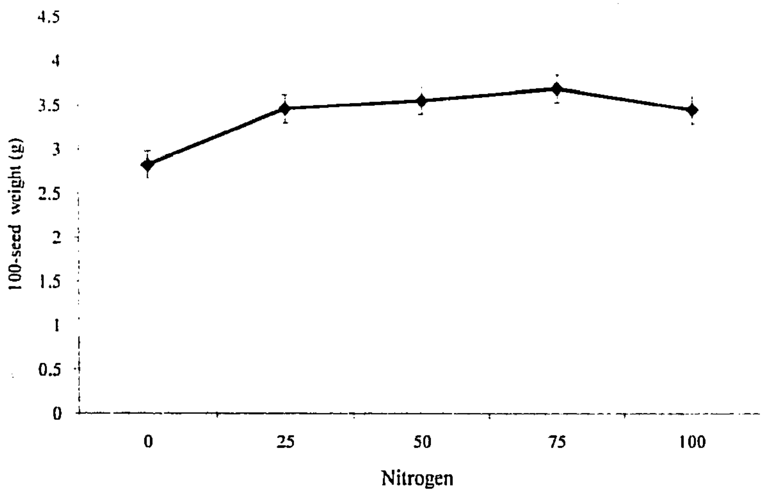

Figure 5. Effect of nitrogen on 100-seed weight in safflower, bars are standard error.

Table 5. Effect of N on safflower capitula diameter and seed number per capitulum

Nitrogen (kg/ha)	Capitula diameter (mm)	Seed number per capitulum
0	24.01a	31.9a
25	24.07a	32.5a
50	24.82a	37.5a
75	24.73a	37.1a
100	24.70a	34.4a
Significance	NS	NS
LSD	1.13	6.62

NS not significant at $P = 0.05$; Means separated using the Least Significant Difference (LSD) at $P = 0.05$, means followed by the same letter within column(s) are not significantly different.

Table 6. Effect of P on safflower capitula diameter and seed number per capitulum

Phosphorus (kh/ha)	Capitula diameter (mm)	Seed number per capitulum
0	23.98a	32.2a
25	24.31a	33.8a
50	24.36a	34.9a
75	24.22a	34.3a
Significance	NS	NS
LSD	1.01	5.83

NS not significant at P = 0.05; Means separated using the Least Significant Difference (LSD) at P = 0.05, means followed by the same letter within column(s) are not significantly different.

4.4 Seed yield

There was no significant ($P > 0.05$) effect of N and P interaction on safflower seed yield, but N and P independently significantly ($P < 0.001$) increased safflower seed yield (Figure 6, 7). Nitrogen application at 25, 50, 75 and 100 kg/ha significantly ($P < 0.001$) increased safflower seed yield compared to plants applied with no N (Figure 6). The response of safflower plants applied with N with respect to seed yield was quadratic, with maximum seed yield obtained with application of 75 kg N/ha (Figure 6). Also P fertilizer application at 25, 50 and 75 kg/ha significantly ($P < 0.001$) increased safflower seed yield compared to plants that received no P application (Figure 7). The response of safflower plants to increasing P application with respect to seed yield was quadratic, with maximum seed yield obtained with application of 50 kg P/ha (Figure 7).

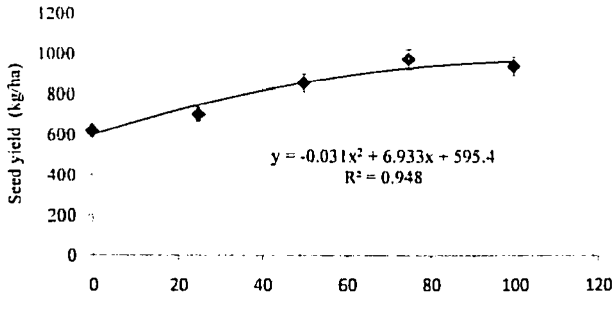

Figure 6. Effects of nitrogen on seed yield in safflower, bars are standard error.

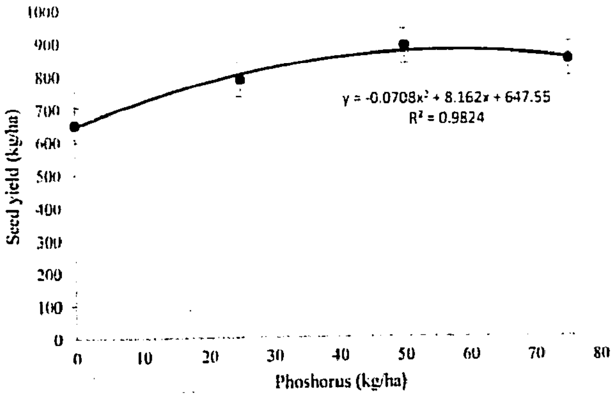

Figure 7.Effect of phosphorus on seed yield in safflower, bars are standard error.

