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Short Communication

EVALUATION OF THE IMPACT OF CHEMICAL AND BIOLOGICAL FERTILISER APPLICATION ON AGRONOMICAL TRAITS OF SAFFLOWER (*Carthamus tinctorius* L.)

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*In order to investigate the influence of biological and chemical fertilisers on morphological traits, yield and yield components of safflower (*Carthamus tinctorius *L.), a field experiment was conducted in Maragheh (37°23' N; 46°16' E), in north western Iran, for one year in the 2014 growing season. The effect of seven treatments was evaluated: T_1 – control (no fertiliser application), T_2 – seed inoculation with P biofertiliser (contains phosphate solubilising bacteria;* Pantoea agglomerans *strain P5 and* Pseudomonas putida *strain P13), T_3 – seed inoculation with N biofertiliser (contains* Azotobacter vinelandii *strain O4), T_4 – foliar application of iron chelate, T_5 – soil application of complete NPK, T_6: foliar application of manganese chelate, and T_7 – foliar application of zinc sulphate. The result showed that although application of N biofertiliser did not have a significant effect on the evaluated traits, P biofertiliser slightly improved grain yield. However, the application of complete NPK fertiliser improved most of the traits, compared to the control and biofertiliser treatment. The best performance was obtained by foliar application of manganese chelate and zinc sulphate. The results showed that micronutrient-deficiencies have to be managed to unlock the potential yield of safflower in semiarid production systems.*

Key words: *iron, manganese, nitrogen, phosphorus, yield components, zinc.*

Safflower (*Carthamus tinctorius* L.) is a highly branched, herbaceous, thistle-like annual plant from the Compositae or Asteraceae family. Safflower production is mainly concentrated in arid and semi arid regions, since it is drought tolerant and has high adaptability to low moisture conditions. This may be due to deep and numerous thin horizontal lateral roots, which allow safflower to survive in periods of moisture shortage. In semi-arid regions, the loss of organic matter and low fertility are of great concern. This is partly due to low biomass production and high rates of organic-matter decomposition. Consequently, nutrient deficiencies or nutrient imbalance as a result of decline in soil organic carbon status in the rainfed areas of semi-arid regions has been reported over for a long period of time (Sahrawat and Wani, 2013). Soils of these areas often have low to medium levels of available phosphorous (P), medium to high levels of potassium (K), and are low in iron (Fe), manganese (Mn) and zinc (Zn). Therefore, nutrient management is one of the important approaches in achieving high productivity of safflower, and one of the most important strategies regarding improvement of safflower yield and quality in semi arid region is balanced plant nutrition.

Micronutrient deficiencies are widespread in semiarid regions. Although micronutrients are required in relatively small quantities for sufficient plant growth and production, their shortage can result in pronounced interruption in physiological and metabolic processes and can significantly affect plant growth. Iron plays an imperative role in photosynthesis, mitochondrial respiration, nitrogen assimilation, phytohormone biosynthesis (ethylene, gibberellic acid, jasmonic acid), scavenging of reactive oxygen species, osmoprotection, and pathogen defence (Hänsch and Mendel, 2009). Moreover, zinc is another important micronutrient and is a component of many enzymes involved in carbohydrate metabolism, maintenance of the integrity of cellular membranes, protein synthesis, regulation of auxin synthesis and pollen formation (Hafeez *et al*., 2013). In addition, manganese plays a direct role in photosynthesis, germination and maturity and formation of aromatic amino acids, lignins, flavonoids, and the phytohormone indole acetic acid (Hänsch and Mendel, 2009; Hebbern *et al*., 2009).

Biofertilisers have the potential to improve health and productivity of crop plants and reduce the need to use synthetic chemical fertilisers. Biofertilisers are substances that include variuos living and beneficial organisms, such as bacteria, which when applied on seed, shoots or soil, colonise the root zone or the inside of the plant and promote growth by improving supply or accessibility of the main nutrients to the host plant (Vessey, 2003). Two major groups of these beneficial bacteria are phosphate-solubilising bacteria and nitrogen-fixing rhizobacteria. Considering the importance of nutrient management, particularly regarding micronutrients (e.g. zinc, iron and manganese), and the significance of biological fertiliser for sustainable agriculture in semi arid regions, this study was carried out to investigate the effect of inorganic and biological fertilisers on growth, yield and yield component of safflower (*Carthamus tinctorius* L.) in a semi-arid highland environment.

