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THE EFFECT OF BIOLOGICAL AND NANOFERTILIZERS ON COWPEA (*Vigna unguiculata* L.) YIELD, QUANTITATIVE AND QUALITATIVE TRAITS IN THE SOUTHERN IRANIAN CLIMATE

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ABSTRACT

Biological and nano fertilizers are alternative and supplements to chemical fertilizers security for the sustainability of agricultural production. The objective of this study was to investigate the effect of biological and nano fertilizers on the quantitative and qualitative yield of cow pea during two crop years (2018-2019 and 2019-2020) in the research farm of the South Agricultural Research and Education Center of Kerman province, Iran. This experiment was carried out factorial in designs of randomized complete blocks with 16 treatments and 3 replications, the studied factors include four levels of biological fertilizers (control, zinc amino chelate, aminoalexin, and bio20) and four levels of nano chelates (control, boron nano chelate, potassium nano chelate and iron nano chelate). The results of the experiment showed that there was no significant difference between the two years of the experiment in terms of all quantitative and qualitative traits. Although the effect of the year on the traits was not significant, the application of biological fertilizers and nano chelate had a significant effect on the quantitative and qualitative traits of cowpea. In other words, the lowest amount of quantitative and qualitative traits related to cowpea was in the absence of biological fertilizers and nano chelate. Based on the obtained results, the application of bio20 biological fertilizer along with iron nano chelate or potassium nano chelate caused a significant increase in quantitative and qualitative traits. In general, and according to the results of the experiment, it was found that the use of biological fertilizers, especially bio20 and aminoalexin, caused a significant improvement in the quality characteristics. The positive effect of bio20 and aminoalexin in improving quality characteristics was significantly higher when used together with nano chelates, especially nano chelates of iron and potassium which is likely due to the positive effect of potassium and iron in various plant metabolic processes. It includes osmotic regulation, increasing chlorophyll, and photosynthesis. Therefore, the interaction of bio fertilizers (Bio20) with nano chelate (iron and potassium) increased seed yield by 50% compared to the control (no fertilizer use).

Keywords: Bio20, biological fertilizer, iron nano chelate, nano chelate, potassium nano chelate, zinc nano chelate

INTRODUCTION

Cow pea (*Vigna unguiculata* L.), is a type of flower bean with the name "Baldi beans", in India "loba", in French Dolic an oeil noir or Oolique an oeil noir and in English, it is called Catjang, Cow peas, Black eye pea (Karimian et al., 2020) in traditional medicine books. It is an herbaceous plant, annual, summer legume, three carbons and considered an important source of nutrition. This plant can fix nitrogen in poor soils and grows well in poor soils in terms of phosphorus content (Karimian et al., 2020). Among legumes, soybeans, beans, and peas rank first to third in terms of cultivated area (FAO, 2019). One of the factors that threaten the health of crops is the excessive use of chemical fertilizers. This problem also causes an increase in harmful and dangerous compounds for the health of people in society in addition to causing instability in soil quality and agricultural production. (Kochaki et al., 2004). One of the most important applications of nanotechnology in agriculture (in the water and soil sector) is the use of nano fertilizers for plant nutrition (Rezaei et al., 2009).

The use of nanotechnology is expanding in all fields, including agriculture. With the use of nano-fertilizers as an alternative to conventional fertilizers, nutrients are released gradually and a controlled manner in the soil (Naderi and

Daneshe Shahraki, 2013). Concerns related to the low. Efficiency of conventional fertilizers (about 30-50%) and little management to improve fertilizers determine the necessity of using nanotechnology for research and development of fertilizers (Derosa et al., 2010). The high absorption speed of nano fertilizers contributes to increasing efficiency and quality of food resources, as well as significant reductions in soil pollution, water reserves, and food products due to reduced leaching of fertilizers, no loss of fertilizers through leaching, and complete absorption of fertilizer by plants. The reason for the release of fertilizer nutrients at optimal rate throughout the growing season was pointed out (Liu and Lal, 2015).

One of the main elements of sustainable agriculture is the use of biological and animal fertilizers in agricultural ecosystems to eliminate or reduce the consumption of chemical fertilizer. Bio-fertilizers, sometimes as a substitute and in most cases as a complement to chemical fertilizers, can ensure the sustainability of the production of agricultural systems. Bio-fertilizers, which are considered an essential part of organic agriculture, contain living organisms or efficient nitrogen-fixing and phosphate-dissolving species that are applied to seeds, soil, or composted areas to increase the number of these microorganisms and acceleration of microbial processes that provide access to nutrients for proper assimilation in plants is used (Venkatashwarlu, 2008; Ozturk and Yildirim, 2013).