4.5 Seed protein

Nitrogen and phosphorus fertilizer application significantly ($P < 0.0002$) interacted to increase the safflower seed protein content (Figure 8). Increasing both N and P application rates significantly ($P < 0.002$) increased safflower seed protein content compared to control plants and plants applied with N or P alone (Figure 8). Maximum seed protein content of 28.9% was obtained with application of a combination of 100 kg N/ha and 50 kg P/ha (Figure 8).

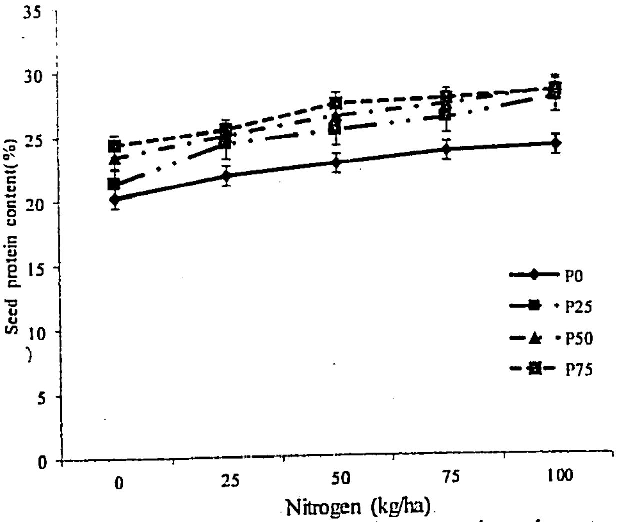

Figure 8. Effect of N and P interaction on seed protein content in safflower, bars are standard error.

4.6 Oil content

Nitrogen and phosphorus fertilizer application significantly ($P < 0.0001$) interacted to increase the safflower seed oil content (Figure 9). Increasing both N and P application rates significantly ($P < 0.001$) increased safflower seed oil content compared to control plants and plants applied with N or P alone (Figure 8). Maximum seed oil content of 39.2% was obtained with application of a combination of 100 kg N/ha and 50 kg P/ha (Figure 8).

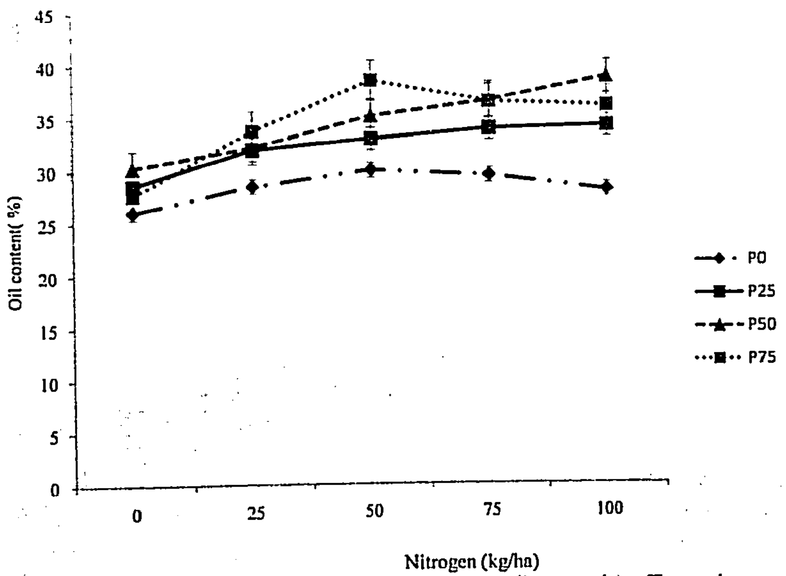

Figure 9. Effect of N and P interaction on oil content in safflower, bars are standard error.

4.7 Leaf N and P contents

Nitrogen and phosphorus fertilizer application significantly ($P < 0.0001$) interacted to increase the leaf N and P contents (Figure 10, 11). Increasing both N and P application rates significantly ($P < 0.001$) increased leaf N content compared to either control plants or plants applied with N or P alone (Figure 10). Increasing both N and P fertilizer application generally increased N leaf content (Figure 10). Maximum leaf N content was observed in safflower plants with a combination of 100 kg N/ha and 75 kg P/ha (Figure 10). Similarly, increasing both N and P application rates significantly ($P < 0.001$) increased leaf P content compared to either control plants or plants applied with N or P alone (Figure 11). Increasing both N and P fertilizer application increased P leaf content (Figure 11). Maximum leaf P content was observed in safflower plants with a combination of 100 kg N/ha and 75 kg P/ha (Figure 11).

