LRF-730
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RESEARCH ARTICLE   Legume Research- An International Journal, Volume Issue : ()

Application of Iron and Zinc Nanochelates Enhances the Productivity and Nutritional Quality of Intercropping Cowpea (*Vigna unguiculata* L.) and Maize (*Zea mays* L.)

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10.18805/LRF-730

ABSTRACT

Background: Nanofertilizers are a new product containing nutrients available in Nano form scale and are mainly preferred due to their effectiveness.

Methods: This research was conducted to assess a three-replicate randomized complete block design (RCBD) factorial experiment in Roudbar area (Kerman province, Iran) in 2018 and 2019 to investigate the effects of Nano iron and zinc chelates on Nano iron and zinc chelates on agronomic and qualitative traits of cowpea in cover crops was investigated. Treatments included the first factor, the cropping system at five levels of cowpea and maize cropping pattern (PC: pure Cultivation, IC1: 75% maize+ 25% cowpea, IC2: 50% maize+ 50% cowpea, IC3: 25% maize+75% cowpea.) and the second factor, four levels of nano-chelate (nano iron chelate, nano zinc chelate and nano iron chelate + nano zinc chelate) and control treatments.

Result: The highest Protein of seed (28.6%), Grain yield (2813 Kg/ha) and Harvest index (44.4%) was measured for cowpea in treatment IC3 under Zn application alone, while the lowest Protein of seed (16.9%) and Grain yield (1618 Kg/ha) was observed in treatment CPC and Harvest index (36%) in treatment IC2 under Control. The highest Protein of seed (22.2%) in treatment IC1 under Zn application, Grain yield (12050 Kg/ha) in treatment MPC under Fe application and Harvest index (54.8%) was measured for maize in treatment MPC under Fe application, while the lowest Protein of seed (7.4%) and Grain yield (9980Kg/ha) was observed in treatment MPC Control and Harvest index (36.5%) in treatment IC1 under Control for maize. Simultaneously applying nano iron and zinc chelates increased all the quantitative and qualitative traits measured in both crops.

Key words: Cereal, Dietary Zn supply, Fe-Zn interaction, Productivity.

INTRODUCTION

In recent years, there have been new approaches and technological advances and innovations in various fields of agriculture (Dwivedi *et al*. 2016). Nanotechnology in particular has the potential to provide effective solutions to many agricultural problems. Nano fertilizers play an important role in the physiological and biochemical processes of crops by increasing the plant's access to nutrients which ultimately leads to improved growth and increased photosynthesis of the plant (Shang *et al*. 2019). Besides, nanocarriers reduce the accumulation of excess chemicals in the plant system and increase the efficiency of nutrient consumption by delivering nutrients at the right place and time (Mahil and Kumar, 2019). Particle size and surface coating are the most important intrinsic factors affecting nanoparticle application efficiency and extrinsic factors such as organic matter, soil texture, or soil pH also strongly influence potential application (Ma *et al*. 2018). There are various types of nanomaterials such as copper (Cu), aluminum (Al), silver (Ag), gold (Au), zinc (Zn) and zinc oxide (ZnO).

Since most crops worldwide are deficient in certain micronutrients (*e.g.* Zn and Fe), nanofertilizers serve as effective and efficient nutritional fortification products Lopez-Valdez *et al*. (2018). Zinc and iron microelements are also important for plants and play an important role in photosynthesis and the accumulation of carbohydrates (Shesh Bahre and Movahedi Dehnavi, 2012). The application of iron and zinc nanofertilizers increased the number of cowpea leaves in a study by Gheyrati-Arani *et al*. (2013), increased photosynthesis, increased the number of pods per plant and improved cowpea harvest index (HI) in a study by Pierre *et al*. (2017) and increased maize grain yield

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How to cite this article: Khalesi, A., Afsharmanesh, G. and Shirzadi, M.H. (2023). Application of Iron and Zinc Nanochelates Enhances the Productivity and Nutritional Quality of Intercropping Cowpea (*Vigna unguiculata* L.) and Maize (*Zea mays* L.). Legume Research. doi: 10.18805/LRF-730.

