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Bio-fortification of Potato Plants with Different Nitrogen Rates and Applied Iron Forms in Presence and Absence of *Azotobacter* sp.

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FIELD investigation during 2018-2019 and 2019-2020 were carried out to study the effect of nitrogen fertilization and iron applications on potato plants in presence or absence of *Azotobacter* sp. The experiment was laid out in split-split plot design with three replications having 16 treatments arrangement as; four rates of nitrogen fertilization (0, 50, 75 and 100% N from recommended dose) as main plot, two forms of iron (soil and foliar) as sub plot and *Azotobacter* sp. inoculation (presence and absence) as sub-sub plot one. The results revealed that application of 75% N from recommended dose had maximum significant effect on vegetative growth and substantially improved the quantitative and qualitative traits of tuber yield. However, with foliar application of Fe-EDTA results increase in vegetative growth parameters, No. of tubers per plant, average tuber weight/ g and total yield; (Mg fed^{-1}) and chemical content of leaves and tubers also, the highest potato tuber yield with priority in presence of *Azotopacter* sp. However, the use of 75% N + Fe-EDTA + *Azotobacter* sp. combination can be agronomically more useful and ecofriendly more feasible to produced good quality and yield of potato.

Keywords: Nitrogen fertilization, Iron, *Azotobacter* sp. and Potato.

Introduction

Potato (*Solanum tuberosum* L.) is the most significant root and tuber crops serving a lot of people in the country as a source of food. It is a decent system for evading food instability particularly in catastrophe circumstances (Tolessa et al. 2017). In Egypt, potato has an important situation among all vegetable crops, where about 20% of total area dedicated for production of vegetables is cultivated with potato. In Egypt, this crop is economically important and any disturbance in its production affects severely its local requirement and more importantly export impact (Kabeil et al. 2008). According to the United Nations Food and Agricultural Organization statistics, in 2018, cultivation area of potato was 163939.00 ha and production was 4325480.00 Mg in Egypt (FAO, 2018). Egypt exported over 759,200 Mg of ware potatoes and became the 5th largest exporter, supplying potatoes primarily to Russia and the EU. In 2019, Egypt took 5% of the global potato export market, Egyptian potato exports were 259.6 million USD in 2019. Egyptian potatoes are notable for their great quality because Egyptian potatoes grown in sandy or clay soil. Additionally, the Egyptian potatoes are preferred by clients for its ability to long shelf life due to the appropriate level of solidity and sugar.

Bio-fortification is aimed at using plant reproduction as an intercession system to address micronutrient malnutrition by producing staple food crops with enhanced levels of bioavailable basic vitamins and minerals that will have measurable effect on improving the micronutrient status of target populations, essentially resource-poor people in the developing world (Saltzman et al. 2014).

Nitrogen fertility management is a key component in potato production because of the quick reaction of potato to fertilization process. The low fertilizer use efficiency (Zebarth et al., 2004) and the low cost of fertilizer lead farmers

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to add lot of fertilizer which may not be fully used by the crop. El-Dissoky (2019) showed that mixed nitrogen fertilizers treatments improved plant growth, tuber yield, quality, nutrient uptake and NUE compared to sole application of conventional forms, with superiority of MF: 75% AN+25% AS, 50% AN+50% AS and 50% U+50% AS. The highest yield of dry matter and starch was obtained by application of mineral fertilization of 50% AN+50% AS. However, the accumulation of NO_3 - in tuber is contrarily corresponding to dry matter; the highest content obtained with 100% Urea, but the lowest content obtained with mineral fertilization of 25% AN+75% AS.

Micronutrients play a very essential role in vital cycle of plants. They increment the leaves chlorophyll content, improve photosynthesis which intensify the absorbing action of the entire plants (Tripathi et al. 2015). Iron is a significant element for plant growth and iron-deficiency induced chlorosis is a widespread nutritional problem. This unsettling influence becomes evident as an ordinary yellowing of young leaves of plants in several crops and affects flower and leaf mineral content and is accountable for significant decreases in crop size, yield and the quality of many species (Husein 2015). Fe-deficiency is a boundless agricultural problem that decreases plant growth and crop yields. Results demonstrated that application of iron increased all plant characteristics relating to yield components, yield and dry matter % of potato crop (Kamil et al. 2014). Iron is important for chlorophyll development in cell without which photosynthesis is not possible. Foliar application of nutrients has many advantages compared with soil application. Foliar fertilization is more economical than root application because of the higher degree of applied nutrient utilization, which makes the nutrients more efficient. It is fast and efficient method of supplying micronutrients in particular. However, it can also be used to satisfy acute needs of macronutrients. Furthermore, some problems of soil fertilization can only be solved by foliar application. Foliar application may likewise overcome the block of nutrient uptake and improve the target organs directly with proper amount of elements (El-Sawy, 2011).

The use of microorganisms as potential bio-fertilizer is one of the principle strategies to execute sustainable farming practices that help improve rhizosphere soil dynamics (Castellanos et al. 2020). The greatest proportion of biological nitrogen in agricultural soils originates from the activity of symbiotic and free-living nitrogen-fixing bacteria living in the relationship with plants. Consequently, it is very important to apply various measures in the plant creation that will increase the number of certain physiological, the total number of soil microorganisms and systematic groups of microorganisms. Bio-fertilization is the best option of mineral fertilizer is important due to its adverse effects on the soil health. *Azotobacter* sp. abundance in the soil is a good indicator of all toxicological and degradational changes in the soil (Dawwam et al. 2013). There are many available alternatives to improve the soil fertility one of them is *Azotobacter* sp. It is a free–living N_2– fixer diazotroph that has many beneficial impacts on the crop growth and yield. It helps in blend of growth regulating substances like cytokinin, auxins, and giberellicacid. In addition, it stimulates rhizospheric microbes, protects the plants from phyto pathogens, improves nutrient uptake and ultimately boost up biological nitrogen fixation. The abundance of these bacteria in soil is related to many factors, mostly soil pH and fertility (Mahato and Kafle, 2018). Under this situation two field experiments were conducted with the objective of adjusting nitrogen dose, foliar and soil application of iron in absence and presence inoculation with *Azotobacter* sp. which may help in optimizing the growth and quality of potato tuber and increasing its yield.

Materials and Methods

Tubers of potato cultivar sponta were used in this study during the winter seasons of 2018-2019 and 2019-2020, the current research was conducted at private farm, El-Mansoura, Dakahlia Governorate, Egypt. At the start of the experiment, soil sample was collected from the experimental field at the beginning of the experiment and analyzed as routine work. Mechanical analysis determined according to the methods of Haluschak (2006) and was loamy soil type described as; 7.77 pH, 2.96 EC/dsm-1 in soil past and 0.11 g.kg-1 organic matter. Available N, P and K were 29.5, 4.45 and 291 mg.kg -1, respectively, were determined according to Reeuwijk (2002). Available Fe was 1.63 ppm was analyzed by electrothermal atomic absorption spectrometery according to Kumpulainen et al. (1983). The experimental design consisted of split-split plot design with three replications having 16 treatments arrangement as; four rates of nitrogen fertilization (0, 50, 75 and 100% of N recommended dose) as main plot, two forms of iron (soil and foliar) as sub plot and *Azotobacter* sp. inoculation (presence and absence) as sub-sub plot one.

Azotobacter sp. inoculum: Cell suspension of Azotobacter chroccoccum as (N-fixation) was kindly provided from the unit of bio-fertilizers, Fac. Agric. Ain shams Univ. Egypt. A mixture of fine calcium carbonate neutralized peat as a carrier was packed into polyethylene bags (200 g carrier per bag), then sealed and sterilized with gamma irradiation (5.0× 106 rads). *Azotobacter chroococcum* was grown on the medium of Hegazy and Neimela (1976), incubated for 48 hr at 28°C to ensure population density of 109 CFU/ml culture and then injected into the bags containing the sterilized carrier to have 108 cell g carrier.

In plots that received of *Azotobacter* sp. inoculation, potato tubers was coated with the inoculum just before planting using a solution of 10% Arabic gum as adhesive agents. N-fertilization were added at two equal doses one after 15 days from planting and the other two weeks later in forms of ammonium sulfate (20.5 % N) at the rates of 0, 50, 75 and 100% from recommended dose for potatoes plant i.e. 0, 60, 90 and 120 kg fed-1, respectively. P and K were supplemented with 75 and 96 kg fed-1 for potatoes through super phosphate (15 % P_2O_5) and potassium sulfate (48 % K2O), respectively. Full dose of P was added to the soil before sowing while; K were added in two equal doses one after 15 days from sowing and the other two weeks later. Iron fertilization was added in two ways one as soil application in form of FeSO4 (20% Fe) at the rate of 5 kg.fed-1, the other one as foliar way in form of Fe-EDTA 12% Fe (300 ppm). On the first week of November during both seasons' seed tubers was planted at 25 cm apart between each other and on one side of ridges (4m long and 70 cm wide). The plot consisted of 4 ridges making an area of about 10 m^2.