The use of biological fertilizers such as Nitroxin includes nitrogen-fixing bacteria of the genus Azotobacter and Azospirillum, which increase the population of beneficial soil microorganisms, and provide plant nutrients such as nitrogen, phosphorus, and potassium, and improving the growth and performance of agricultural plants (Arancon et al., 2004). Plant nutrition and the use of organic and chemical fertilizers are one of the most important and practical tools to increase production and the quality of products (Malkoti et al., 2009). One of these effective fertilizers is various chelating substances that have wide uses in plant nutrition. Scientific research on corn, tomato, apple, potato, and wheat plants shows that amino acid chelates are well absorbed and improved crop yield (Ghasemi et al., 2013).

For sustainable agriculture, biological fertilizers can be used as a supplement to chemical fertilizers or as an alternative to them (Fathi et al., 2013). Aminochelates are amino acid based fertilizers, which are widely used in the fertilizer and agriculture market today. One of them is humic acid which is proposed as the new generation of fertilizers, which are gradually being replaced by the previous simple forms in the market. In many studies, the improvement of plant growth and development has been reported due to the use of amino chelates (Datir et al., 2012). Amino acid chelates based chemical fertilizers were first developed by American chemist to improve plant access to nutrients (Bradley, 2010). Foliar spraying of the black bean plant (*Vigna unguiculata*) with iron nanoparticles at a concentration of 500 mg caused a significant increase in the number of pods, the weight of 1000 seeds, the amount of iron and chlorophyll in the leaves compared to the control and caused a higher yield of the product compared to the application of iron (Became normal with similar concentrations). In addition, iron nanoparticles significantly improved the beneficial effect of other nano fertilizers (magnesium nanoparticles) on black beans (Delfani et al., 2014). Similar results of significant improvement in the weight of leaves, aerial organs, and pods in soybean (*Glycine max* L.) have been reported with the application of nano-iron fertilizer (Sheykhbaglou et al., 2012). The application of nano iron oxide powder compared to normal iron oxide caused a significant increase in spike length, plant height, grain weight per spike, dry weight of straw and stubble, and thousand-seed weight in wheat (Mazaherinia et al., 2010).

Delfani et al. (2014) reported that foliar application of iron nanoparticles at a concentration of 500 mg/liter for black bean (*Hassk vigna sinensis* (L.) savi. Ex) significantly increased the amount of chlorophyll (10%) and the witness. It was reported in another experiment that foliar spraying of iron and zinc sulfate caused a significant increase in the number of photosynthetic pigments, phenols, and plant performance (Miransari et al., 2014). Iron chelate nano fertilizer is a reliable fertilizer base for iron release due to its proper stability and controlled release power. The most important way to protect iron against increased stabilization in the soil is to raise pH (Jokar and Ronaghi, 2016).

Mazaherinia et al. (2010) found in a greenhouse experiment that the use of iron oxide nano powder compared to normal iron oxide significantly increased the plant iron concentration, spike length, plant height, seed weight per spike, dry weight of straw and stubble in wheat. It was weighed of 1000 seeds and the weight of seeds per pot was in wheat. This situation may be due to the properties of nanoparticles and their greater solubility, lightness and smallness, and the possibility of roots encountering nanoparticles more than ordinary iron oxide particles. It has been reported that the effect of iron nano chelate foliar application on plant weight, leaves number, and sesame plant height is significant (Boghoriet al., 2014). In research, the use of potassium fertilizer caused a change in the average traits of plant height, number of branches, number of leaves, number of pods, seed yield, and biological performance of sesame plants (RamRao et al., 2007). Their use has been banned for a long time due to the harmful effects of conventional chemical fertilizers on the cultivation environment and the quantity and quality of agricultural products. In nano fertilizers, nutrients are released gradually and controlled manner in the soil, and therefore, the use of nano-fertilizers leads to increasing the efficiency of the use of nutrients, decrease soil toxicity, and minimizing the negative effects caused by excessive consumption. Fertilizers reduce the frequency of fertilizer use (Nadri and Danesh Shahraki, 2018).

The cowpea is one of the oldest plants of tropical and subtropical regions, especially in the south of Kerman, which grows well in temperate regions and has a great ability to biologically stabilize soil nitrogen; on the other hand, it has the ability to stabilize nitrogen in poor soils. It grows well in poor soils in terms of phosphorus content. Their roots coexist with nitrogen-fixing bacteria, which is effective in soil fertility. Beans occupy the largest cultivated area due to their high protein percentage and other favorable agricultural characteristics dedicated (Karimian et al., 2020).

Considering the positive and significant effects of biological fertilizers and nano chelates on different products, the current study was carried out to investigate the effect of zinc amino chelate, aminoalexin, and bio20 biological fertilizers as well as boron, potassium, and iron nano chelates on yield, quantitative and qualitative properties of Cowpeas.