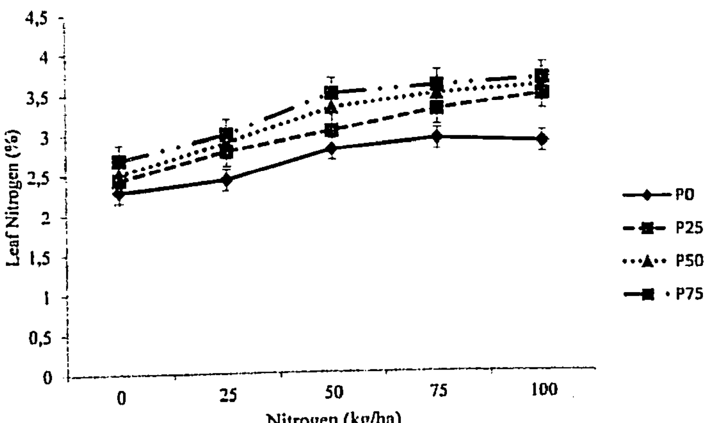

Figure 10. Effect of N and P interaction on leaf N content in safflower, bars are standard error.

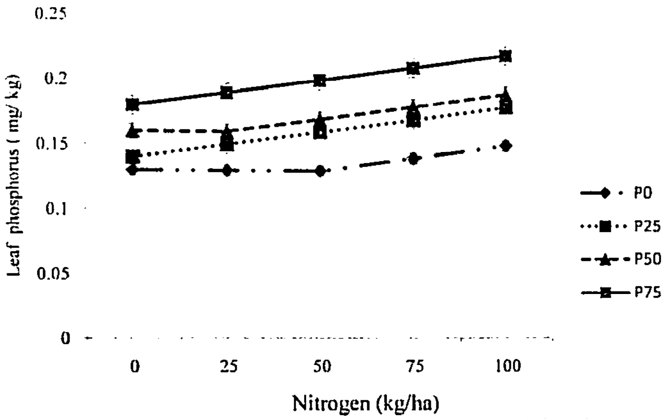

Figure 11 . Effect of N and P interaction on leaf phosphorus content in safflower, bars are standard error.

CHAPTER 5

5.0 DISCUSSION

Safflower is a newly introduced oil crop in Botswana, there are no specific crop management packages for this crop. These field trials were carried out to optimize N and P fertilization for production of safflower under semi-arid conditions of Botswana.

5.1 Soil analysis

The soil analysis done at the start of the experiment showed that the soil pH was slightly acidic with low N, P, CEC and organic carbon. Application of fertilizers, limestone ammonium nitrate (LAN) and single superphosphate (SSP) had no significant ($P > 0.05$) influence on the soil pH, N, P, OC and CEC, at the end of the experiment, but application of SSP significantly ($P < 0.05$) increased soil P at the end of the experiment. The increase in soil P due to single superphosphate application was attributed to the 10.5% P in the fertilizer. The higher P in plots applied with SSP fertilizer than control plots could also be explained by the low P leached beyond the root zone unlike N (Marschner, 2005). Application of LAN fertilizer had no significant effect on total soil N at the end of the experiment compared to the start of the experiment. Emongor *et al.* (2004) and Emongor *et al.* (2012) reported that Sebele soils are deficient in P and N, but low in CEC and organic carbon. Ademba (2008) and Zhu and Zhu (2015) reported that N fertilizers do not only enhance soil N when applied, but also beneficial to soil fauna which are important in the mineralization and nitrification of soil N.

5.2 Effects of N and P on vegetative growth

Nitrogen is one of the mineral nutrients that are required in larger quantities than any other element for the completion of the plant's life cycle (Mengel and Kirkby, 2001; Marschner, 2005), affecting the growth, development, yield and quality of crops (Eckert, 2010). Plants respond quickly to increased availability of N (Mengel and Kirkby, 2001; Marschner, 2005). Nitrogen application in the current study significantly ($P < 0.0017$) increased safflower plant height and had a non-significant ($P > 0.05$) increase in primary branches. The increase in vegetative growth (plant height and primary branch number) due to N fertilizer application was attributed to the role of N in cell division, cell elongation as well as photosynthesis since leaf N content significantly ($P < 0.001$) increased with increase in both N and P fertilizer application in the current study. Nitrogen is also a major structural cell component of protoplasm, proteins, enzymes, coenzymes, vitamins, chlorophyll and other cell components essential for safflower growth and development (Salisbury and Ross, 1992; Mundel *et al.*, 1997; Mengel and Kirkby, 2001; Marschner, 2005; Weinberg *et al.*, 2007). Tunçtürk and Yildirim (2004) reported that application of N at 40, 80, and 120 kg/ha significantly ($P < 0.05$) increased safflower plant height compared to control plants. While Siddiqui and Oad (2006) reported that application of N to safflower at 30, 60, 80, 120, 150, and 180 kg/ha increased plant height and branches number per plant than control plants, but that these variables peaked at 120 kg N/ha. In the current study, safflower plant height and primary branch number peaked at 75 kg N/ha. However, Strašil and Vorlíček (2002) in the Czech Republic reported that N application at 40 or 80 kg/ha had no effect on safflower vegetative growth. Similar results were also reported by Malek and Ferri (2014) and Haghighati (2010) who reported that application of 30, 60 or 90 kg N/ha had no significant ($P > 0.05$) effect on safflower plant height of safflower. Increased supplies of N are reported to increase leaf area index and thereby increased light absorption, enhanced total dry matter production and accumulation in various plant parts, explaining the increase in safflower vegetative growth (Mündel *et al.*, 1997; Weinberg *et al.*, 2007).