After 70 days from sowing; five plant samples were randomly taken from each plot and carried immediately to the laboratory. Plant growth parameters in expression of plant height (cm), fresh and dry weight of plant foliage (g/plant) as well as chlorophyll contents (mg/g F.W) were determined. Samples of 100 g from each sample were oven dried at 70 °C tell constant weight was reached. Then, dry matter was calculated (g/plant) and the dried parts were thoroughly ground and stored for chemical analysis of N, P, K % and Fe mg kg-1.

At full maturity stage (125 days from sowing); total tuber yield was collected and calculated as Mg/fed. A random sample of 20 tubers from each experimental plot were taken for determination of tuber measurement i.e, fresh weight of tuber (g/plant), number of tubers per plant, average tuber weight (g) and tuber day matter (%) Chemical constituents of tubers expressed as N was obtained using the Kjeldahl method. P and K were measured using spectrophotometers and flame photometer, respectively as descripted by Rukun (1999), Fe was analyzed by electrothermal atomic absorption spectrometery according to Kumpulainen et al. (1983). Chlorophyll content was measured by spectrophotometric method of Gavrilenko and Zigalova (2003). Quality parameters of fresh tubers i.e., For estimation of total carbohydrates in fresh tubers; the ethanol extract was used for the determination of total carbohydrates was estimated in hot acisic medium using antheron method. The green colours was measured spectrophotometrically at 630 nm. Sadasivam and Manickam, (1996). Starch was determined by the method of Antheron reagent as described by Thymanavan and Sadasivam, (1984). Tuber samples were treated with 80 % ethanol to remove sugars and then starch was extracted with percholoric acid. The intensity of green to dark green colour was estimated photometrically at wave length of 630 nm. Glucose content was calculated in the sample using standard curve and the value was multiplied by a factor of 0.9 to arrive the starch content. Vitamin C was determined according to the method described by Mazumdar and Majumder (2003) using titrimetric estimation with 2, 6 dicholoro phenol dye solution as well as NO3-N content (mgkg-1) were determined according to Singh (1988). Results from identical experiments of the 2 years were combined for analysis. Significant differences among treatments means were determined at P ≤ 0.05 by using LSD test and Duncan's Multiple Comparisons Test. Data of the present study were statistically analyzed using CoSTATE Computer Software, according to Gomez and Gomez (1984).

Results

Vegetative growth parameters

Data at Table 1 indicated the effect of nitrogen fertilization rates, forms of iron fertilization as well as their interactions in presence or absence of *Azotobacter* sp. inoculation on the vegetative growth parameters of potato plants as plant height, fresh and dry weight during both seasons of the experiments 2018-2019 and 2019-2020. As for the effect of nitrogen fertilization data in Table 1 indicated that plant height, fresh and dry weight were significantly affected with all rates of nitrogen (0, 50, 75 and 100% recommended doses of N). The highest mean values were recorded using 75% N comparing with the control during both seasons then decrease with 100% N. The same table showed the plant height, fresh and dry weight as affected by forms of iron fertilization whether in soil ($FeSO_4$) or foliar application (Fe-EDTA). The data were significantly affected with both applications but the highest mean values recorded with foliar application with Fe-EDTA (76.44 & 84.10 cm for plant height), (434.66 & 460.70 g/plant for fresh weight) and (50.42 &52.68 g/plant for dry weight), respectively during both seasons. *Azotobacter* sp. inoculation significantly increased all vegetative growth parameters comparing with non-inoculation and recorded the highest values (79.41 & 87.35 cm for plant height), (445.01 & 469.61 g/plant for fresh weight) and (51.91 & 54.18 g/plant for dry weight), respectively during both two seasons.

The statistical analysis of variance in Fig. 1, 2 & 3 revealed that there were highly significant differences for the plant height, fresh and dry weight (g) owing to the interaction effect among all treatments. In this respect, the highest values recorded with using 75% of N recommended dose + foliar application with FE-EDTA in presence of *Azotobactersp*. during both seasons.

TABLE 1. Vegetative growth parameters of potato plants as affected by N-fertilization, iron forms, *Azotobacter* sp. and their interactions during 2018-2019 and 2019-2020

Treatments	Plant height cm		Fresh weight of plant foliage g/plant		Dry weight of plant foliage g/plant	
	1st	2nd	1st	2nd	1st	2nd
	Nitrogen fertilization rates					
0% N	59.36d	64.12d	375.31d	409.09d	40.89d	43.97d
50% N	75.92c	83.21c	432.40c	458.45c	50.15c	52.38c
75% N	80.55a	91.29a	449.05a	473.23a	52.73a	54.83a
100% N	79.35b	89.71b	442.07b	467.23b	51.74b	53.87b
LSD at 5%	1.08	0.93	3.19	0.57	0.15	0.29
	Forms of iron application					
FeSO4 (soil)	70.77b	77.31b	414.12b	442.55b	47.19b	49.74b
Fe-EDTA (foliar)	76.81a	86.85a	435.29a	461.45a	50.57a	52.78a
LSD at 5%	0.31	0.33	1.20	1.02	0.05	0.19
	Azotobacter sp. inoculation					
Without	67.80b	76.55b	403.77b	433.63b	45.70b	48.24b
With Azotobacter	79.78a	87.61a	445.63a	470.36a	52.06a	54.28a
LSD at 5%	0.72	0.69	0.93	1.21	0.07	0.17

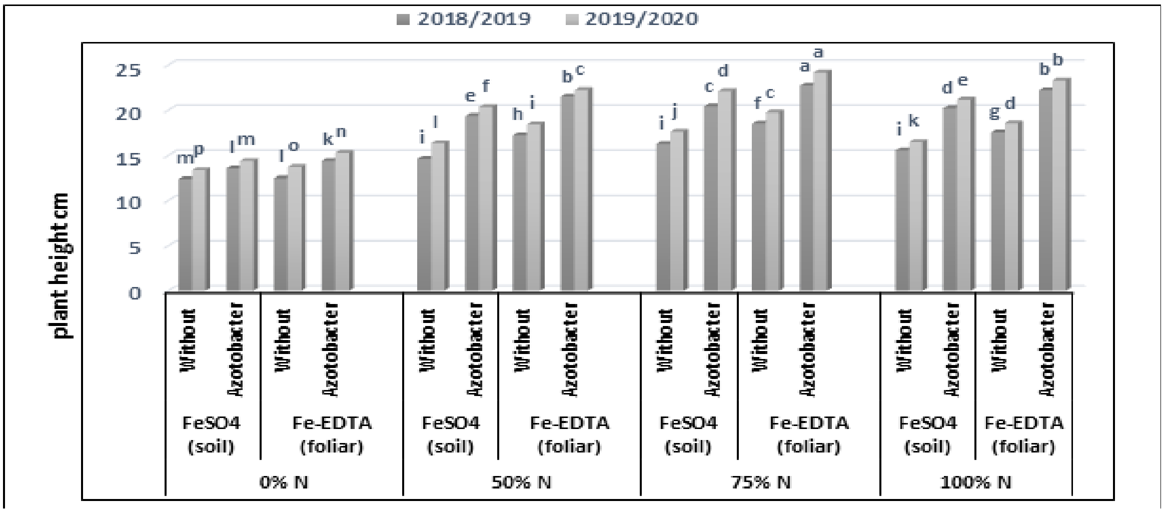

Fig. 1. Interaction effect among N-fertilization, iron forms and *Azotobacter* sp. on plant height during 2018/2019 and 2019/2020

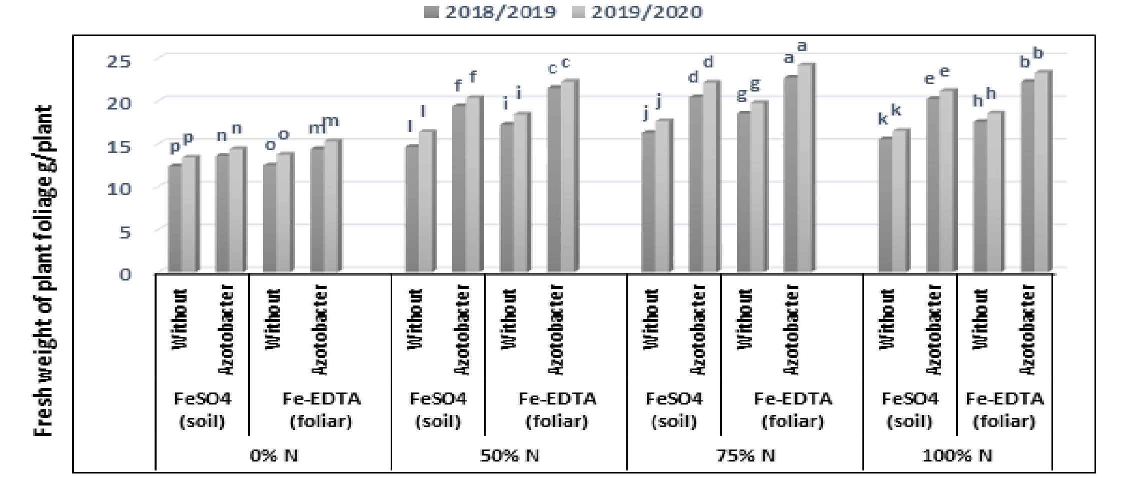

Fig. 2. Interaction effect among N-fertilization, iron forms and *Azotobacter* sp. on Fresh weight of plant foliage g/plant during 2018/2019 and 2019/2020