Submitted: 05-12-2022 **Accepted:** 06-03-2023 **Online:** 21-04-2023

in a study by Goodarzi *et al*. (2014). Using nanofertilizers can be synthesized according to the nutrient requirements of intended crops Kah *et al*. (2018).

Limited resources and rapid population growth, expected to reach 9.6 billion by 2050, are driving the industry forward by requiring the development of highly efficient agriculture while helping reduce poverty and hunger in the world (Zhang *et al*. 2015). Legumes are one of the important sources of food, rich in protein, for human and animal nutrition, which after cereals are considered as the most important human food source, especially in terms of protein. Nowadays, intercropping is common in a large number of countries (Baumann *et al*. 2002). In the intercropping pattern, cereals have more competitiveness for soil mineral nitrogen (SMN) than legumes due to faster and deeper root growth as well as higher nitrogen requirements (Lithourgidis *et al*. 2011). There are many studies in the field of mixed cropping of cereals and legumes showing that the yield of both crops is increased in the case of intercropping compared to monoculture of these crops (Yu *et al*. 2015; Martin-Guay *et al*. 2018). In this regard, it can be mentioned the mixed cultivation of maize and cowpea. Given the importance of iron and zinc nanofertilizers in improving crop yields, increasing crop resistance and protecting the environment, intercropping is part of crop rotation programs to achieve sustainable crop ecosystems. This study was conducted to investigate the effect of foliar application of nano iron and zinc chelates on quantitative and qualitative traits of cowpea (*Vigna unguiculata* L.) and maize (*Zea mays* L.) in different patterns of intercropping in line with sustainable agricultural goals.

MATERIALS AND METHODS

Site description and planting

A factorial experiment was carried out in a randomized complete block design (RCBD) with the application of nano iron and zinc chelates alone or together in different ratios intercropping of maize and cowpea and in three replications in 2018 and 2019 in Roudbar area (Kerman province, Iran) whit a hot and arid climate (28.22°N, 58.9°E).

Treatments included five levels of cowpea and maize cropping pattern (MPC: Maize pure Cultivation or CPC: Cowpea pure Cultivation, IC1: 75% maize+ 25% cowpea, IC2: 50% maize+ 50% cowpea, IC3: 25% maize+75% cowpea) and four levels of nano-chelate (nano iron chelate, nano zinc chelate and nano iron chelate + nano zinc chelate) and control treatments.

Before the experiment, 2 composite soil samples from 0-30 and 30-60 cm depths of soil profiles were prepared for physical and chemical soil tests. Based on soil test results (Table 1) and the recommendations of soil experts In maize, all triple superphosphate fertilizers, potassium sulfate and one-third of urea fertilizers were distributed in a strip under the seeds of the row spacing at the same time as cropping. The remaining one-third of urea fertilizer was applied as a top dress in the 6-Leaf stage and the remaining one-third was applied when the tassel was formed. In cowpea, 50% of urea fertilizer and all triple superphosphate fertilizer and potassium sulfate fertilizer were distributed at the same time as sowing under seeds and the remaining urea fertilizer was distributed in 20 days, 30 days and 40 days, one-third each time, after cropping as a top dress between row spacing. In total, there were 60 plots with dimensions of 6 $\times$ 3 m. The sowing date for both crops was 31 July and maize KCS703 and local cowpea species were selected. This Late maize hybrid produces one ear per plant. The row spacing for both crops was 75 cm, the distance between plants per row was 16 and 8 cm for maize and cowpea, respectively and the sowing depth was 5 cm for maize and 3 cm for cowpea. Each plot has 4 rows with a space of 75 cm and a length of 6 m (18 m^2). 3 rows of cowpea and one row of maize were sawed in intercropping of 75% cowpea + 25% maize, 2 rows of cowpea and 2 rows of maize were sowed in intercropping of 50% cowpea + 50% maize and one row of cowpea and 3 rows of maize were sowed in intercropping of 25% cowpea + 75% maize. The distance between the plots was 120 cm in sole cropping of cowpea and maize sole cropping and 100 cm in intercropping (Rostami *et al*. 2009). In all treatments, sawing was done by hand after the preparation of ridges and furrows. The first irrigation was done immediately after sowing. The irrigation cycle in sole cropping and intercropping of both crops was considered every 6 days based on soil moisture conditions.