5.3 Effects of N and P on yield components

The yield components of safflower are seed weight, plant height, first branch height, number of branches, capitula diameter, number of seed per capitulum and number of capitula per plant (Gonzalez *et al.*, 1994; Omidi-Tabrizi, 2000; Bagheri *et al.*, 2001; Camas and Esendal, 2006; Emongor *et al.*, 2013; Emongor *et al.*, 2015; Moatshe *et al.*, 2016). Chaundry (1990) reported that for selection of high yielding varieties, number of seeds per capitulum, number of capitula per plant and 1000-seed weight could be used as a primary selection criterion while Ahmadzadeh *et al.* (2012) reported a positive correlation between the number of seeds per head, 100-seed weight, days to 50% flowering on grain yield of safflower genotypes.

In the current study, application of N, P and the interaction of N and P had no significant ($P > 0.05$) effect on capitula diameter and seed number/capitulum, but the interaction of N and P significantly ($P < 0.001$) increased safflower capitula number/plant. Nitrogen and P interaction increased safflower capitulum number/plant because of the role of these nutrients in crop physiology (Mengel and Kirkby, 2001; Marschner, 2005; Emongor *et al.*, 2017). Nitrogen affects growth, development, yield and quality of crops (Eckert, 2010) and it is a major component of many essential plant organic compounds such as chlorophyll and coenzymes (Green *et al.*, 1995; Raven *et al.*, 1999; Heidari and Mohammad, 2012), nucleic acids, 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.*, 1995; Mengel and Kirkby, 2001; Marschner, 2005). A good supply of N stimulates root growth and development, the uptake of other nutrients, encourages vegetative growth, giving leaves a deep green colour (Marschner, 2005; Heidari and Mohammad, 2012). While, P is an essential part of phospholipids which are important in cellular membrane structure (Marschner, 2005; Sanchez, 2007). Phosphorus is also an essential component of ATP (Mengel and Kirkby, 2001; Marschner, 2005; Havlin *et al.*, 2013). Synthesized through respiration and photosynthesis, ATP contains a high energy phosphate group that drives most energy-requiring biochemical processes in plants (Marschner, 2005). Adequate P nutrition enhances many aspects of plant physiology, including the fundamental processes of photosynthesis, flowering, fruiting (including seed production), and maturation (Raven *et al.*, 1999; Marschner, 2005; Adebooye *et al.*, 2010). Phosphorus also promotes the growth and development of lateral roots and rootlets (Brady and Weil, 1996; Mengel and Kirkby, 2001; Marschner, 2005; Adebooye *et al.*, 2010). Phosphates form the rails of the nucleic acids, DNA and RNA (Terrence, 2009). Application of 75 kg N/ha plus 50 kg P/ha in the current study significantly ($P < 0.001$) increased capitula number/plant than any other N and P combination or N and P application alone. El-Nakhawy (1991) and Golzarfar *et al.* (2012) reported that application of P or N alone resulted in lower yield components than when N and P are added together. El-Nakhawy (1991) reported that application of 46 N + 46 P, 92 N + 46 P, 138 N + 46 P kg/ha increased 100-seed weight of safflower by 8, 18.7, and 19.2 %, respectively, compared to control plants where no fertilizer was applied. They further reported that application of 46 N + 46 P, 92 N + 46 P, 138 N + 46 P kg/ha increased capitula number/plant and seed weight/capitulum 10.6, 43.2, 35.7%, and 17.6, 54.4, 32.4%, respectively, compared to control plants. 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, 150 N + 100 P_2O_5 kg/ha significantly ($P < 0.05$) increased capitula number/primary branch, capitula number/plant, capitula number/secondary branch, seed number/secondary capitulum, and 1000-seed weight compared to safflower plants applied with no fertilizer or N or P alone.