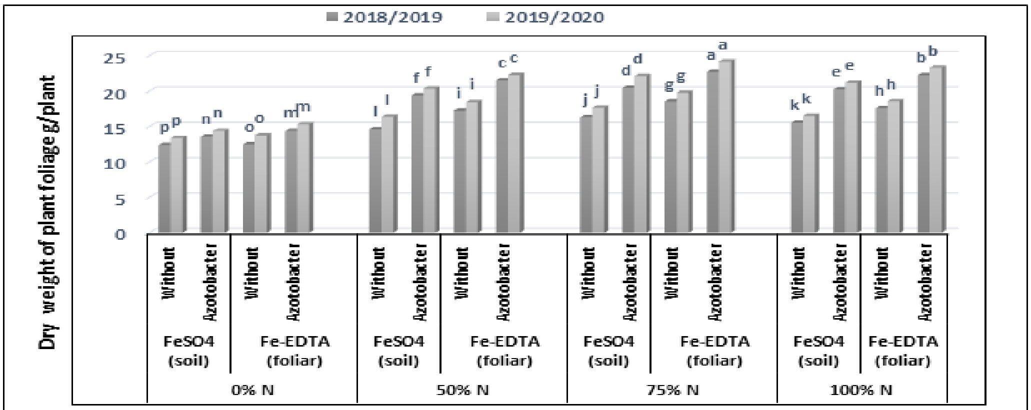

Fig. 3. Interaction effect among N-fertilization, iron forms and *Azotobacter* sp. on dry weight of plant foliage g/plant during 2018/2019 and 2019/2020

Chemical composition

Chlorophyll content in leaves

Results presented in Table 2 declared the content of photosynthetic pigments as affected by nitrogen fertilization rats, iron forms, *Azotobacter* sp. and their interactions during 2018-2019 and 2019-2020.Chlorophyll a, b and total recorded at Table 2 as affected by nitrogen fertilization rates. photosynthetic pigments increased with increasing N-fertilization rates up to 75% from recommended dose and recorded the highest values comparing with control treatment without fertilization. Iron forms weather using in soil or foliar way effect on the chlorophyll a, b and total as the result in Table 2 revealed. Both forms caused a significant increase in chlorophyll content, but foliar application with Fe-EDTA recorded the highest mean values of chlorophyll a (0.693 and 0.683 mgg^{-1}), chlorophyll b (0.406 and 0.418 mgg^{-1}) and total chlorophyll (1.119 and 1.125 mgg^{-1}) during two seasons, respectively.

Data presented at Fig. 4, 5 & 6 showed the mean values of chlorophyll content as affected by presence or absence of *Azotobacter* sp. inoculation. Data revealed that addition of *Azotobacter* sp. increased chlorophyll content comparing with the un-inoculated plants during both seasons. Figures 4, 5 & 6 showed the interaction between previous treatments on chlorophyll content of potato plants. It can be found that chlorophyll a, b and a+b significantly increased in N-fertilization, plants fertilized with Fe forms in presence of *Azotobacter* sp. It can be noticed that the plants grown under 75%RD N fertilization and foliated with 300 ppm Fe-EDTA in presence of *Azotobacter* sp. attained the highest chlorophyll a, b, and a+b as compared with other treatments.

TABLE 2. Chlorophyll content of potato plants as affected by N-fertilization, iron forms, Azotobacter sp. and their interactions during 2018-2019 and 2019-2020

Treatments	Chlorophyll a (mg g^{-1}) FW		Chlorophyll b (mg/g) FW		Total chlorophyll (mg g^{-1}FW)	
	1st	2nd	1st	2nd	1st	2nd
Nitrogen fertilization rates						
0% N	0.614d	0.488d	0.357c	0.367d	0.971d	0.855d
50% N	0.689c	0.680c	0.403b	0.416c	1.092c	1.096c
75% N	0.710a	0.740a	0.417a	0.432a	1.126a	1.172a
100% N	0.700b	0.694b	0.412ab	0.424b	1.112b	1.118b
LSD $_{at\ 5\%}$	0.006	0.006	0.008	0.004	0.007	0.008
Forms of iron application						
$FeSO_4$ (soil)	0.663b	0.617b	0.388b	0.401b	1.051b	1.019b
Fe-EDTA (foliar)	0.693a	0.684a	0.406a	0.418a	1.100a	1.102a
LSD $_{at\ 5\%}$	0.002	0.002	0.001	0.001	0.003	0.003
Azotobacter sp. inoculation						
Without	0.650b	0.603b	0.380b	0.391b	1.030b	0.994b
With Azotobacter	0.706a	0.698a	0.415a	0.428a	1.121a	1.126a
LSD $_{at\ 5\%}$	0.003	0.003	0.001	0.003	0.003	0.004

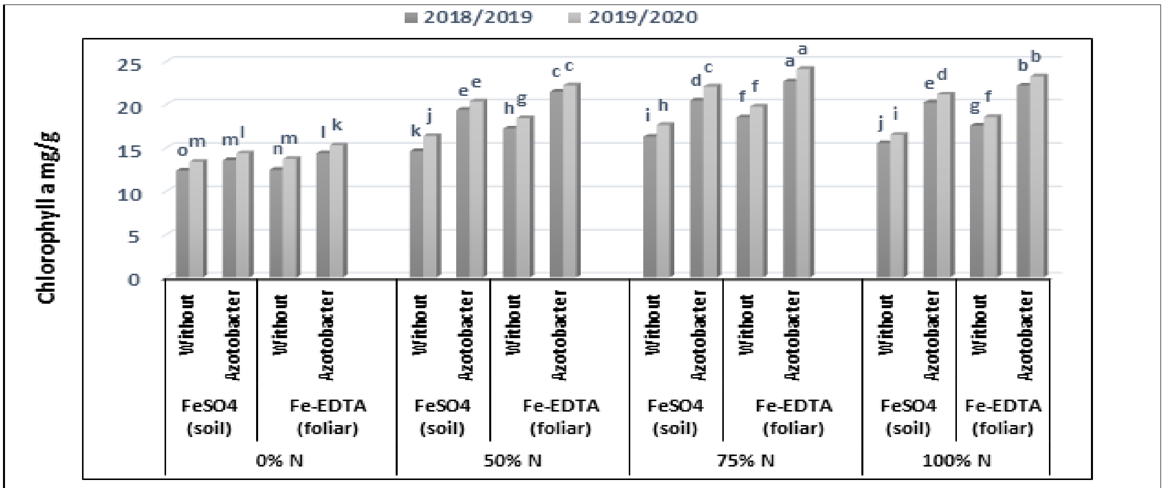

Fig. 4. Interaction effect among N-fertilization, iron forms and Azotobacter sp. on chlorophyll a mg/g FW during 2018/2019 and 2019/2020

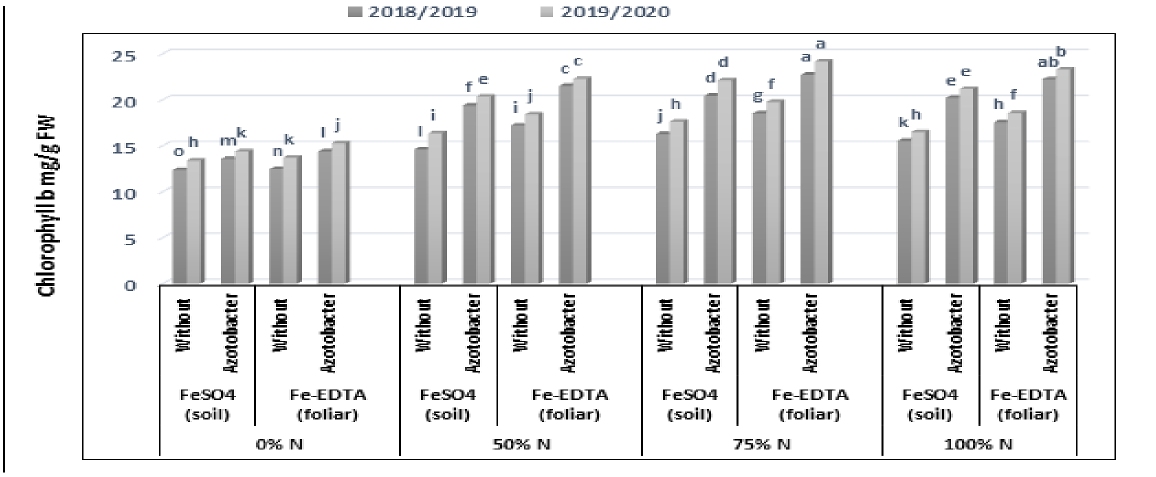

Fig. 5. Interaction effect among N-fertilization, iron forms and Azotobacter sp. on chlorophyll b mg/g FW during 2018/2019 and 2019/2020