Treatments of nano iron chelate fertilizer (khazra, 9%) and nano zinc chelate fertilizer (khazra, 12%) were prepared by foliar application with concentrations of 1 and 1.5 per thousand, respectively, in two stages: 6-Leaf stage and tassel formation for maize and 10-Leaf stage for cowpea. In the control treatment, a foliar application was done on the leaves with distilled water. The foliar application was done before sunrise to better absorb the solution and minimize evaporation. Sampling was performed in all treatments to measure the yield and yield components of cowpea in intercropping and monocropping at the end of the growing season by removing the side effect from the beginning and end of each plot. From each plot, 10 plants were randomly selected to measure the traits and the desired traits were evaluated on them.

Laboratory analyses

Important crop traits were measured, such as 1000-seed weight (TGW), biological yield (BY), grain yield (GY), grain protein content (P), harvest index (HI), Iron concentrations in leaves (Leaf Fe) and Zinc concentrations in leaves (Leaf Zn) were measured. The amount of grain protein content by digestion method using the automatic Kajeldal device was measured (Bremner and Breitenbeck, 1983). Besides, the amount of iron and zinc in the leaves was determined with an ICP (Inductively Coupled Plasma) device (GBC Integra XL sequential model) made in Australia.

Statistical analysis

The analysis of variance (ANOVA) was performed for each year to verify statistical differences between two plants and different Nano-chelate and interaction. Treatment means were compared using the using the Duncan testat the 5% and 1% level of probability. The statistical analysis applied on the data using MSTAT-C software.

RESULTS AND DISCUSSION

The results showed that the 1000-Grain weight (TGW) of cowpea and maize were affected by different intercropping ratios (Table 2 and 3). The highest 1000-Grain weight (TGW) of cowpea (240g) and the same in the 1000-Grain weight (TGW) of maize (272 g) was observed in IC2 under Fe+Zn nano-chelate and the lowest (100.6 g) was observed for cowpea in treatment IC1 control and for maize (184g) in treatment MPC under control (Table 2 and 3). Different intercropping ratios affected cowpea and maize Biological yield (Table 2 and 3). The maximum biological yield (19580kg/ha^{-1}), leaf Fe (68.63 mg/kg^{-1}) and leaf Zn (168.6 mg/kg^{-1}) of cowpea was produced in treatment CPC under Fe+Zn application. Moreover the maximum biological yield was produced for maize (28129kg/ha^{-1}) under Fe+Zn application, leaf Fe (51.6 mg/kg^{-1}) under Zn and leaf Zn (173 mg/kg^{-1}) under Fe in IC1 application which was not significantly different from biological yield produced in intercropping with other levels. The minimum biological yield (10083kg/ha^{-1}) and leaf Zn (95.4 mg.kg^{-1}) was obtained in IC1 and leaf Fe (27.3 mg/kg^{-1}) IC3 of cowpea under Control (Table 2). The minimum biological yield (23586 kg/ha) in IC2 of maize under Fe application and leaf Fe of maize (22.7 mg.kg^{-1}) in IC3 under Control and leaf Zn (63.6 mg.kg^{-1}) in MPC under control (Table 3).

The highest protein of seed (28.6%), grain yield (2813 Kg/ha^{-1}) and harvest index (44.4%) was measured for cowpea in treatment IC3 under Zn application alone, while The lowest protein of seed (16.9%) and grain yield (1618 Kg/ha^{-1}) was observed in treatment CPC and harvest index (36%) in treatment IC2 under control (Table 2). Moreover,

Table 1: Physical and chemical properties of the soil of the study area in 2018 and 2019.