In the current study, N fertilizer application significantly ($P < 0.05$) increased 100-seed weight. Increasing N application up to 75 kg/ha significantly ($P < 0.05$) increased 100-seed weight compared to control plants. Application of N above 75 kg/ha resulted in a significant ($P < 0.05$) decrease in 100-seed weight. The increase in 100-seed weight due to N application was attributed to increased N and P uptake observed in the current study. With higher application of

N and P, the uptake of each of these nutrients increased, suggesting substantial accumulation of photosynthates in the vegetative organs (leaves). At the vegetative phase of crop development, vegetative organs behave as sinks, but at the onset of grain filling all the older leaves shift from being sinks to source, remobilizing N and P to the grains (Marschner, 2005). Wang *et al.* (1999) and Sadras and Egli (2008) reported that the final grain weight was related to the grain filling rate and grain filling duration. Ali and Noorka (2013) reported that phosphorus and nitrogen accumulation led to the development of adequate leaf area necessary for the interception and utilization of photosynthetically active radiation, this in turn led to higher dry matter which was then translocated to the sinks, being the seeds. In safflower, weight per seed is reported to be negatively affected by a decrease in photosynthetically active radiation for the period of seed filling (Aguirrezabai *et al.*, 2003). The increase in fruit yield 100-seed weight due to N application was also attributed to the role of N in increasing vegetative growth and total dry matter. Nitrogenous compounds make up a significant part of total dry weight of plants and increase in N and P supply leads to efficient utilization of carbohydrate to form protoplasm hence increase in yield components (Lamido, 1994; Marschner, 2006). Sabo *et al.* (2013), Agba and Enga (2006) and Lawal (2000) reported that improved supply of nutrients to plants leads to better utilization of carbon and subsequent synthesis of photo-assimilates leading to higher yield components and yield. Similar results on safflower have been reported in literature (Arani *et al.*, 2011; Golzarfar *et al.*, 2012; Abd El-Mohsen and Mahmoud, 2013; Singh and Singh, 2013). Abd El-Mohsen and Mahmoud (2013) reported that application of 0, 40, 80 and 120 kg N/ha to safflower plants significantly ($P < 0.05$) increased 1000-seed weight, capitula number/plant and days to 50% flowering and the response was quadratic to increasing N application rate. Application of 80 kg N/ha gave the highest 1000-seed weight and capitula number/plant, beyond which a decrease resulted in all the yield components traits (Abd El-Mohsen and Mahmoud, 2013). Increasing P application to safflower plants is reported to increase yield components of safflower (Arani *et al.*, 2011; Golzarfar *et al.*, 2012; Singh and Singh, 2013). Singh and Singh (2013) reported that application of 0, 40 and 80 kg P_2O_5/ha significantly ($P < 0.05$) increased capitula number/plant, seed weight/capitula, 1000-seed weight and harvest index. Application of P to safflower plants at 50 and 100 kg/ha significantly ($P < 0.05$) increased capitula number/plant and 1000-seed weight compared to control plants (Golzarfar *et al.*, 2012), while Arani *et al.* (2011) reported that P application at 0, 50, 75 and 100 kg P_2O_5/ha significantly ($P < 0.01$) increased a 1000-seed weight and harvest index of safflower.

5.4 Effects of N and P on seed yield

Nutrient management is one of the critical inputs in achieving high productivity of safflower (Mündel *et al.*, 2004). Safflower is reported to require adequate supply of nutrients for its full production potential even under limiting moisture conditions (Rao, 1985; Reddy *et al.*, 1996; Kubsad *et al.*, 2001). Nitrogen and P are reported in literature to be two key essential nutrients for safflower growth and development, therefore, optimizing their rate of application will increase safflower seed yield and oil content with minimum environmental pollution risk (Belandger *et al.*, 2000; Antoniadou and Wallach, 2002; Henke *et al.*, 2007). Nitrogen and P independently significantly ($P < 0.001$) increased safflower seed yield. The response of safflower plants to applied N with respect to seed yield was quadratic, with maximum seed yield obtained with application of 75 kg N/ha. The response of safflower plants to increasing P application with respect to seed yield was quadratic, with maximum seed yield obtained with application of 50 kg P/ha. The increase in seed yield in response to increasing N and P fertilizer application was attributed to N and P induced increase in plant height, primary branch numbers, capitula number/plant and 100-seed weight which are yield components of safflower. The increase in safflower seed yield due to N and P fertilizer application because of the role of these nutrients in promoting root growth and development, uptake of other nutrients, vegetative growth and chlorophyll biosynthesis (Marschner, 2005; Heidari and Mohammad, 2012). Nitrogen and P are important in seed set, seed growth, seed filling and final seed yield of crops (Marschner, 2005). Seed set, seed growth, seed filling, and seed yield are determined primarily by the size of N and P pool in the vegetative parts of plants (Schilling and Trobisch, 1970; Mengel and Kirkby, 2001; Marschner, 2005).