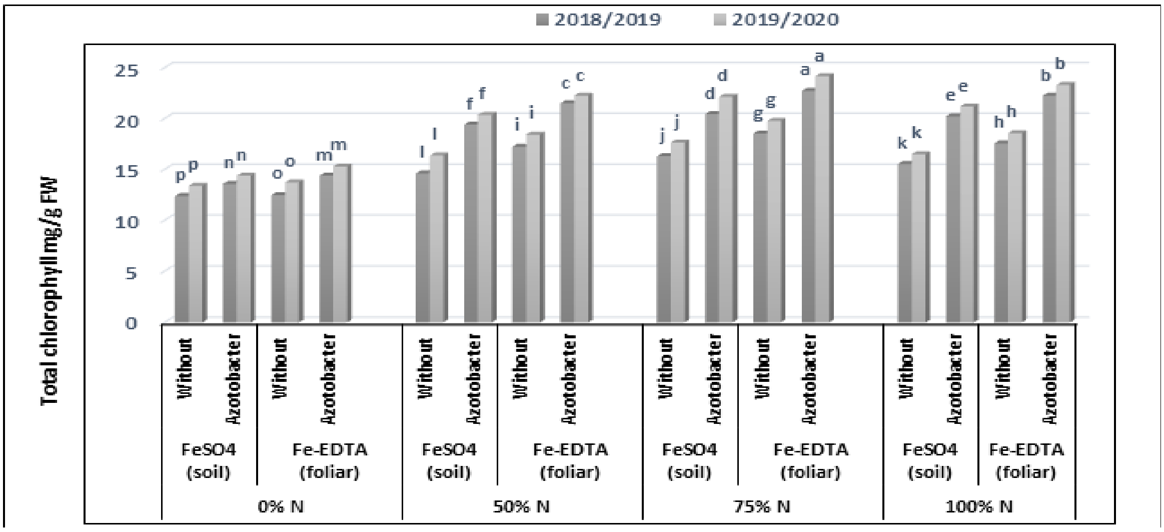

Fig 6. Interaction effect among N-fertilization, iron forms and Azotobacter sp. on total chlorophyll mg/g FW during 2018/2019 and 2019/2020.

Nutrient content in leaves and tuber

Data recorded at Table 3 and 4 showed the effect of nitrogen fertilizer rates on N, P, K and Fe in leaves and tuber of potato plants. The result indicated that all nutrient increased significantly with increasing application of nitrogen fertilization comparing with the untreated plants. The highest mean values of N, P, K % and Fe mg.kg^{-1} recorded with 75% N- fertilization from N-fertilization. Application of iron forms weather in soil or foliar way affected significantly in elements of leaves and tuber as indicated at Table 3 and 4. In addition, found that iron in foliar way in forms of Fe-EDTA was more superior to recorded the highest values of N, P, K and Fe content in leaves and tubers. In the same Tables, using *Azotobactersp.* inoculation affected in N, P, K and Fe content in leaves and tubers of potato plants. The increase was significant comparing with the un-inoculated plants. The increase in nitrogen concentration and all other treatments can be attributed to the positive role of the used *Azotobactersp.* bacteria as a inoculum with a wide range of positive properties that promote plant growth, including its ability to stabilize the nitrogen and then increase the plant concentration of nitrogen.

It is obvious from the same data in Figures (7 to 14) that all nutrient in leaves and tuber of potato plant are affected by interaction. In general, plants received 75% N with Fe-EDTA in presence of *Azotobactersp.* gave the highest values of N, P, K % and Fe mg.kg^{-1} investigated in during the two seasons.

TABLE 3. N, P, K and Fe content of potato leaves as affected by N-fertilization, iron forms, Azotobactersp. and their interactions during 2018-2019 and 2019-2020

Treatments	N, %		P, %		K, %		Fe, mg kg^{-1}	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Nitrogen fertilization rates								
0% N	2.42c	2.95c	0.316d	0.348d	3.11d	3.39c	12.39d	13.34d
50% N	2.84b	3.24b	0.379c	0.399c	3.43c	3.70b	13.90c	14.97c
75% N	3.00a	3.33a	0.398a	0.416a	3.52a	3.78a	14.06a	15.13a
100% N	2.87b	3.28b	0.389b	0.410b	3.47b	3.75a	13.47b	14.47b
LSD $_{at\ 5\%}$	0.04	0.04	0.004	0.003	0.03	0.03	0.08	0.12
Forms of iron application								
$FeSO_4$ (soil)	2.68b	3.14b	0.359b	0.383b	3.33b	3.60b	13.34b	14.35b
Fe-EDTA (foliar)	2.88a	3.25a	0.382a	0.403a	3.44a	3.71a	13.57a	14.61a
LSD $_{at\ 5\%}$	0.02	0.01	0.002	0.004	0.03	0.01	0.11	0.03
*Azotobacter*sp. inoculation								
Without	2.58b	3.09b	0.348b	0.372b	3.28b	3.54b	13.01b	14.02b
With Azotobacter	2.98a	3.31a	0.393a	0.414a	3.49a	3.77a	13.90a	14.94a
LSD $_{at\ 5\%}$	0.02	0.02	0.003	0.002	0.02	0.02	0.04	0.07

TABLE 4. N, P, K and Fe content of potato tubers as affected by N-fertilization, iron forms, *Azotobacter* sp. and their interactions during 2018-2019 and 2019-2020

Treatments	N, %		P, %		K, %		Fe,mg kg $^{-1}$	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
				Nitrogen fertilization rates				
0% N	1.06d	1.26d	0.196d	0.207d	2.47d	2.62d	9.16d	9.88d
50% N	1.36c	1.52c	0.224c	0.236c	2.73c	2.88c	9.87c	11.10c
75% N	1.45a	1.60a	0.232a	0.245a	2.80a	2.96a	9.99a	11.22a
100% N	1.40b	1.57b	0.229b	0.242b	2.77b	2.92b	9.65b	10.77b
LSD at 5%	0.01	0.03	0.003	0.005	0.02	0.02	0.12	0.10
				Forms of iron application				
FeSO4 (soil)	1.27b	1.43b	0.215b	0.228b	2.65b	2.79b	9.60b	10.64b
Fe-EDTA (foliar)	1.37a	1.54a	0.225a	0.237a	2.73a	2.89a	9.73a	10.84a
LSD at 5%	0.03	0.03	0.003	0.003	0.02	0.02	0.06	0.09
				*Azotobacter*sp. inoculation				
Without	1.21b	1.40b	0.210b	0.223b	2.60b	2.76b	9.45b	10.41b
With Azotobacter	1.43a	1.57a	0.230a	0.243a	2.78a	2.92a	9.89a	11.07a
LSD at 5%	0.02	0.01	0.002	0.002	0.02	0.03	0.06	0.05

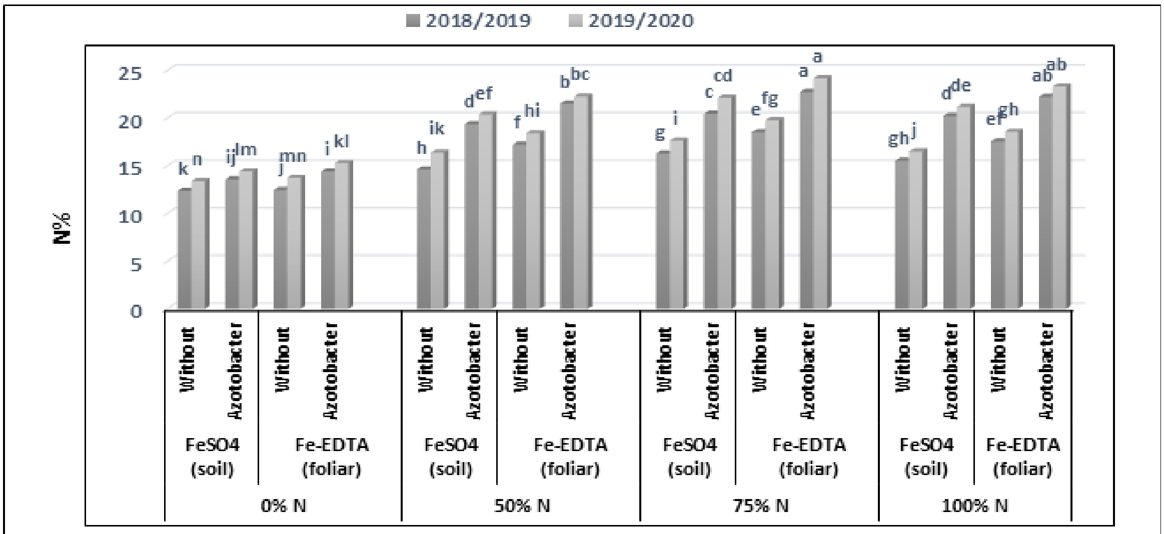

Fig. 7. Interaction effect among N-fertilization, iron forms and *Azotobacter* sp. on N% in potato leaves during 2018/2019 and 2019/2020