Year	Depth (cm)	EC (ds/m)	pH	N (%)	P (mg/kg)	K (mg/kg)	Zn (mg/kg)	Fe (mg/kg)	Soil texture
2018	0-30	2.14	8.1	0.04	5	185	1.70	12.25	Sandy-loam
	30-60	2.01	8.0	0.02	3.8	115	0.68	10.01	Loamy-sand
2019	0-30	1.8	8.1	0.05	5	143	1.60	11.25	Sandy-loam
	30-60	1.4	8	0.02	4	104	0.44	10.02	Loamy-sand

Table 2: Mean comparison of the triple interaction of plant, planting systems and type of fertilizer on iron and zinc concentration in cowpea.

Treatment	Fertilizer	TGW (g)	P (%)	BY (kg/ha)	GY (kg/ha)	HI (%)	Leaf Fe (mg.kg^{-1})	Leaf Zn (mg.kg^{-1})
IC3	Fe	170.85ab	23.20ab	16797b	2673a	44.30a	33.70bc	135.80b
IC3	Zn	170.82ab	28.60a	16056b	2813a	44.40a	30.29c	127.60c
IC3	Fe+Zn	190.89ab	25.50a	16503b	3118a	43.20a	37.40bc	150.00a
IC3	Control	140.74b	20.01b	14468c	1634c	41.20ab	27.30c	124.20c
IC2	Fe	230.94a	24.30ab	16000b	2383ab	43.30a	35.70bc	126.51c
IC2	Zn	220.93a	27.50a	16378b	2292ab	42.10a	31.70bc	119.53c
IC2	Fe+Zn	240.95a	26.60a	16646b	2287ab	42.30a	43.70b	139.20b
IC2	Control	140.76b	20.20b	12298d	1336d	36.00b	32.80bc	120.50c
IC1	Fe	130.70bc	23.30ab	14681c	1633c	41.40ab	42.48a	122.30c
IC1	Zn	130.69bc	25.50a	14083c	1656c	40.30ab	35.27bc	112.81d
IC1	Fe+Zn	140.70b	26.60a	12907d	1770c	40.40ab	49.51a	108.30d
IC1	Control	100.60c	20.04b	10083d	1099d	39.30b	38.62bc	95.40d
CPC	Fe	170.79ab	18.90bc	17420b	2581ab	42.10a	49.50a	154.68a
CPC	Zn	170.80ab	23.10ab	17577b	2736ab	43.30a	43.70b	145.70b
CPC	Fe+Zn	160.73b	22.30ab	19580a	2753ab	42.60a	68.63a	168.60a
CPC	Control	110.50 c	16.90c	16640b	1618c	38.00b	44.80b	140.20b
	Mean squar	17181.90**	414.94**	7549382.12**	8498131.51**	174.25**	3294**	1203.7**
	LSR1%	61.00	5.04	6624.05	2253.76	17.30	0.45	1.25
	CV (%)	9.99	14.05	10.15	7.70	14.50	0.5	1.4

TGW: 1000-Grain weight, GY: Grain yield, BY: Biological yield, HI: Harvest index, P: Protein of seed , Leaf Fe: Iron concentrations in leaves , Leaf Zn: Zinc concentrations in leaves . CPC: Cowpea pure Cultivation, IC1: 75% maize + 25% cowpea, IC2: 50% maize + 50% cowpea, IC3: 25% maize +75% cowpea. Mean with the same letter(s) is not significantly different using Duncan (P≤0.05). ** and *: significant at 1% and 5% probability levels, respectively and ns: not significant.

the highest protein of seed (22.2%) in treatment IC1 under Zn application, grain yield (12050 Kg/ha^{-1}) in MPC under Fe application and harvest index (54.8%) was measured for maize in treatment MPC under Fe application, while the lowest protein of seed (7.4%) and grain yield (9980 Kg/ha^{-1}) was observed in treatment MPC Control and Harvest index (36.5%) in treatment IC1 under control for maize (Table 3). The present study demonstrates that intercropping of common cowpea with maize affect the total performance of culture. According to the results, the application of nano zinc and iron chelates alone or together increased the yield and improved all quantitative and qualitative traits of cowpea. Intercropping also increased the 1000-seed weight of cowpea. According to Nassary *et al*. (2020), the 1000-seed weight of cowpea increased in intercropping with maize. The increase in 1000-seed weight in intercropping with equal proportions of legumes and grains is related to intraspecific competition. On the other hand, the high proportion of high cereals and broad leaves with shading leads to reduced photosynthesis and reduced accumulation of carbohydrates. The application of micronutrients such as zinc increases the 1000-seed weight in mung beans (Shojaei and Makarian 2014; Kanwal *et al*. 2020). Iron, zinc and the 1000-seed weight in soybean (Shesh Bahre and Movahedi Dehnavi, 2012) due to improved photosynthesis and accumulation of carbohydrates (Shojaei and Makarian, 2014). These results were consistent with the results of this study.