The research was carried out in the research field of the College of Agriculture, University of Maragheh (37°23' N; 46°16' E), Maragheh, in northwest Iran, for one year in the 2014 growing season. Maragheh is located at an altitude of 1485 m above sea level in the highland semi-arid zone. The experiment was carried out in randomized complete block design with three replicates to investigate effects of chemical and biofertilisers on morphophysiological traits of safflower (*Carthamus tinctorius* L.). The previous crop on the plots was Lentil (*Lens culinaris*). Safflower grows best on deep, fertile, well-drained loam soils with good water-holding capacity. The soil texture was sandy loam containing 0.14% organic matter (OM); pH was 7.57. The soil texture was 53% sand, 31% silt, and 16% clay. Other soil parameters were electrical conductivity (EC) = 1.96 $ds \cdot m^{-1}$, 0.058% nitrogen (N), 5.67 available phosphorus ($mg \cdot kg^{-1}$), and 342 $mg \cdot kg^{-1}$ available potassium (K). Soil pH was determined in 1:1 (V/V) soil:water extract. Spring safflower (*Carthamus tinctorius* L.) CV. 'Esfahan' was used in the experiment. Seeds were hand planted on 21 April. Each plot had area 4 m^2 and consisted of eight rows, 2 m long and 25 cm apart. Seeds were sown 4 cm apart at 5 cm depth. There was no incidence of pests or disease on plants during the experiment. Weeds were controlled over the growth period with hand hoeing. Irrigations was applied regularly towards late June, when 60 percent of the total available soil water was depleted. The treatments comprised of T_1 — control (no fertiliser application), T_2 – seed inoculation with P biofertiliser (contains phosphate solubilising bacteria; *Pantoea agglomerans* strain P5 and *Pseudomonas putida* strain P13), T_3 — seed inoculation with N biofertiliser (contains *Azotobacter vinelandii* strain O4), T_4: foliar application of iron chelate (2000 $mg \cdot kg^{-1}$ or 0.2%; Fe-EDTA), T_5 – soil application of complete NPK 20-20-20 (90 kg $fertiliser \cdot ha^{-1}$), T_6: foliar application of manganese chelate (0.2 %; Mn-EDTA), and T_7 — foliar application of zinc Sulphate (0.2 %;). All of the soil and foliar applications were made at the stage of stem elongation (BBCH scale; 32, according to Flemmer *et al.*, 2014) and repeated at pre-bloom stage. All necessary cultural practices and plant protection measures were followed uniformly for all the plots during the entire period of experimentation. Ground cover was evaluated at the flowering stage. The ratio of ground area (%) covered by the crop canopy was estimated visually. Crop phenology was monitored at 1–2-day intervals throughout the season and number of days from sowing to initiation of flowering (BBCH scale: 61; first florets open, upper portion of florets emerges through the bracts), reproductive phase duration (BBCH scale 61–95) and day to maturity were determined. At maturity stage (BBCH scale 95), most of the leaves were coloured brown and very little green remained on the bracts of the latest flowering heads. Yield and yield component were evaluated at maturity stage.

The climate of the location is semi arid and cold temperate with average annual rainfall 375 mm, consisting of 73% rain and 27% snow falling through winter and early spring. Rainfall is not generally well-distributed through the year and the occurrence of rainfall during the late winter and early spring is frequent, with about 10 rainy days per month on the average. Rainfall from June through October is relatively rare, when the highest rate of evapotranspiration is recorded. The precipitation was 77.3 mm during the cropping season. The relative humidity ranges between 28–52% during the growing season. All data were subjected to an analysis of variance (ANOVA) for each character. The MSTATC software package was used for statistical analysis. The least significant difference (LSD) at 5% was used to compare between means. Principal component analysis (PCA) was used to describe several inter-correlated quantitative dependent variables (Everitt and Dunn, 1992). Also, correlation coefficients were computed via plotting of the first two principal component analysis using Minitab version 14.