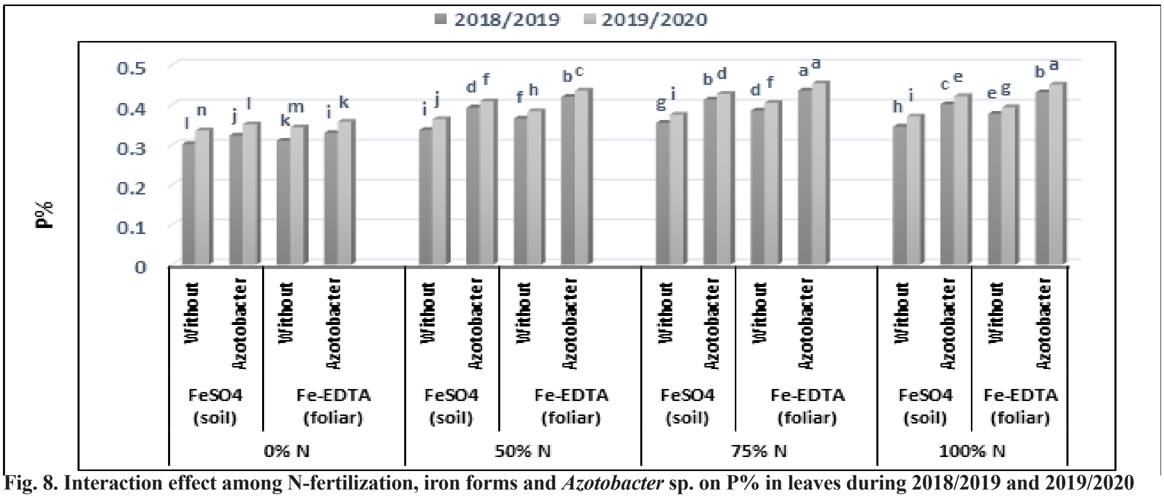

Fig. 8. Interaction effect among N-fertilization, iron forms and *Azotobacter* sp. on P% in leaves during 2018/2019 and 2019/2020

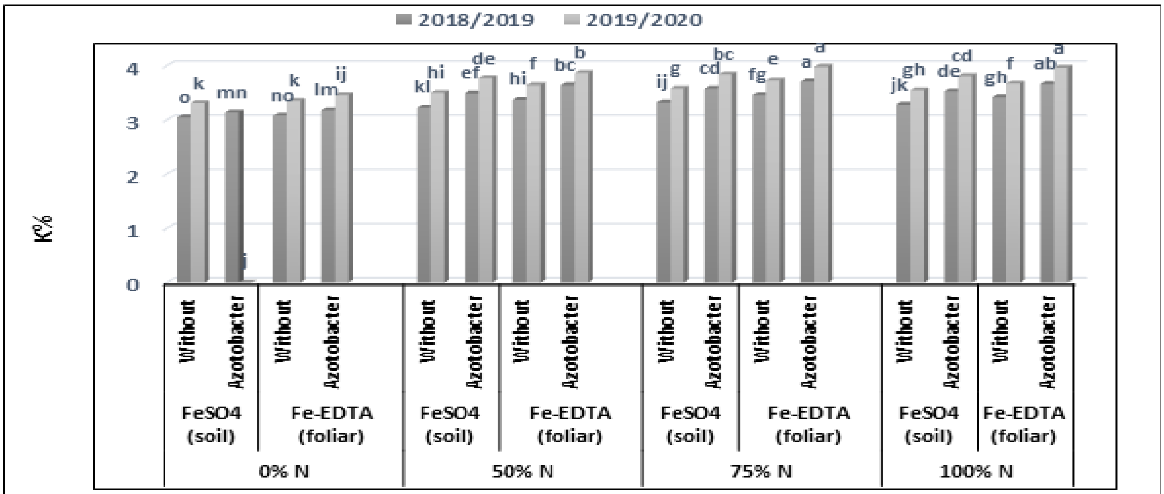

Fig. 9. Interaction effect among N-fertilization, iron forms and *Azotobacter* sp. on K% in leaves during 2018/2019 and 2019/2020

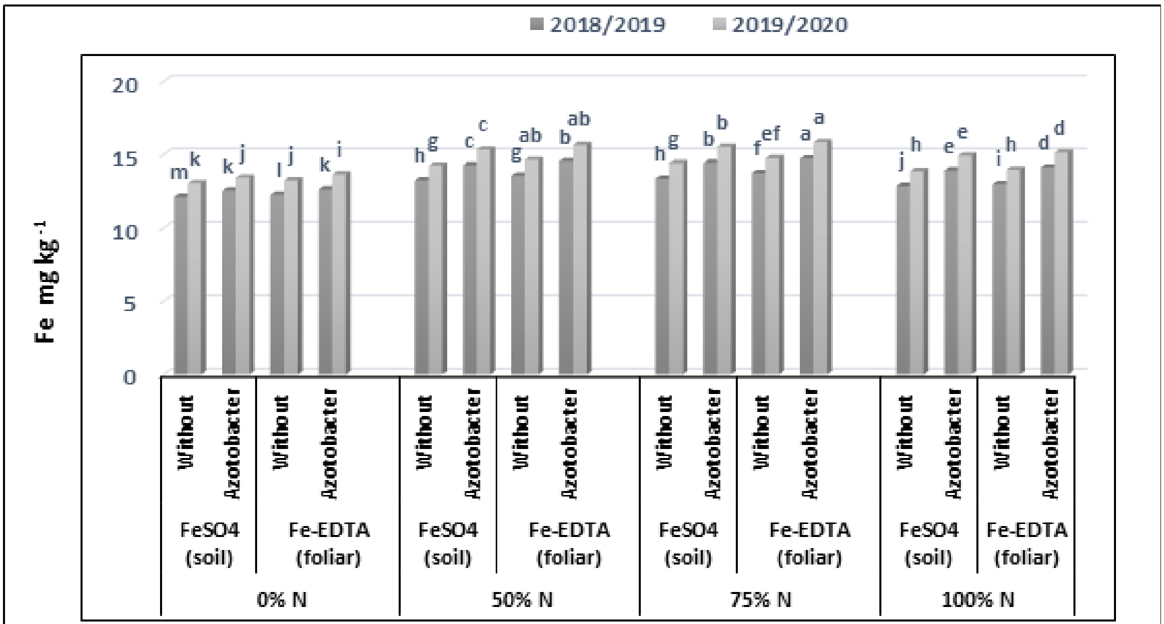

Fig. 10. Interaction effect among N-fertilization, iron forms and *Azotobacter* sp. on Fe mg.kg -1 in potato leaves during 2018/2019 and 2019/2020

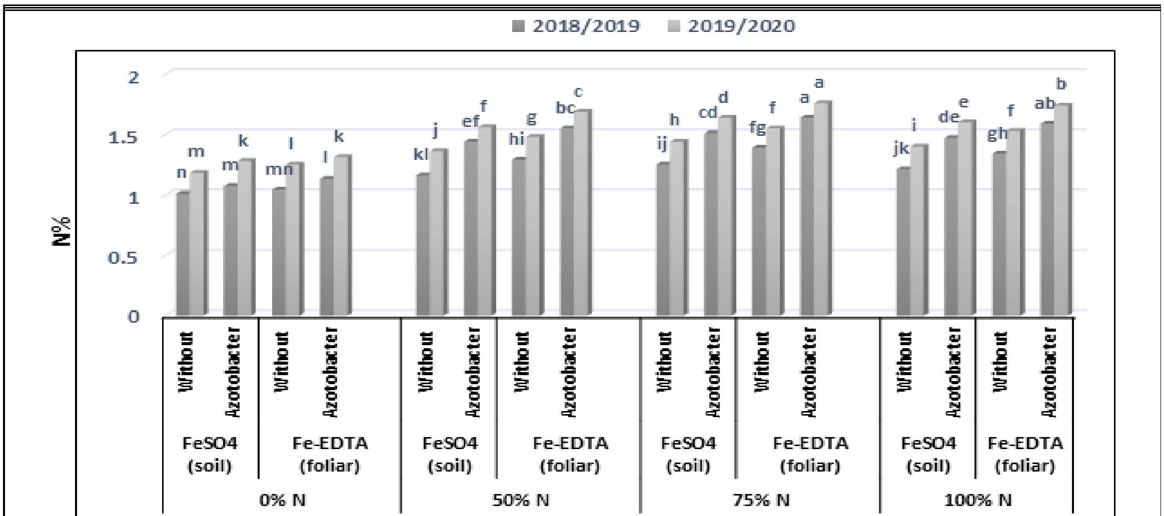

Fig. 11. Interaction effect among N-fertilization, iron forms and *Azotobacter* sp. on N% in potato tubers during 2018/2019 and 2019/2020