Nanofertilizers have a significant impact in the agriculture sector for achieving enhanced productivity and resistance to abiotic stresses. Intercropping of 75% cowpea + 25% maize and application of nano iron or zinc chelates in the region increased the yield of the crops. According to Nord *et al*. (2020), in intercropping of maize and cowpea, cowpea biological yield was increased in monocropping compared to intercropping. In the study of evaluation of intercropping of cowpea and maize, Nassary *et al*. (2020) and Hosseinzadeh *et al*. (2018) provided similar results on a significant increase in biological yield in sole cropping of cowpea compared to intercropping with maize. In monocropping and intercropping with a high portion of cowpea, the number of cowpea plants per unit area increases and, therefore, cowpea biological yield increases. Ahmadi Dastgerdi *et al*. (2015) reported that the application of 5 g/l nano-iron chelate fertilizer in the 3-4-Leaf stage significantly increased the capsule bean biomass. Iron and zinc are essential elements in photosynthesis. Therefore, foliar application of iron and zinc nano-chelates leads to increased leaf yield during photosynthesis and increased yield by rapidly providing nutrients during plant growth stages, increasing vegetative growth, improving photosynthesis, increasing greenness and leaf area duration (Monica and Cremonini, 2009).

Intercropping also increased cowpea protein content. (Shaker-Koohi and Nasrollahzadeh, 2014) also reported an

Table 3: Mean comparison of the triple interaction of plant, planting systems and type of fertilizer on iron and zinc concentration in maize.

Treatment	Fertilizer	TGW (g)	P (%)	BY (Kg/ha)	GY (Kg/ha)	HI (%)	Leaf Fe (mg.kg^{-1})	Leaf Zn (mg.kg^{-1})
IC3	Fe	232ab	16.6ab	25277b	10574 bc	42.6ab	30.6ab	150.4a
IC3	Zn	243ab	19.9a	25623b	11251b	45.8ab	40.6a	81.1c
IC3	Fe+Zn	250a	18.8b	26142b	11743b	49.6a	38.7a	130.5ab
IC3	Control	188c	11.6b	26602b	10175c	42.8ab	22.7b	83.0c
IC2	Fe	256a	11.6b	23586c	10854bc	49.5a	33.2ab	161.1a
IC2	Zn	269a	21.1a	23835c	10678 bc	45.9ab	44.7a	88.1c
IC2	Fe+Zn	272a	20.1a	24681c	10451c	43.7ab	42.7a	142.7a
IC2	Control	215b	10.5b	24182c	10365c	42.6ab	30.9ab	93.7b
IC1	Fe	246a	20.1a	27210a	11649b	45.1ab	39.8a	173.1a
IC1	Zn	256a	22.2a	26750b	11598b	45.1ab	51.6a	95.2b
IC1	Fe+Zn	257a	21.2a	28129a	12993a	43.9ab	47.5a	155.3a
IC1	Control	189c	12.6b	26060b	10908bc	36.5b	36.6ab	97.2b
MPC	Fe	227ab	16.8ab	21955d	12050a	54.8a	22.6b	141.7a
MPC	Zn	224ab	16.7ab	21913d	11892b	53.8a	33.6ab	72.4c
MPC	Fe+Zn	228ab	15.7ab	23795c	11880b	54.7a	31.8ab	120.7b
MPC	Control	184c	7.4c	23637c	9980d	47.1a	19.6b	63.6c
	Mean squar	184.83**	215.84**	49208486.37**	5753417.82**	67.87**	1090.6**	2085.7**
	LSR1%	7.15	8.99	2097.48	703.99	4.56	1.33	31.54
	CV (%)	16.73	15.10	16.35	12.53	4.23	1.20	9.00