MATERIALS AND METHODS

This study aims to investigate the effect of biological fertilizers and nano chelate fertilizers on the yield and morphological characteristics of cowpea during two crop years 2017-2018 and 2018-2019 in a farm located in the Center for Research and Education of Agriculture and Natural Resources in South Kerman (Jiroft) with a geographical length of 57 41 degrees north and 28 degrees 37 minutes east latitude, 627 meters above sea level. Jiroft city has a hot and dry climate with mild winters and hot and dry summers with an average annual rainfall of 140 mm, a maximum temperature of 48 degrees Celsius and a minimum temperature of 4 degrees Celsius and a relative humidity of 0 to 65% was done (Table 1).

Table 1. Mean, maximum and minimum temperature and total monthly Rainfall registered 2020 in Kerman South

Month	Temperature						Total monthly rainfall (mm)	
	Average °C		Maximum °C		Minimum °C			
	2018 year	2019 year	2018 year	2019 year	2018 year	2019 year	2018 year	2019 year
March	26.9	27.3	39.6	40	14.2	14.6	3.2	2.8
April	33.1	34.3	46.2	46.6	20	22	6.3	5.9
May	37.2	37.8	47.8	48.4	26.6	27	0	0
June	37.1	37.6	45.9	46.4	28.4	28.8	1.1	0.8
July	34.6	36.7	44.2	44.4	36	28	2.1	1.8
August	31.9	33.1	42.8	43.2	21	23	0	0
September	28.3	28.9	39	39.8	17.6	18	0	0
October	21.3	21.9	33.2	34.1	9.4	9.8	6	6
November	17.9	18.6	31	33.1	3.9	4.1	0	0
December	13.5	13.6	18.1	18.2	8.9	9.1	12.1	11.2
January	18.9	20.1	23.9	24.1	14	16	24.1	22.9
February	20.3	20.9	30.6	30.9	10	11	64.5	63.8

The studied factors include four levels of biological fertilizers (control, zinc amino chelate, aminoalexin, and bio20) and four levels of nano chelates (control, boron nano chelate, potassium nano chelate and iron nano chelate) which included a total of 16 treatments and 48 experimental plots. Each plot was planted in 6 planting lines with a length of 4 meters and 50 cm between the rows and 7 cm on the planting line, and the date of planting was 20 July, The general characteristics of biological fertilizers (use method, time of use and amount of use) are included in tables (2 and 3).

Table 2. General characteristics of biological fertilizers

Fertilizer name	Ingredient	method of use	consumption time	amount of use
Zinc aminochelate	Contains 7% zinc - 10% free amino acid - 20% organic matter	Spraying	Early in the growing season and repeat as needed	1 to 2 liters per thousand liters of water
aminoalexin	Contains 30% phosphorus-20% potassium-4% free amino acid	Spraying	It should be done when the humidity and temperature conditions favor the growth of pathogenic agents or pathogens. The number of times of use is between 2 and 4 times, and the time interval between each use is 7 to 14 days	3 to 4 liters per hectare
bio20	Contains 20% useful phosphorus - 20% potassium - 20% total nitrogen - 11.40% nitrogen	Spraying	The beginning of the growing season	1 to 2 liters per hectare

The soil of the field was analyzed by the soil science laboratory in two crop years before the implementation of the project (Tables 4 and 5). Fertilizers including phosphorus, potash and nitrogen fertilizers in the amount of 40 weight of pure nitrogen and 45 weight of phosphorus (P_2O_5) and 35 weight of potash (K_2O) were added to the soil from the source of potassium sulfate based on the soil test, and nitrogen fertilizer with ammonium sulfate source in three stages. It is fed in the same way for all plots. All the phosphorus and potassium fertilizers along with half of the nitrogen fertilizer, which was considered as the basic fertilizer, were applied before planting and the rest of the nitrogen fertilizer was used in two stages, 20 and 30 days after planting.

Table 3. General characteristics of nano chelate fertilizers

Fertilizer name	Ingredient	method of use	consumption time	amount of use
boron nano chelate	Contains 9% boron - powder and completely soluble in water and absorbable by plants	Spraying	First stage: before flowering Second stage: before the seed ripens	2 grams per liter or 2 in 1000
Potassium nano chelate	Contains 27% potassium - powdery and completely soluble in water and absorbable by plants	Spraying	First stage: early plant growth The second stage: when the seed is filled	2 grams per liter or 2 in 1000
Iron nano chelate	Contains 9% iron- powder and completely soluble in water and absorbable by plants	Spraying	In the stages of stemming and bunching	2 grams per liter or 2 in 1000