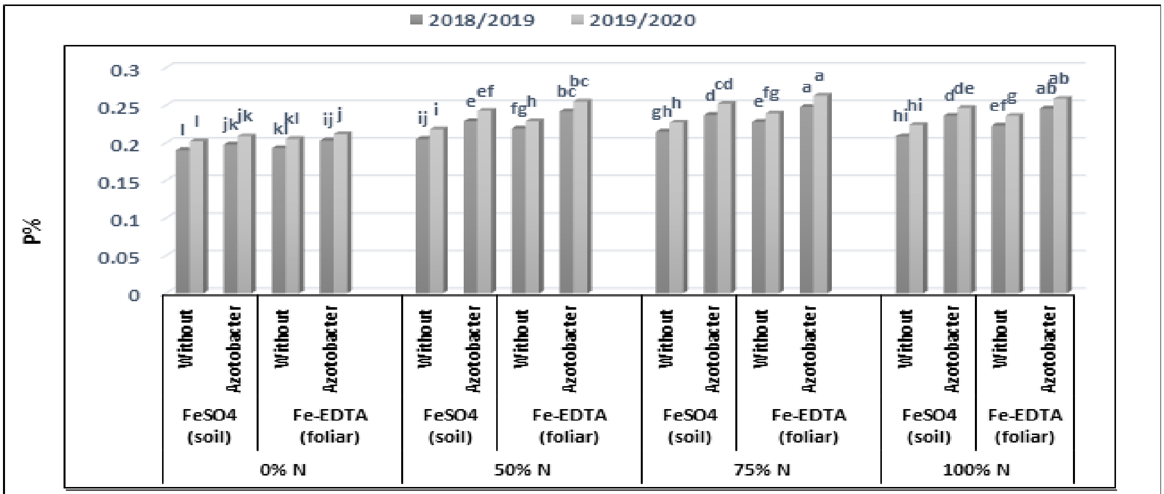

Fig. 12. Interaction effect among N-fertilization, iron forms and *Azotobacter* sp. on P% in potato tubers during 2018/2019 and 2019/2020

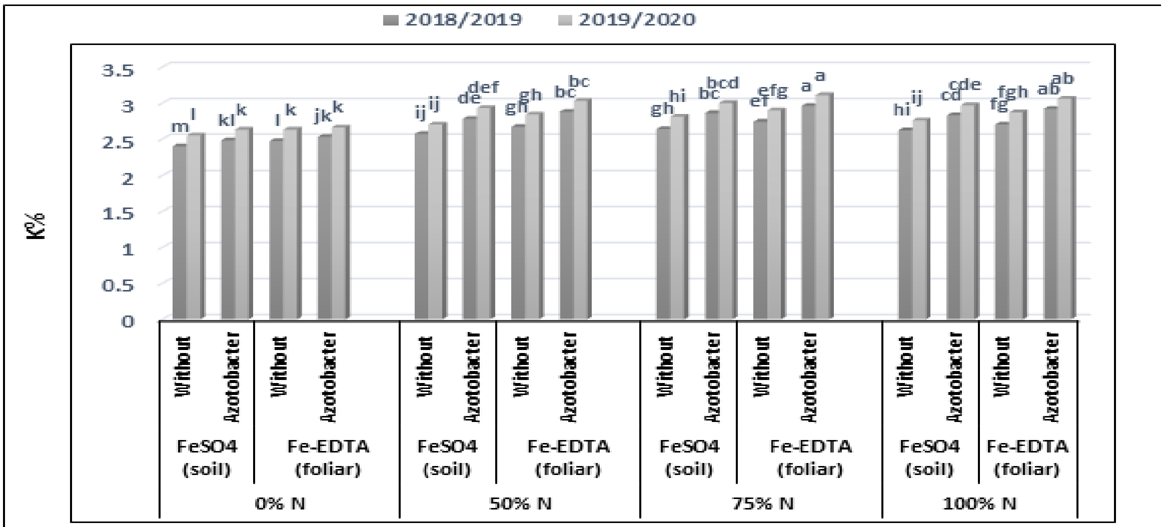

Fig. 13. Interaction effect among N-fertilization, iron forms and *Azotobacter* sp. on K% in potato tubers during 2018/2019 and 2019/2020

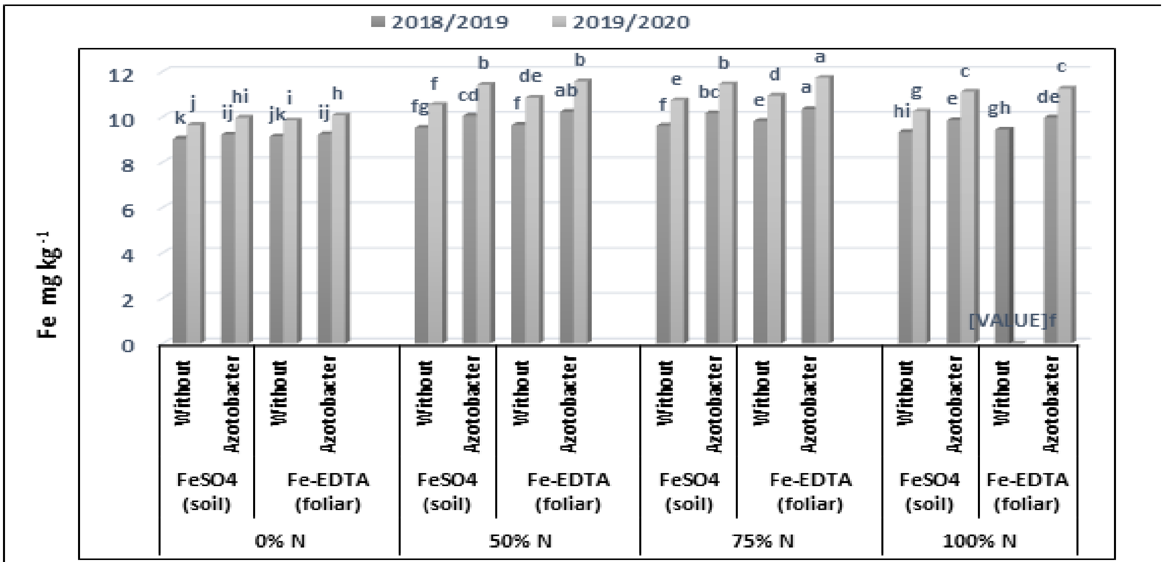

Fig. 14. Interaction effect among N-fertilization, iron forms and *Azotobacter* sp. on Fe mg kg -1 in potat tubers during 2018/2019 and 2019/2020

Yield and its components

Yield components of potato tubers in expression of fresh weight of tuber (g plant $^{-1}$), No. of tubers per plant, average tuber weight/ g and dry matter of tubers (%) as well as total yield; (Mg fed^{-1}) as affected by different rates of N-fertilization, iron forms, *Azotobacter* sp. and their interactions during 2018-2019 and 2019-2020 are presented in Table 5. Statistical analysis of the data presented in Table 5 showed that; within the different rates of N-fertilization studied it can be observed that; using of 75% N from recommended dose was superior for recording the highest values of all the aforementioned traits, following 100% N and lastly 50% N comparing with the untreated plants. For example, the average values for the fresh weight of tubers are 824.92, 817.80 and 807.84 (g plant^{-1}) in 2018-2019 for the treatments of 75, 100 and 50% NPK, respectively. The same trend was realized for the other yield components and tuber yield (Mg fed $^{-1}$) during both seasons. Comparing with the control treatment; the average values of yield and its components for the plants treated with any of the studied N-fertilization in single form at different rates were over than obtained for the control treatment and such effect was significant for all parameters.

Regarding to the effect of iron forms in soil or foliar way on yield and its components as indicated in Table 5, we found that application of iron increased significantly effect on yield and its components, but foliar application with Fe-EDTA was more superior than soil application with $FeSO_4$ during both seasons. It is clear from data in Table 5 that the positive effect was happened due to inoculation with *Azotobactesp*. and recorded the highest mean values of fresh weight of tuber (g plant $^{-1}$), No. of tubers per plant, average tuber weight/ g and dry matter of tubers (%) as well as total yield; (Mg fed^{-1}) comparing with the un-inoculated plants.

It is evident from the results indicated in Fig. 15 - 19 that treating potato plants with all treatments interactions significantly increased all yield and its components as indicated at the same table. The highest mean values of all traits previse recorded with 75% N-fertilizer+ Fe-EDTA+ *Azotobactersp*. during 2018-2019 and 2019-2020.