Analysis of variance for ground cover showed a significant difference among fertiliser treatments at the 5% level (Table 1). Application of NPK, iron chelate, manganese chelate and zinc sulphate significantly improved ground cover over control and biological fertilisers. Analysis of variances for days to maturity showed a significant effect of fertiliser application on this attribute. The Fisher's least significant difference (LSD) test at 5% level of probability showed that plants grown with manganese chelate and zinc sulphate had later maturity in comparison with other treatments (Table 1). Application of fertiliser had a significant effect ($p < 0.01$) on plant height (Table 1). Plant height increased with foliar application of micronutrients fertilisers (Fe, Mn, and Zn). Moreover, application of manganese chelate significantly increased the first capitula height (cm) from ground level (first branch height). However, plants grown with N biofertiliser showed the lowest height of first capitula.

Fertiliser application affected the number of branches $plant^{-1}$. The highest number of branches was recorded in plants grown with zinc sulphate, manganese chelate and P biofertiliser. A similar trend was observed for capitula diameter. The smallest capitula were observed in control plants and those grown with N biofertiliser (Table 1).

Fertiliser treatments extensively affected yield and yield components of safflower (Table 2). The highest number of capitula per plant was obtained by application of manganese chelate and followed by NPK, zinc sulphate and P biofertiliser. Plants grown with manganese chelate had the highest seed number per capitula, significantly differed from the other treatments (Table 2). Mean comparisons for seed weight per capitula showed that foliar application of micronutrient fertilisers produced larger and heavier seeds, in comparison with control and other fertiliser treatments. A similar trend was observed for thousand seed weight.

Table 1

EFFECT OF FERTILISER TREATMENTS ON MORPHO-PHYSIOLOGICAL TRAITS OF SAFFLOWER (*Carthamus tinctorius* L.) PLANTS

Treatment	GC	DF	RPD	DM	PH	FCH	NBP	SD	CD
Control (No. fertiliser)	65.3	73.2	35.1	125	64.2	33.5	5.1	6.9	1.8
N biofertiliser	65.2	74.3	38.9	118	64.6	29.8	4.6	6.8	1.7
P biofertiliser	69.5	72.4	32.3	123	67.3	33.4	6.2	6.6	2.3
Iron chelate	77.6	75.4	39.9	130	69.3	36.2	5.3	7.2	2.0
NPK	75.6	77.3	35.4	128	66.4	33.2	4.4	7.4	2.1
Manganese chelate	83.0	78.2	42.2	138	71.8	38.5	6.7	7.5	2.4
Zinc sulphate	81.3	79.1	39.0	134	73.2	34.2	7.1	7.7	2.1
LSD	7.9	5.6	5.0	5.3	5.7	3.6	0.4	0.7	0.2
Level of significance	*	NS	*	**	*	**	**	NS	**

GC, ground cover percentage; DF, number of day from sowing to initiation of flowering; RPD, reproductive phase duration; DM, day to maturity; PH, plant height (cm); FCH, first capitula height (first branch height); NBP, number of branches plant^{-1}; SD, stem diameter (mm); CD, capitula diameter (mm). If the difference between two treatment means is greater than the least significance difference (LSD), then those treatment means are significantly different at the 5% level of significance. NS, not significant; * significant at 5% level of probability; ** significant at 1% level of probability.

Table 2

MEAN COMPARISON OF YIELD AND YIELD COMPONENTS OF SAFFLOWER (*Carthamus tinctorius* L.) IN RELATION TO FERTILISER TREATMENT

Treatment	NCP	NSC	SWC	USP	TSW	STY	SY	BY	HI
Control (No. fertiliser)	5.8	21.3	0.68	8.4	31.2	3715	1567	5670	27.6
N biofertiliser	5.1	20.5	0.66	7.5	30.2	3992	1623	6123	26.5
P biofertiliser	6.1	21.3	0.71	8.0	34.1	3607	1804	5916	30.4
Iron chelate	5.8	22.0	0.75	7.7	33.2	4148	1741	6455	26.9
NPK	6.7	21.5	0.70	6.8	31.2	3785	2049	6298	32.5
Manganese chelate	7.3	24.1	0.88	5.6	33.3	3973	2396	6947	34.4
Zinc sulphate	6.2	22.2	0.77	6.1	34.5	3852	2131	6653	32.0
LSD	0.5	2.1	0.07	0.5	2.8	464	185	742	2.8
Level of significance	**	*	*	**	**	NS	**	*	*

NCP, number of capitula per plant; NSC, number of seed per capitula; SWC, seeds weight per capitula; USP, unfilled seeds numbers per plants; TSW, thousand seeds weight (g); STY, straw yield (kg·ha^{-1}); SY, seed yield (kg·ha^{-1}); BY, biological yield (kg·ha^{-1}); HI, harvest index. If the difference between two treatment means is greater than the least significance difference (LSD), then those treatment means are significantly different at the 5% level of significance. NS, not significant; * significant at 5% level of probability; ** significant at 1% level of probability.