TGW: 1000-Grain weight, GY: Grain yield, BY: Biological yield, HI: Harvest index, P: Protein of seed, Leaf Fe: Iron concentrations in leaves, Leaf Zn: Zinc concentrations in leaves. MPC: Maize pure Cultivation, IC1: 75% maize + 25% cowpea, IC2: 50% maize + 50% cowpea, IC3: 25% maize + 75% cowpea. Mean with the same letter(s) is not significantly different using Duncan (P≤0.05). ** and *: significant at 1% and 5% probability levels, respectively and ns: Not significant.

increase in grain protein content of cereals in intercropping compared to monocropping. Increased nitrogen fixation from legumes increases the grain protein content. Krishnasree *et al*. (2022) reported that Foliar nutrition of macro and micro nutrients enhanced the crude protein content in bush type vegetable cowpea. Suman (2018) revealed that foliar application of Zn and B enhanced the N metabolism leading to increase in the protein content of rice grain. Zinc plays a major role in the translocation of starch from source to sink and N metabolism. Hence, adequate availability of Zn improved the quality of seed (Taliee and Sayadian, 2000). Shruthi *et al*. (2013) revealed that foliar application of water-soluble fertilizers enhanced the protein content of pod. Marzouk *et al*. (2019) reported an increase in cowpea grain protein content (27.8% in the first crop season and 26.9% in the second crop season) as a result of the application of zinc nano-micronutrients compared to other micronutrients. This increase can be attributed to the appropriate concentration of amino acids and other nutrients in it, which stimulated plant metabolism. Therefore, sufficient amounts of these elements are necessary to increase the metabolism of carbohydrates, RNA, DNA and the synthesis of oils and proteins and foliar application and elimination of deficiency of these elements improve the physiological reactions involved in protein synthesis (Thomas *et al*. 2009). Sheikh-(Baghloo *et al*. 2010) reported that soybean grain yield was significantly increased due to the application of iron oxide nanoparticles. (Rafique *et al*. 2015) and (Marzouk *et al*. 2019) reported that foliar application of nano zinc and iron improved the yield of pea and cowpea, respectively. The results of the study by Jamal *et al*. (2018) indicated that the simultaneous application of iron and zinc increased grain yield in mung bean. It was also found that grain yield and Harvest index in intercropping of 75% cowpea + 25% maize were maximum. According to Soleimanehpur *et al*. (2017), the maximum Harvest index was obtained in sole cropping of cowpea (46.46%) and also in sole cropping of pea (26.03%) in comparison with inter with intercropping with barley or triticale. They also found that the harvest index in intercropping treatments was similar to or superior to Harvest index in monocropping (weed). On increasing the cowpea Harvest index as a result of the application of nano-iron chelate fertilizer (Pierre *et al*. 2017). Since it has been suggested that higher densities of legumes are used in combination with cereals to increase yield Lithourgidis *et al*. (2011), on the other hand, nanoparticles such as zinc increase yield by increasing chlorophyll durability and improving photosynthesis (Monica and Cremonini, 2009), the combination of these two treatments can increase harvest index.

CONCLUSION

As a result, by decreasing the ratio of cowpea to maize, the intercropping advantage decreased. In the intercropping advantage of the mixture of these two plants, the factor of light and increasing the efficiency of light consumption has also been effective, so that from the top to the bottom of the canopy of the maize plant, the available light for photosynthesis decreases, which in some levels or layers, the amount of light reaches below the compensation point of the plant, that is the reason why cowpeas with a lower compensation point can be placed there (Dhima *et al*. 2007).

ACKNOWLEDGEMENT

Authors thank the esteemed management of the Water and Soil Department and the hardworking staff of the Agricultural and Natural Resources Research center of Kerman Province who helped to complete and improve the quality of this research.

Conflict of interet: None.

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