TABLE 5. Yield and its components of potato tubers as affected by N-fertilization, iron forms, *Azotobacter* sp. and their interactions during 2018-2019 and 2019-2020

Treatments	Fresh weight of tuber g/plant		Number of tuber/ plant		Average weight of tuber g/plant		DM of tuber %		Total tuber yield (Mg fed-1)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Nitrogen fertilization rates										
0% N	748.69d	795.17d	3.42b	5.67b	116.15c	130.62d	15.82d	17.10d	13.20d	14.19c
50% N	807.84c	846.73c	5.25a	8.25a	129.22b	150.00c	17.66c	19.23c	18.18c	19.34b
75% N	824.92a	862.00a	6.08a	8.75a	133.05a	155.65a	18.19a	19.85a	19.49a	20.92a
100% N	817.80b	855.93b	5.83a	8.42a	131.14ab	153.34b	18.00b	19.53b	18.88b	19.88ab
LSD at 5%	1.74	1.99	0.91	0.73	2.04	1.04	0.13	0.05	0.50	1.40
Forms of iron application										
FeSO4 (soil)	789.32b	830.12b	4.79b	7.38b	124.88b	143.80b	17.08b	18.54b	16.55b	17.73b
Fe-EDTA (foliar)	810.31a	849.79a	5.50a	8.17a	129.89a	151.00a	17.76a	19.31a	18.32a	19.43a
LSD at 5%	1.40	1.34	0.21	0.60	0.79	1.52	0.02	0.08	0.37	0.55
*Azotobacter*sp. inoculation										
Without	778.58b	820.83b	4.25b	7.08b	122.93b	140.23b	16.76b	18.15b	15.57b	16.79b
With Azotobacter	821.04a	859.09a	6.04a	8.46a	131.84a	154.58a	18.08a	19.70a	19.31a	20.38a
LSD at 5%	0.92	1.02	0.45	0.42	0.98	0.77	0.07	0.07	0.76	0.50

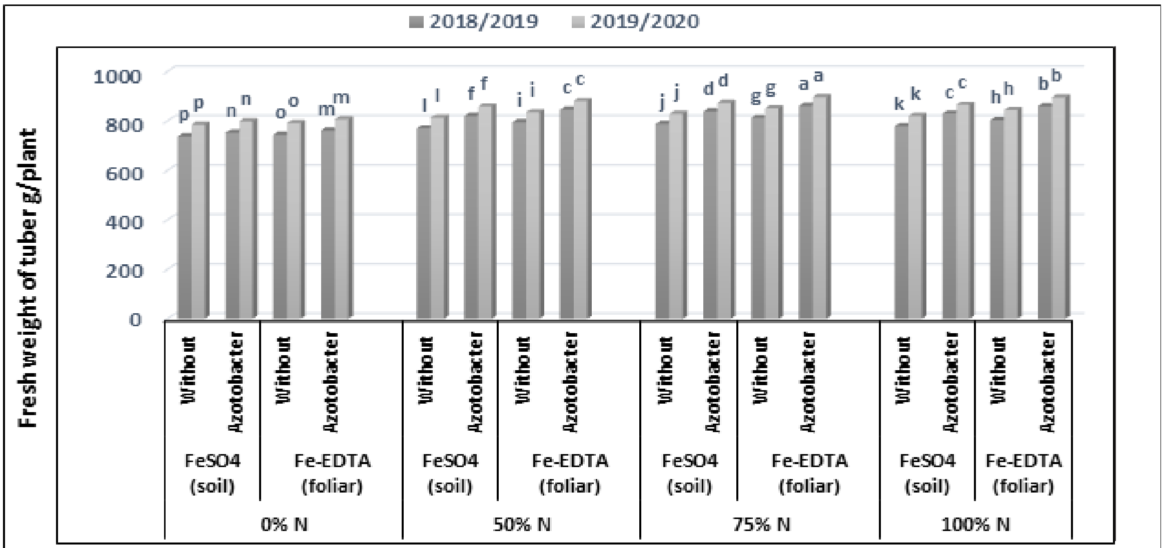

Fig. 15. Interaction effect among N-fertilization, iron forms and *Azotobacter* sp. on fresh weight of tuber g/plant during 2018/2019 and 2019/2020.

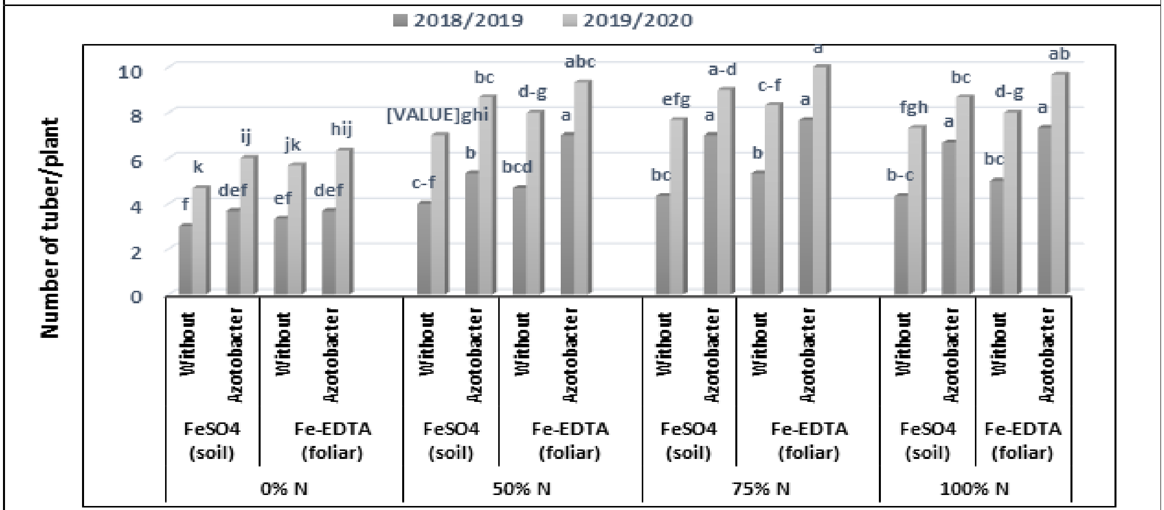

Fig. 16. Interaction effect among N-fertilization, iron forms and *Azotobacter* sp. on number of tuber/plant during 2018/2019 and 2019/2020

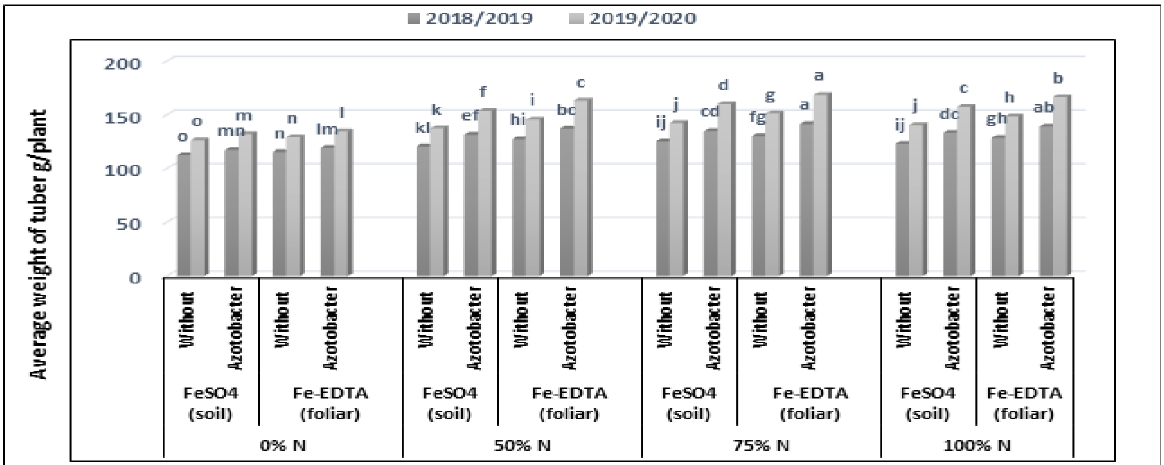

Fig. 17. Interaction effect among N-fertilization, iron forms and *Azotobacter* sp. on average weight of tuber g/plant during 2018/2019 and 2019/2020