Table 4-Soil analysis of the studied farm in 1st year

Texture	A.O.K (ppm)	A.O.P (ppm)	Total N (%)	O.M (%)	(ds/m)	pH	Soil depth (cm)
S-L	210	8	0.003	0.22	2.28	8.1	0-25
S-L	170	6	0.002	0.19	1.95	8	25-50

Table 5- Soil analysis of the studied farm in 2nd year

Texture	A.O.K (ppm)	A.O.P (ppm)	Total N (%)	O.M (%)	(ds/m)	pH	Soil depth (cm)
S-L	180	5	0.003	0.22	2.28	8.0	0-25
S-L	140	4	0.003	0.19	1.95	8.1	25-50

The local cultivar Jiroft is suitable for cultivation in the south of Kerman. Also, the length of the growing season (vegetative and reproductive) of this plant in the climatic conditions of this region is 75 to 80 days, and when about 70-80% of its pods turn yellow, it is the time for the plants to mature, in fact, it can be harvested in 100 days they are taken.

The evaluated traits included the amount of protein, potassium, and seed starch, the amount of chlorophyll a and b, and the total, hundred seed weight, and yield. At the time of physiological treatment, considering the marginal effects, the number of 10 plants selected from the 2 middle lines was counted and measured. To measure chlorophyll, the method Arnon (1949) was used, and the amount of seed protein was measured by the method Bradford, (1976) with the following formula: 6.25 × nitrogen percentage = protein percentage.

The method of McCready et al (1950) was used to measure starch, and the method of Rowell (1995) was used to measure sodium.

Bartlett's test was first performed for the uniformity of the data, and composite analysis was performed to investigate the effect of the year. For this purpose, the normality of the data was checked and the normality of the data was done using SAS software (ver. 9.2).

RESULTS AND DISCUSSION

As the analysis of variance table (3) showed, the effect of the year and the interaction effect of the year with other studied factors on the measured quantitative and qualitative traits were not significant. The effect of different treatments of the biological fertilizer and nano chelate alone on all traits studied except chlorophyll a was significant at the 1% probability level. A significant difference between biological fertilizer and nano chelate treatments was observed at the 5% probability level in terms of leaf chlorophyll content. The interaction effect of biological fertilizer and nano chelate on seed protein and hundred seed weight was significant at the five percent probability level and on other qualitative and quantitative traits studied at the one percent probability level (Table 6).

Chlorophyll a, b, and total

The results of a two-year composite analysis of chlorophyll a, b, and total chlorophyll showed that the effect of year on these traits was not significant (Table 6). The results of comparing the average traits evaluated in cowpea (Table 7) showed that the year had a significant effect on the amount of leaf chlorophyll. The highest amount of chlorophyll a (0.498 mg/g fresh weight) and the total was observed in the second year (0.742 mg/g fresh weight) and chlorophyll b (0.269 mg/g fresh weight) in the first year. Also, regarding the effect of biological fertilizer, the highest amount of chlorophyll a, b, and total improvement, respectively (0.506, 0.269, and 0.775 mg/gram of fresh weight) in Bio20 treatment and the lowest amount of chlorophyll a, b, and total improvement, respectively (0.447, 0.223 and 0.671 mg/g of fresh weight) were observed in the control treatment. Of course, no significant difference was observed in terms of chlorophyll between the control and aminochelate and zinc aminoalexin treatments. Also, in terms of chlorophyll b, no significant difference was observed between bio20 with zinc aminochelate and aminoalexin treatments (Table 7). Among the nano chelate fertilizers used, iron nano chelate had the most positive effect on these traits. Thus, with the use of this fertilizer, the amount of chlorophyll a (0.475 mg/gr of fresh weight), chlorophyll b (0.289 mg/gr of fresh weight), and total chlorophyll (0.764 mg/gr of fresh weight) was maximum.

Table 6. Variance analysis of some morphological traits in cowpea under the influence of year, biological fertilizer, and nano-fertilizer

Sources of changes	df	Hundred seed weight	Seed yield	Seed potassium	Total chlorophyll	Chlorophyll b	Chlorophyll a	Seed starch	Seed protein
Year	1	0.009 ns	0.002 ns	0.028 **	0.015**	0.083 **	0.193 ns	5.378 ns	3.425 ns
repeat (year)	4	0.154	0.002	0.002	0.001	0.001	0.142	4.368	3.975
Bio-fertilizer	3	52.323 **	1.831**	0.044 **	0.012 **	0.016 *	168.223**	20.893**	61.857 **
Year x biological fertilizer	3	0.012ns	0.000 ns	0.004 ns	0.002 ns	0.007 ns	0.55 ns	0.482 ns	0.261 ns
Nano fertilizer	3	26.019 **	0.422 **	0.026 **	0.012 **	0.005 *	22.0895**	48.494 **	95.216**
Year × nano fertilizer	3	0.025 ns	0.002 ns	0.002 ns	0.002 ns	0.010 *	0.049n.s	1.060 ns	1.153*
Biological fertilizer × nano fertilizer	9	0.936*	0.018**	0.013 **	0.004 **	0.013 **	197.691**	2.648**	3.370*
Year × biological fertilizer × nano fertilizer	9	0.007ns	0.001 ns	0.003 ns	0.003ns	0.002 ns	0.037 ns	0.364 ns	0.301 ns
error	60	0.111	0.005	0.001	0.001	0.001	0.057	1.55	1.084
CV		14.44	18.91	9.66	18.09	14.36	6.58	5.08	4.96