Fertilisers considerably affected the unfilled seeds number per plant and the lowest number of unfilled seeds was recorded in plants grown with foliar application of manganese chelate, followed by zinc sulphate and NPK (Table 2). Significant effect of fertiliser application on straw yield and biological yield were observed. The highest straw yield was observed in plants grown with iron and manganese chelate and lowest in the control (no applied fertiliser) and P biofertiliser (Table 2). A similar trend was observed for biological yield. Plants grown with manganese chelate, zinc sulphate and NPK produced the highest seed yield compared to other treatments. Although the application of iron chelate produced high biological yield, this status was not reflected in seed production, and for this reason these plants had a relatively low harvest index (Table 2). The highest harvest index was recorded in plants grown with manganese chelate, zinc sulphate and NPK. The results suggest that partitioning of assimilated carbon among the various sink organs was largely affected by application of fertilisers.

The PCA ordination described a significant amount of the total variation. In the ordination, the correlation coefficient between any two traits is approximated by the cosine of the angle between their vectors. Positive correlation was shown (Fig. 1) among seed yield, number of seeds per capitula, ground cover, day to maturity, and plant height; among harvest index, number of capitula per plant, seeds weight per capitula, and thousand seeds weight; and among biological yield, stem diameter and number of the days from sowing to flowering. There was a negative correlation between the number of unfilled seeds per plants and grain yield (Fig. 1), indicated by location of these traits on opposite sides of the ordination.

Fertiliser improved safflower plant establishment by promoting vegetative growth. Although the number of days from sowing to flowering was not significantly affected by fertiliser treatment, mean comparisons by LSD showed a slight increase of this period by application of chemical fertiliser (Table 2). However, Namvar and Sharifi (2011) re-

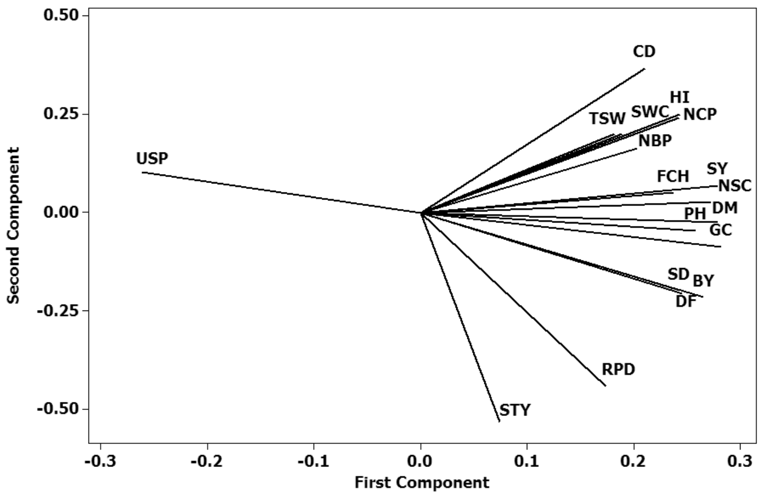

Fig. 1. Plot of the first two PCA axes showing relation among various agronomical traits of safflower. GC, ground cover percentage; DF, number of days from sowing to initiation of flowering; RPD, reproductive phase duration; DM, day to maturity; PH, plant height; FCH, first capitula height (first branch height); NBP, number of branches plant^{-1}; SD, stem diameter; CD, capitula diameter; NCP, number of capitula per plant; NSC, number of seed per capitula; SWC, seeds weight per capitula; USP, unfilled seed numbers per plants; TSW, thousand seed weight; STY, straw yield; SY, seed yield; BY, biological yield; HI, harvest index.

ported that flowering was significantly delayed by increased application of nitrogen fertiliser. In semi arid regions, excessively delayed flowering is not a suitable trait and can lead to a higher temperature in the reproductive stages and a subsequently negative effect on grain yield.