The increase in seed yield due to N and P fertilizer application in the current study was also attributed to the roles of N and P as constituents of protein structure, nucleic acids (DNA and RNA), apoenzymes, coenzymes (ATP and FAD) and prosthetic groups (chlorophyll and cytochromes) (Mengel and Kirkby, 2001; Marshner, 2005). Greater N supply increases leaf expansion resulting in increased interception and efficient utilization of solar radiation leading to greater accumulation of dry matter in the leaves and shoots, hence high safflower seed yield (Ahmed *et al.*, 2007). Application of P might have resulted in better root penetration and proliferation contributing to the production of photosynthates and their translocation to sinks (seeds) (Hemalatha *et al.*, 2013). Ali and Noorka (2013) reported that N and P accumulation led to the development of adequate leaf area necessary for the interception and utilisation of photosynthetically active radiation, this in turn led to higher dry matter translocated to the sinks (seeds). Variation in dry matter and seed yield of safflower in response to N and P nutrition has been reported from differences in the amount of photosynthetically active radiation intercepted by the crop canopy, and how efficiently the plants use this radiation (Papakosta and Gagianas, 1991; Koutroubas *et al.*, 2004). Grain growth is supported by photosynthetic activities of leaves and inflorescences and also by translocation of stored photosynthates in the plant canopy. Dry matter, seed yield, and N and P accumulation in the safflower plants prior to anthesis is an important source of photosynthetic products, and N and P compounds for grain growth and development (Papakosta and Gagianas, 1991; Koutroubas *et al.*, 1998a, b; Koutroubas *et al.*, 2004). In the current study, increasing N and P application increased the leaf N and P content which might have increased safflower seed yield as explained above. Zaman and Das (1990) and Dordas and Sioulas (2008) reported that N application to safflower of up to 200 kg/ha plants led to increased leaf N and chlorophyll contents leading to an increase in photosynthetic rate and stomatal conductance. The increase in photosynthetic rate and stomatal conductance resulted in enhanced water use efficiency and net increase in safflower yield (Zaman and Das, 1990; Dordas and Sioula, 2008). Nitrogen and P fertilization is also reported to affect dry matter, as well as N and P accumulation and partitioning into various parts of safflower plants (Dordas and Sioulas, 2009).

Findings similar to those from the current study are reported in literature (El-Nakhlawy, 1991; Mirzakhani *et al.* 2009; Haghighati, 2010; Golzarfar *et al.*, 2011). For instance, Golzarfar *et al.* (2011) reported that application of 75 N + 50 P_2O_5, 75 N + 100 P_2O_5, 150 N + 50 P_2O_5, 150 N + 100 P_2O_5 kg/ha significantly ($P < 0.05$) increased safflower seed yield. These researchers observed that maximum safflower seed yield was produced by application of 150 N + 100 P_2O_5 kg/ha, constituting an increase of 325% compared to the control plants. Haghighati (2010) reported that application of N and P at 30 N + 30 P, 30 N + 60 P, 60 N + 30 P, 60 N + 60 P, 90 N + 30 P Kg/ha increased the seed yield with a maximum being observed at 90 N + 30 P. Furthermore, Mirzakhani *et al.* (2009) observed that with application of 50 N + 25 P, 100 N + 50 P, and 150 N + 75 P kg/ha to safflower significantly ($P < 0.05$) increased seed yield by 16.4%, 55.4% and 28.3%, respectively, compared to the control plants. Maximum safflower seed yield of 1462 kg/ha was produced by plants fertilized with 100 N + 50 P kg/ha (Mirzakhani *et al.*, 2009). El-Nakhlawy (1991) reported that application rate of 46 N + 46 P, 92 N + 46 P and 138 N + 46 P kg/ha significantly ($P < 0.05$) increased safflower seed yield, by 25.5%, 70%, 74% , respectively, compared to the control plants. In the current study maximum seed yield of 984 and 895 kg/ha was observed by application of 75 kg N/ha and 50 kg P/ha, respectively. Nasir *et al.* (1978) found that 75 kg N/ha was adequate for optimum seed, oil, and protein yield, and higher N rates (up to 600 kg/ha) did not improve safflower seed yield and yield components. Haby *et al.* (1982) reported that 275 kg N/ha which came both from fertilizer and N mineralization from the soil was enough to produce high safflower seed yield. Malek and Ferri (2014) reported that application of 0, 30, 60 and 90 kg N/ha significantly ($P < 0.01$) increased safflower seed yield. Singh and Singh (2013) reported that application of N at 40 or 80 kg/ha to safflower plants significantly ($P < 0.05$) increased seed yield compared to seed yield from control plants. Abd El-Mohsen and Mahmoud (2013) reported that N fertilizer application at 0, 40, 80, and 120 kg/ha increased safflower seed yield and the response was quadratic with increase in N application rate. The best seed yield was obtained with 80 kg N/ha, beyond which safflower seed yield significantly ($P < 0.05$) decreased (Abd El-Mohsen and Mahmoud, 2013). In California (USA), it is recommended to apply 110-170 kg N/ha to irrigated safflower depending on soil type (Kaffka and Kearney, 1998). While in Germany the recommended N and P fertilizer rates are 50-70 and 50 kg/ha, respectively (Elbassam, 1998, Elfadl *et al.*, 2009). Phosphorus application to safflower plants has been reported to increase seed yield (Malek and Ferri, 2014; Singh and Singh, 2013; Golzarfar *et al.*, 2012; Golzarfar *et al.*, 2011; Arani *et al.*, 2011; Haghighati, 2010; Purvimath *et al.*, 1993). Singh and Singh (2013) reported that application of 40 and 80 kg P_2O_5/ha to safflower plants significantly ($P < 0.05$) increased seed yield compared to control plants, however, there was no significant ($P > 0.05$) difference in seed yield of plants fertilizer either with 40 or 80 kg P_2O_5/ha. Golzarfar *et al.* (2012) reported that safflower plants fertilized with 50 and 100 kg P_2O_5 kg/ha increased seed yield compared to control plants. Phosphorus application significantly ($P < 0.05$) increased seed yield by 23.5 and 36.5%, respective to 50 and 100 kg P_2O_5/ha compared to control plants (Golzarfar *et al*., 2012). However, no significant N and P interaction effects on safflower seed yield has was reported by Haghighati (2010) and Malek and Ferri (2014). The variability observed in literature and the results of the current study on optimum N and P to optimize safflower seed yield was attributed to differences in climate, soil type, genotypes, and crop management issues such as N and P fertilization regimes.