** and *, respectively, the existence of a significant difference at the probability level of one and five percent, and ns the absence of a significant difference.

Table 7. The simple effect of year, biological fertilizer and nano chelate on yield, quantity and quality of cowpea.

Sources of changes	**Seed protein (%)**	**Seed starch (%)**	**Chlorophyll a (mg/gFW)**	**Chlorophyll b (mg/gFW)**	**Total chlorophyll (mg/gFW)**	**Seed potassium (%)**	**Seed yield (t/ha)**	**Hundred seed weight (g)**
year								
first year	20.76a	20.91a	0.439b	0.269a	0.708b	2.61a	1.47a	11.26a
second year	21.14a	21.37a	0.498a	0.244b	0.742a	2.70a	1.45a	11.23a
Bio-fertilizer								
control	18.82d	19.88c	0.447b	0.223b	0.671c	2.62a	1.08d	9.29d
Zinc aminochelate	20.81c	21.28b	0.458b	0.261a	0.720b	2.47b	1.46c	11.07c
Aminoalexin	21.55b	21.24b	0.463b	0.271a	0.734b	2.41c	1.53b	11.84b
bio20	22.63a	22.14a	0.506a	0.269a	0.775a	2.39d	1.75a	12.77a
Nano-fertilizer								
control	18.68d	19.48d	0.452c	0.240b	0.691c	1.85c	1.28c	9.97d
Boron nano chelate	20.27c	20.49c	0.461b	0.243b	0.704c	2.22b	1.43b	10.82c
Potassium nanochelate	21.46c	21.93b	0.468ab	0.254b	0.740b	3.19a	1.55a	11.99b
Iron nano chelate	22.40a	22.65a	0.475a	0.289a	0.764a	2.25b	1.57a	12.20a

Of course, in terms of the amount of chlorophyll, no significant difference was observed between potassium and iron nano chelate treatments. The lowest amounts of these traits were observed in the control treatment (no application of nano chelate). The results of comparing the average traits of cowpea evaluated under the influence of the mutual effects of biological fertilizer and nano chelate fertilizer showed that the lack of biological fertilizers (biological control) and nano fertilizers caused a significant decrease in biochemical traits. Thus, in the absence of fertilizer application, the amount of total chlorophyll (0.657 mg/g of fresh weight) showed a significant decrease compared to other treatments (Table 7).

Based on the results of this research, the application of Bio20 caused the maximum increase in leaf chlorophyll compared to the biological control. Zinc aminochelate and aminoalexin also caused a significant improvement in the amount of chlorophyll compared to the control. As micronutrients, iron, and zinc play a very important role in the growth and development of plants. Among the nano-fertilizers used, iron nano chelate had the most positive effect on the biochemical traits of cowpea plants. With the use of this fertilizer, the amount of chlorophyll in the leaves showed a significant increase compared to the non-use of biological fertilizers. In general, with the increase in the use of biological fertilizer and nano chelate, the amount of chlorophyll a and b and the total chlorophyll of cowpea increased. The high levels of fertilizers are used to lead to the improvement of vegetative growth, increasing the number of leaves and increasing the level of light absorption, chlorophylls, and photosynthesis level in the plant (Ajeng et al., 2020). It has been reported that the use of iron and nano-iron fertilizers in basil plants increased the total chlorophyll compared to the control treatment (Peyvandi et al., 2011). Carotenoids are auxiliary pigments that affect light absorption and transmission and are considered chlorophyll protectors during the photooxidation process (Akbarian et al., 2012) Yadavi et al (2014) reported that the amount of chlorophyll b, the amount of total chlorophyll and the number of carotenoids showed a significant increase under the influence of zinc amino chelate, aminoalexin, and bio20 along with potassium nano chelate and iron nano chelate.

Zhao et al. (2007) stated that the chlorophyll index per unit leaf area of plants is genetic, but it is strongly influenced by various environmental factors. Photooxidation of chlorophyll due to destruction of chloroplast thylakoid membranes and reactive oxygen types and increased activity of Chlorophylase enzyme have been reported as one of the reasons for the reduction of chlorophyll in moisture deficiency conditions (Alonso et al., 2001). Iron deficiency leads to a decrease in the concentration of photosynthetic pigments such as chlorophyll and carotenoids per unit of leaf surface, and as a result, the amount of leaf photosynthesis decreases.