Our study confirmed that complete micronutrient fertilisers are essential for rapid vegetative growth and increased ground cover. This is a high priority in the semiarid regions where efficient soil moisture management considerably improves crop yield. A high percent of ground cover formed early can create a layer between the soil and the atmosphere, which prevents sunlight from reaching the soil surface, thus reducing evaporation. This observation is consistent with the observation of Nie and Zollinger (2012) that application of fertilisation increases ground cover.

Our results revealed that application of micronutrients fertiliser improves vegetative growth, plant height and number of the branches. Ali and Mahmoud (2012) observed also that application of both micro- and macronutrient increased number of branches of safflower. Micronutrients are required in small amounts. They directly or indirectly affect vital processes in plant, such as photosynthesis, respiration, protein synthesis and reproduction (Marschner, 2012). Even though many soils contain sufficient micronutrients, these are often unavailable to the plants.

The height of first capitula height was affected by fertiliser application. Although Ēamaș and Esendal (2006) suggested that first branch height was the least affected trait by environmental factors, our result showed that this trait considerably responded to fertiliser treatment. This trait is important in mechanized harvesting and thus fertiliser application can improve harvest practice.

All of the examined yield components responded to fertiliser application, as observed by Kohnaward *et al.* (2012), who showed that foliar spray of manganese and zinc in cropping systems with medium input significantly promoted the yield and yield component of safflower. It has been revealed that the most important yield component in safflower is the number of capitula per plant. This implies that fertilisation management can significantly affect safflower productivity in semi arid regions. Foliar spraying of Mn, Zn and Fe increased both seed number and seed weight (size). Reduced crop yields can often be explained by photosynthesis (the source of assimilates) or in the sink (the site of assimilate utilisation). Here it seems that application of micronutrients affected the sink size or sink strength. Seed number is related to canopy photosynthesis and assimilate availability during flowering and seed set, while seed size depends on assimilate supply during seed filling (Egli and Bruening, 2003). The results may suggest that manganese chelate and zinc sulphate provided favourable conditions during flowering and seed filling.

Our experiment showed some effectiveness of NPK fertiliser in some traits, while the lack of effectiveness of biofertiliser was quite evident. This finding corroborates the ideas of Roberts (2007) and Janmohammadi *et al.* (2014), who suggested limited effectiveness of biofertiliser in semi-arid regions, due to soil and climatic conditions of this area. However, the low influence of nitrogen fertiliser also can partly be due to the previous crop, since lentils are capable of fixing atmospheric nitrogen. Overall, the best performance of safflower was obtained by foliar spraying of micronutrient fertilisers. Mn and Zn showed the greatest positive impact on growth and yield of safflower. Manganese is necessary for plant metabolism and it has two functions in proteins a) as a catalytically active metal, or b) having an activating role on enzymes (Hänsch and Mendel, 2009). Also zinc is an essential micronutrient and plays a key role as a structural constituent or regulatory cofactor in a wide range of different enzymes and proteins in many important biochemical pathways.

Generally, in the semi-arid regions with Mediterranean climate, apart from water shortage, productivity is limited by poor fertility of the soils. Results of the current study showed that micronutrient deficiency is one of the main causes for decline in safflower production systems. Soil fertility management is a fundamental agricultural practice for sustained increase in safflower productivity and needs to be considered. In this regard, both macronutrients and micronutrients require attention simultaneously along with other management practices.

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ĶĪMISKĀ UN BIOLOĢISKĀ MĒSLOJUMA PIELIETOJUMA IETEKME UZ SAFLORA (*Carthamus tinctorius* L.) AGRONOMISKĀM PAZĪMĒM

Pētīta dažādu bioloģisko un minerālo mēslojumu veidu ietekmē uz saflora (*Carthamus tinctorius* L.) agronomiskām pazīmēm pustuksnešu zonā (Irāna). Parādīts, ka bez ūdens ierobežotības ražības limitējošais faktors ir arī nabadzīgās augsnes, īpaši mikroelementu trūkums. Makro un mikroelementu balanss ir būtisks priekšnoteikums labai agronomiskai praksei.