5.5 Effect of N and P on seed oil and protein contents

Oil content of safflower seeds is a very important economic trait and is considered one of the most important factors affecting the success of safflower introduction in new areas or regions (Bassil and Kaffka, 2002; Camas *et al*., 2007). Safflower oil seed content is influenced by cultivar (genotype), location, soil type, climate and agronomic practices such as plant mineral nutrition (Rahamatalla *et al*., 2001; Zhang and Chen, 2005; Koutroubas and Papadoska, 2005; Gawand *et al*., 2005; Moatshe *et al*., 2016).

In the current study, N and P fertilizer application significantly ($P < 0.0001$) interacted to increase safflower seed oil and protein contents. Maximum seed oil and protein contents of 39.2 and 28.9% was obtained, respectively, with a combined application of 100 kg N/ha and 50 kg P/ha. The increase in safflower seed oil content due to N and P fertilizer application was attributed to the role of N and P in the synthesis of storage lipids and oils. The content of storage lipids and oils in plants is closely related to the supply of both N and P (Baringer, 1966; Bahl *et al*., 1997; Gugel and Falk, 2006; Johnson and Gesch, 2013; Jiang *et al*., 2013; 2014). Nitrogen application in the absence of P decreases seed oil content of some oil crops such as sunflower (Bahl *et al*., 1997; Abbadi and Gerendas; 2009), camelina (Gugel and Falk, 2006; Urbaniak *et al*., 2008; Jiang *et al*., 2014) and canola (Taylor *et al*., 1997; Kockings *et al*., 1997; Saleem *et al*., 2001; Jan *et al*., 2002; Tahir *et al*., 2003). The inverse relationship of N application and seed oil content in absence of P is attributed to the degradation of carbohydrates in the tricarboxylic acid (TCA) cycle due to the application of N which are further degraded to acetyl coenzyme A (Acetyl CoA) and thus, there would be more protein in plant cells with increasing supply of N (Bahl *et al.*, 1997; Tahir *et al.*, 2003; Aminpanah, 2013; Jiang *et al.*, 2014). Similar inverse relation between oil and protein content has been reported (Bahl *et al.*, 1997; Tahir *et al.*, 2003; Aminpanah, 2013; Jiang *et al.*, 2014). Fatty acids and amino acids biosynthesis are generally known to compete for carbon skeletons and energy metabolism (Gehringer *et al.*, 2006; Jiang *et al.*, 2014) and the availability of carbohydrates reduces oil synthesis with increasing N supply (Rathke *et al.*, 2005: Jiang *et al.*, 2013). In safflower, high N application in the range higher than 80 kg/ha and without P application depending on climatic conditions, locality, soil type and genotype is reported to lower seed oil content (Dordas and Sioulas, 2004; Tunctürk and Yildirim, 2004; Soleimani, 2010; Bitaranfan *et al.*, 2013). Safflower plants applied with 40 and 80 kg N/ha significantly ($P < 0.05$) produced higher seed oil content of 33.3 and 33.5%, respectively, than control plants that produced seed oil content of 32.4% (Singh and Singh, 2013). Application of N at 40, 80 and 120 kg/ha to safflower plants was also reported to significantly ($P < 0.01$) increase safflower seed oil content and oil yield compared to seed oil content and oil yield from control plants (Abd El-Mohsen and Mahmoud, 2013). Maximum seed oil content (33.9%) and oil yield (594.7 kg/ha) was produced with fertilizer application to safflower plants of 80 kg N/ha (Abd El-Mohsen and Mahmoud, 2013). Bitarafan *et al.* (2011) reported that application of N at 50, 100 and 150 kg/ha significantly ($P < 0.05$) increased the seed oil content of safflower compared the seed oil content from control plants, but application of N beyond 100 kg N/ha decreased the seed oil content. Soleimani (2010) reported that application of 50, 75, 100, 125, and 150 kg N/ha to safflower plants increased the seed oil content and oil yield, but application of 100 kg N/ha gave the highest seed oil content and oil yield of 29.9% and 755 kg/ha. Elfadl *et al.* (2009) reported that application of 0, 40 and 80 kg N/ha had no significant ($P > 0.05$) effect on safflower seed oil content and oil yield. Dordas and Sioulas (2008) did not find any relationship between rates of N and safflower seed oil content. Tunçtürk and Yildirim (2004) reported that N application at 40 and 80 kg/ha significantly ($P < 0.05$) increased safflower seed oil content compared to seed oil content of control plants, however, application of N beyond 80 kg N/ha significantly ($P < 0.05$) decreased the seed oil content.