The reason for the reduction in the number of photosynthetic units and the reduction in the photochemical efficiency of the photosynthetic system is reduced. Foliar spraying with iron nano chelate increases the photosynthesis of the plant due to the role of iron in the production of chlorophyll; therefore, more carbohydrates are transferred to the roots, thus the growth and absorption of elements. Food by the root is increased and as a result the concentration of elements in the plant also increases the range of fertilizer. Mir (2014) the deficiency of the nutrient iron increases with the increase of the iron element, and the chlorophyll index of the leaf also increases, which is consistent with the results obtained from this experiment. The advantages of using nano K Iron chelate include increased the metabolism of plants and more and more effective absorption of the main elements and codes, as well as the targeted delivery of micronutrients to the specific tissues of plants (Rasoli et al., 2013). Also iron plays an important role in the synthesis of chlorophyll and it is one of the main components of chlorophyll (Taiz and Zeiger, 2002; Babaeian et al., 2012). On the other hand, it has been mentioned in some reports that iron deficiency causes the loss of chlorophyll and decreases the activity of oxidase enzymes such as catalase and peroxidase, which causes a sharp decrease in plant photosynthesis (Ahmadi et al., 2019).

Starch, protein, and potassium seeds

The results of a two-year composite analysis of starch, protein, and seed potassium showed that the effect of year on these traits was not significant (Table 6). Based on the results of the table comparing the average of (7) bio-fertilizers, the highest amount of protein and starch in the seeds is respectively (22.63 and 22.14%) related to Bio20 treatment, and regarding seed potassium, related to the control treatment is 2.62% and the lowest amount of protein and starch. It was observed that the seeds were better (18.82 and 19.88%) related to the control treatment and regarding the seed potassium related to the bio20 treatment, it was observed to the extent of 2.39%. Of course, no significant difference was observed in terms of grain starch between aminochelate and zinc aminoalexin treatments (Table 7). Among the nano chelate fertilizers used, iron nano chelate had the most positive effect on the percentage of protein and starch in seeds. Thus, with the use of this fertilizer, the amount of protein (22.40%), and starch (22.65%) was the maximum, and the lowest amount of these traits were observed in the control treatment (no use of nano chelate), respectively (18.68 and 19.48%), and regarding seed potassium, the highest amount of application of potassium nanochelate fertilizer (3.19%) and the lowest amount was observed in the control treatment (1.85%).

According to the results of this research, the application of bio20 caused the maximum increase of protein and starch of the seed by 22.63 and 22.14% respectively, compared to the control (no use of bio-fertilizer). As micronutrients, iron, and zinc play a very important role in the growth and development of plants. Among the nano-fertilizers used, iron nano chelate had the most positive effect on the amount of protein and starch of cowpea plant. With the use of this fertilizer, the amount of protein and starch in the seeds showed a significant increase compared to the non-use of bio-fertilizers, but the opposite is true for potassium, in bio-fertilizers, the amount of seed potassium (2.62%) in the control treatment (no use of Bio-fertilizer) is the highest and in nano chelate fertilizers. In general, the application of bio-fertilizer and nano chelate increased the protein and starch content of cowpea seeds (Table 8).

Table 8. Comparison of the average morphological traits evaluated in cowpeas under the influence of the mutual effects of biological fertilizer and nano chelate

Bio-fertilizer	nano chelate	Seed protein (%)	Seed starch (%)	Chlorophyll a (mg/gFW)	Chlorophyll b (mg/gFW)	Total chlorophyll (mg/gFW)	Seed potassium (%)	Seed yield (ton.ha^{-1})	Hundred seed weight (g)
control	control	17.45h	18.55g	0.426cd	0.231cd	0. 657i	1.30j	0.98j	8.12j
	Boron nano chelate	18.58gh	19.68efg	0.425cd	0.222cd	0.6487i	1.91h	1.11i	8.93i
	Potassium nanochelate	19.22fg	20.36def	0.480bcd	0.221cd	0.702efgh	2.75a	1.13i	9.98g
	Iron nano chelate	20.04ef	20.92de	0.456cd	0.219cd	0.675ghi	2.21d-h	1.13i	10.16g
Zinc aminochelate	control	18.45gh	19.45fg	0.472cd	0.206d	0.678fghi	2.11f-i	1.30h	9.42h
	Boron nano chelate	19.46fg	19.64efg	0.447cd	0.246bcd	0.694efgh	2.55bc	1.42fg	11.02f
	Potassium nano chelate	21.46cd	22.56ab	0.454d	0.279ab	0.734cde	2.71b	1.56de	11.67de
	Iron nano chelate	23.87b	23.47a	0.459cd	0.312a	0.771c	2.51bcd	1.57cd	12.19bc
Amino alexi	control	18.45gh	18.84g	0.502abc	0.267bc	0.769bc	1.85i	1.36gh	11.18f
	Boron nano chelate	20.80de	20.90de	0.470cd	0.242bcd	0.712d-g	2.01ghi	1.49ef	11.36ef
	Potassium nano chelate	22.63e	22.44ab	0.462cd	0.264bc	0.726c-f	2.41b-f	1.64bc	12.48b
	Iron nano chelate	24.20ab	22.80ab	0.417d	0.312a	0.729c-f	2.02c-g	1.66b	12.36b
Bio20	control	20.63d-f	21.08cd	0.407d	0.254bc	0.661hi	2.15e-i	1.50d-f	11.20f
	Boron nano chelate	22.23c	21.72b-d	0.501a-c	0.259bc	0.761b-d	2.43b-e	1.71b	11.98cd
	Potassium nano chelate	22.53c	22.33a-c	0.547ab	0.250b-d	0.796b	2.60bc	1.88a	13.85a
	Iron nano chelate	25.41a	23.41a	0.568a	0.311a	0.879a	2.39b-f	1.93a	14.08a

In each column, the treatments that have at least one letter in common were not significantly different from each other.

The results of comparing the average traits evaluated in cowpea under the influence of the mutual effects of biological fertilizer and nano chelate fertilizer showed that the lack of use of biological fertilizers (biological control) and nano fertilizers caused a significant decrease in biochemical traits. In the absence of fertilizer application, the amount of seed protein (17.45%), seed starch (18.55%), and seed potassium (1.30%) showed a significant decrease compared to other treatments (Table 8). Among the investigated treatments, the application of aminoalexin and bio20 along with iron nano-chelate caused a significant increase in grain protein content (24.20% and 25.41%, respectively). Also, the use of biological fertilizers (amino chelate zinc, aminoalexin, and bio20) along with nano chelate potassium and nano chelate iron caused a significant increase in the amount of grain starch (22.33 to 23.47 percent compared to 18.55 percent in the control). The highest amount of seed potassium (2.75) was also observed in the application of potassium nano chelate in interaction with the biological control (Table 5). researchers stated that the highest percentage of protein was obtained from the use of Fertilizer Phosphate-2 bio fertilizer and the lowest from the control treatment (no use of bio-fertilizer) which is consistent with the results of this research (Dehmardeh et al., 2017). The results of the survey (Zarabpoor et al., 2011) showed that there is a significant difference between different levels of bio-fertilizer use on seed protein traits, and these fertilizers had the greatest effect on seed protein. The results of Afrasiabi et al. (2011) 's study also showed that the protein percentage of scotalata one-year alfalfa had the highest values in the presence of Fertilizer Phosphate-2 biological fertilizer.

Khoshvaghti and Tajbakhsh-Shishavan (2022) stated that fertilizers containing different elements have different effects on the biochemical quality of corn seeds, aminochelate micro mix, aminochelate iron and nano iron chelate fertilizers increased the starch and protein content of corn seeds. It had a good effect on the amount of starch (81%) and protein (12.7%) of corn seeds, which is consistent with the results of this research. Ibrahim & Mohamed (2012) reported, by using foliar nutrition, photosynthesis activity is stimulated and leads to an increase in chemical compounds such as protein and starch. researchers stated that the potassium element activates enzymes involved in photosynthesis, carbohydrate, and protein metabolism, helps to transport carbohydrates, and plays a key role in stimulating the activity of the enzyme starch synthetase and the synthesis of simple glucose molecules as well as. It catalyzes starch complexes, therefore, in the experiment, if the amount of starch and protein increases, the number of potassium decreases, which is consistent with the results of this research (Hawkesford et al., 2012).

Seed yield and hundred seeds weight

The results of a two-year composite analysis of the yield and hundred seeds weight showed that the effect of year on these traits was not significant (Table 6). Based on the results of the comparison of the average effect of bio-fertilizer, the highest yield and hundred seeds weight are respectively (1.75 ton/ha and 12.77 gr) related to bio20 treatment and the lowest yield and hundred seeds weight are respectively (1.08 ton/ha and 9.29 gr) related to the control treatment was observed (Table 4). Among the nano chelate fertilizers used, iron nano chelate had the most positive effect on seed yield and hundred-seed weight. So, with the application of this fertilizer, the yield of seeds (1.57 ton/ha), the weight of one hundred seeds (12.20 gr) was the maximum, and the lowest amount of these traits in the control treatment (no use of nano chelate) was (1.28 ton/ha and 9.97 gr), respectively was observed (Table 7).