Lipids comprise of a group of naturally occurring molecules such as fats, waxes, sterols, fat-soluble vitamins (vitamins A, D, E, and K), monoglycerides, diglycerides and phospholipids (Subramaniam *et al.*, 2011; Mashaghi *et al.*, 2013). Fats are a subgroup of lipids called triglycerides (Vance and Vance, 2002). Phosphorus is required for the formation of fats, being a component of the glycerol unit and it is needed for conversion of acetate units into fatty acids (Salisbury and Ross, 1992). Phosphorus being a component of the glycerides explains why application of P increased the oil content of safflower yield. Phosphorus is also known to play a role in carbohydrate metabolism and helps in the conversion of carbohydrate into oil (Salisbury and Ross, 1992; Bahl *et al.*, 1997; Vance and Vance, 2002; Subramaniam *et al.*, 2011; Mashaghi *et al.*, 2013). Singh and Singh (2013) reported that application of 40 and 80 kg P_2O_5/ha to safflower plants significantly ($P < 0.05$) increased the oil seed content compared to control plants. While, Arani *et al.* (2011) reported that P application at 0, 50, 75, and 100 kg/ha significantly ($P < 0.05$) increased safflower seed oil yield. The response of safflower seed oil yield to increasing P application was linear (Arani *et al.*, 2011). El-Nakhlawy (1991) reported that application of 46 N + 46 P, 92 N + 46 P and 138 N + 46 P kg/ha decreased the oil content of safflower seed by 2.8%, 3.3% and 4.9%, respectively, compared to the control plants.

Nitrogen and P fertilizer application interacted to increase the safflower seed protein content in the current study. Nitrogen and P are essential nutrients for growth and are key limiting factors in agrosystems. Nitrogen is a constituent of amino acids, which are required for the synthesis of proteins and other related compounds hence explaining the increase in seed protein content with increasing N (Walch-Liu *et al.*, 2000; Mengel and Kirkby, 2001; Marschner, 2005; Aminpanah, 2013; Jiang *et al.*, 2013). Nitrogen and P play important roles in all plant metabolic processes (Mengel and Kirkby, 2001; Marschner, 2005). Nitrogen and P supply is reported in literature to improve efficient utilization of carbohydrates to form protoplasm hence increase in seed protein content (Lamido, 1994; Marschner, 2006).

5.6. Effects of N and P on leaf N and P contents

Nitrogen and phosphorus fertilizer application significantly ($P < 0.0001$) interacted to increase the leaf N and P contents. Maximum leaf N and P contents was observed in safflower plants with a combination of 100 kg N/ha and 75 kg P/ha. Application of N and P fertilizer increased the uptake and partitioning of N and P to the safflower leaves hence explaining the increase in leaf N and P contents in plants applied with fertilizers. Inorganic and organic fertilizers are sources of essential mineral nutrients such as N and P (Mengel and Kirkby, 2001; Marchner, 2005). Nitrogen and P fertilizer application improves the uptake and partitioning of these nutrients within plants (Mengel and Kirkbery, 2001; Marschner, 2005; Emongor *et al.*, 2012).

CHAPTER 6

6.0 CONCLUSION AND RECOMMENDATIONS

Based on vegetative growth, yield, yield components and oil content of safflower, it was concluded that under sandy loam soils of Botswana, the optimal N and P fertilizer application rate to optimize safflower seed yield and oil content was 75 kg N/ha and 50 kg P/ha. Application of N and P beyond 75 and 50 kg/ha, respectively, resulted in decrease in safflower yield, yield components and oil content. It is recommended that for wider application of these fertilizer rates in Botswana, N and P trials be undertaken in various parts of the country under different soil types, irrigation regimes and rain-fed agriculture to confirm the current findings.

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