According to the results of this research, the application of bio20 caused the maximum increase in grain yield and hundred seed weight by 1.75 ton/ha and 12.77 gr, respectively, compared to the control (no application of biological fertilizer). Among the nano-fertilizers used, iron nano chelate had the most positive effect on seed yield and hundred seeds weight of cowpea plants. With the use of this fertilizer, the amount of seed yield and the weight of one hundred seeds showed a significant increase compared to the non-use of biological fertilizers. In general, with the increase in the use of biological fertilizer and nano chelate, the seed yield and hundred seed weight of cowpea increased (Table 8).

The results of the mutual effects of biological fertilizer and nano chelate fertilizer showed that the non-use of biological fertilizers (biological control) and nano fertilizers caused a significant decrease in biochemical traits. So, in the absence of fertilizer application, the amount of seed yield (0.98 ton/ha) and the weight of one hundred seeds (8.12 gr) showed a significant decrease compared to other treatments. Also, the use of biological fertilizers (amino chelate zinc, aminoalexin, and bio20) along with nano chelate potassium and nano chelate iron caused a significant increase in grain yield (1.88 and 1.93 ton/ha compared to 1.50 ton/ha in the control) and hundred-seed weight (1385 and 14.08 gr compared to 11.20 gr in the control) (Table 8). Researchers reported an increase in sunflower seed yield due to the use of biological fertilizer (Babaeian et al., 2012). Also, Some researchers reported that the yield of chickpeas was significant with the use of iron nano chelate foliar application at the level of 1% (Hamzeei et al., 2014). Researchers found that the effect of iron concentration on chickpea plants with increasing iron concentration, the amount and percentage of nitrogen absorption increased significantly (Singh et al., 2004).

The use of iron nanoparticles has been reported to increase the longitudinal growth of beans (*Vigna sinensis*) due to the specific surface of the iron nanoparticles and their ability to absorb and move more in the plant (Khalaj et al, 2019). The researchers reported that there is a significant linear relationship between iron concentration and plant yield; As a result of iron consumption, the amount of chlorophyll, photosynthesis, and vegetative growth of the plant increased, and this increases the level of carbonation and, the amount of dry matter produced in the plant (Amaliotis et al., 2002).

Tomar (1998) stated that the effect of phosphate-dissolving bacteria and bio fertilizers on the performance of cowpea increased the weight of one hundred seeds and the yield of this plant, which is consistent with the results of this research. Also, some researchers reported that the highest seed yield and hundred seed weight were obtained from the use of Fertilizer-2 phosphate bio-fertilizer and the lowest from the control treatment (no use of bio-fertilizer), which is consistent with the results of this research (Dehmardeh et al., 2018). According to (Ismail-khanbehbin et al., 2011), the use of nitrogen fertilizer increases the speed of grain filling and the speed of dry matter accumulation, so the increase of nitrogen causes the transfer of nutrients to the seed and the weight of one hundred grains increases. However, if nitrogen is available at the right time due to the creation of favorable vegetation and the development of the plant surface, the ability to photosynthesis and store photosynthetic materials in the seed increases, and heavier seeds are produced.

In each column for each factor, the treatments that have at least one letter in common were not significantly different from each other.

CONCLUSION AND SUGGESTION

In general, and according to the results of this experiment, we determined that the use of biological fertilizers, especially Bio20 and Aminoalexin, caused a significant improvement in the quality and performance of cowpea. Also, it was significantly higher due to the increase of protein, starch, and other qualitative characteristics with the combined use of nano chelates, especially nano chelates of iron and potassium, and the positive effect of potassium and iron in various plant metabolic processes. Osmotic regulation includes an increase in chlorophyll and photosynthesis. It seems likely that the use of these fertilizers can be effective in improving and maintaining the yield and quality of cowpea seeds under stress conditions, so a positive interaction between the use of bio-fertilizers, especially bio20, and aminoalexin with nano chelates (mainly iron and potassium) was observed to increase the quantitative and qualitative characteristics of cowpea, therefore, the application of these elements along with the bio-fertilizers under study seems to be a suitable solution to increase yield and quality due to the lack of iron and potassium in agricultural soils,. The product will be produced and on the other hand, the environmental pollution caused by the use of chemical fertilizers in the soil and other adverse effects related to them will be reduced. The economic study of the Moore treatment recommended (use of fertilizer) with the witness (no use of fertilizer) showed that according to the price and the cost of the consumption of fertilizers and deducting it from the price of approximately 1 ton of cowpea (use of treatments), the farmer's net income increased and In terms of added value and economic efficiency, it is also justified and recommended considering the increase and development of the cultivated area of this product in the region.

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