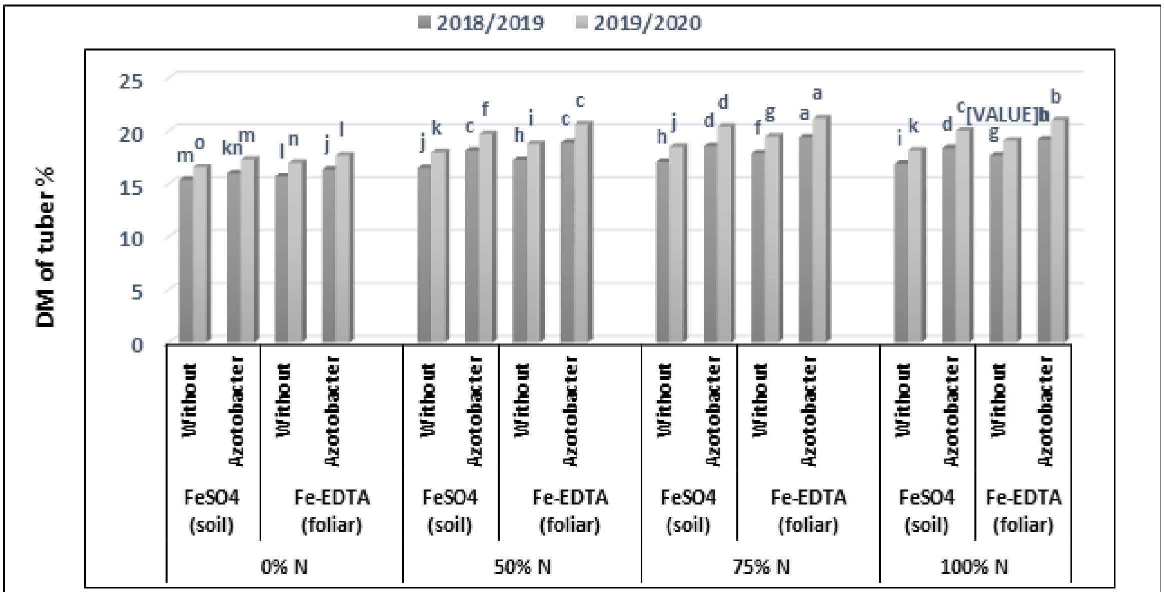

Fig. 18. Interaction effect among N-fertilization, iron forms and *Azotobacter* sp. on DM of tuber % during 2018/2019 and 2019/2020

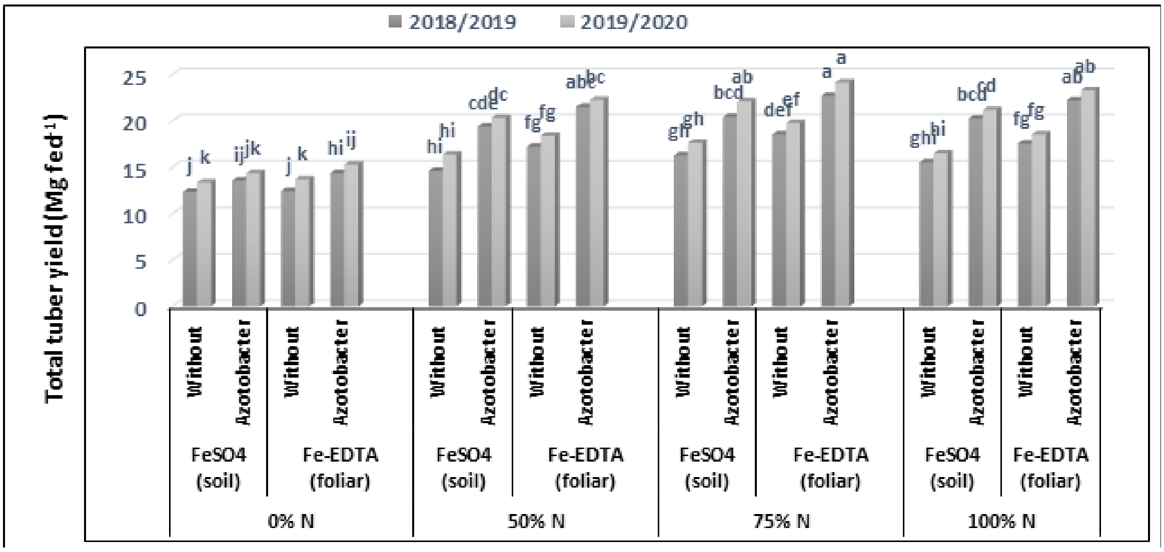

Fig. 19. Interaction effect among N-fertilization, iron forms and *Azotobacter* sp. on total tuber yield (Mg fed-1) during 2018/2019 and 2019/2020

Quality parameters

Quality parameters of potato tubers in expression of total carbohydrates, starch (%), vitamin C (mg 100g $^{-1}$) and NO_3-N;(mg kg $^{-1}$) are presented in Table 6 during both seasons of the experiment. Data obtained in Table 6 indicated that,with different rates of N-fertilization studied can be observed that; using of 75% N from recommended dose was superior for recording the highest values oftotal carbohydrates, starch (%) and vitamin C (mg 100g $^{-1}$), while NO_3-N;(mg kg $^{-1}$) decreased with increasing N-fertilization rates during both seasons.All the above-mentioned traits found in the same Table were significantly affected due to using forms of iron fertilization and the highest values were recorded with FE-EDTA in foliar way, except NO_3-N ppm.On the contrary, of this trend; sharply and significantly increased were happened in the mean values of all parameters in potato tubers due to the addition of *Azotobecter* sp., except NO_3-N which decreased. The same traits was happened during both seasons.

It is obvious from the data revealed in Fig. 20 - 23 that, interaction between all treatments significantly affected in total carbohydrates, starch (%), vitamin C (mg 100g $^{-1}$) and NO_3-N;(mg kg $^{-1}$) are presented in Table 6 during both seasons of the experiment. In the same way found that, application of 75% N-fertilization+ Fe-EDTA +*Azotobacter* sp. recorded the highest values of mentioned parameters except NO_3-N ppm, which recorded the lowest values in the same treatments. This trend was true in the second season.

TABLE 6. Quality parameters of potato tubers as affected by N-fertilization, iron forms, *Azotobacter* sp.and their interactions during 2018-2019 and 2019-2020.

Treatments	Total carbohydrate%		Starch %		V.C mg/100 g		NO3-N ppm	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Nitrogen fertilization rats								
0% N	22.37d	25.07d	18.21d	19.76d	18.28d	19.74d	46.86a	54.58a
50% N	23.36c	25.86c	19.78c	21.57c	19.87c	21.24c	34.49b	39.56b
75% N	23.62b	26.07b	20.22b	22.05b	20.32b	21.71b	38.00c	44.03c
100% N	23.51a	25.96a	20.01a	21.85a	20.13a	21.51a	34.00d	39.43d
LSD at 5%	0.12	0.08	0.09	0.16	0.08	0.06	0.96	0.85
Forms of iron application								
FeSO4 (soil)	23.03b	25.60b	19.28b	20.98b	19.37b	20.77b	39.25a	45.33a
Fe-EDTA (foliar)	23.40a	25.88a	19.83a	21.63a	19.93a	21.33a	37.42b	43.47b
LSD at 5%	0.08	0.08	0.04	0.07	0.09	0.04	0.76	0.85
Azotobacter sp. inoculation								
Without	22.85b	25.44b	19.02b	20.68b	19.08b	20.48b	41.63a	48.56a
With Azotobacter	23.57a	26.04a	20.09a	21.93a	20.22a	21.62a	35.04b	40.24b
LSD at 5%	0.04	0.07	0.07	0.06	0.05	0.07	0.55	0.45

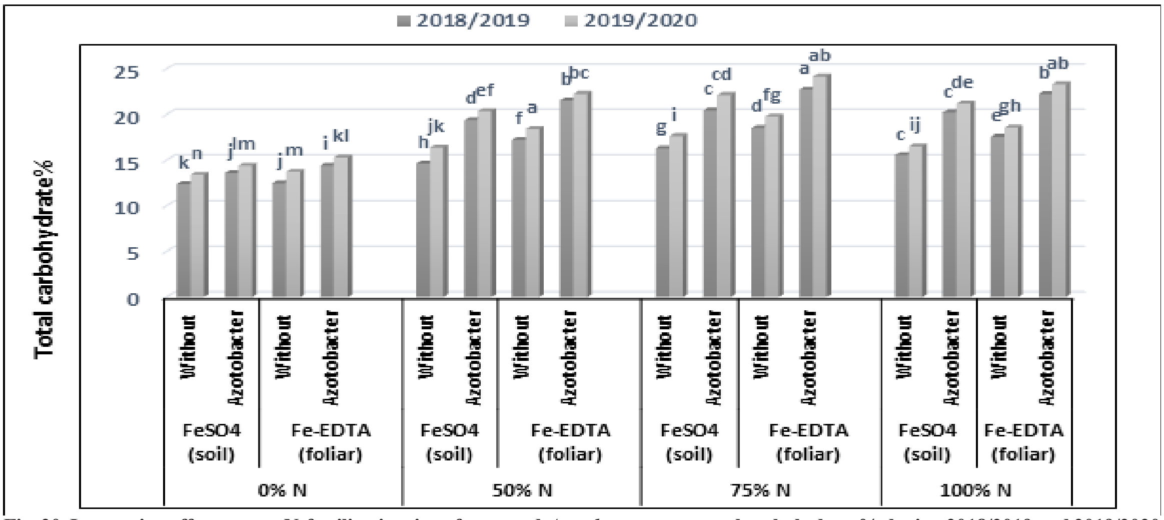

Fig. 20. Interaction effect among N-fertilization, iron forms and *Azotobacter* sp. on total carbohtdrate% during 2018/2019 and 2019/2020

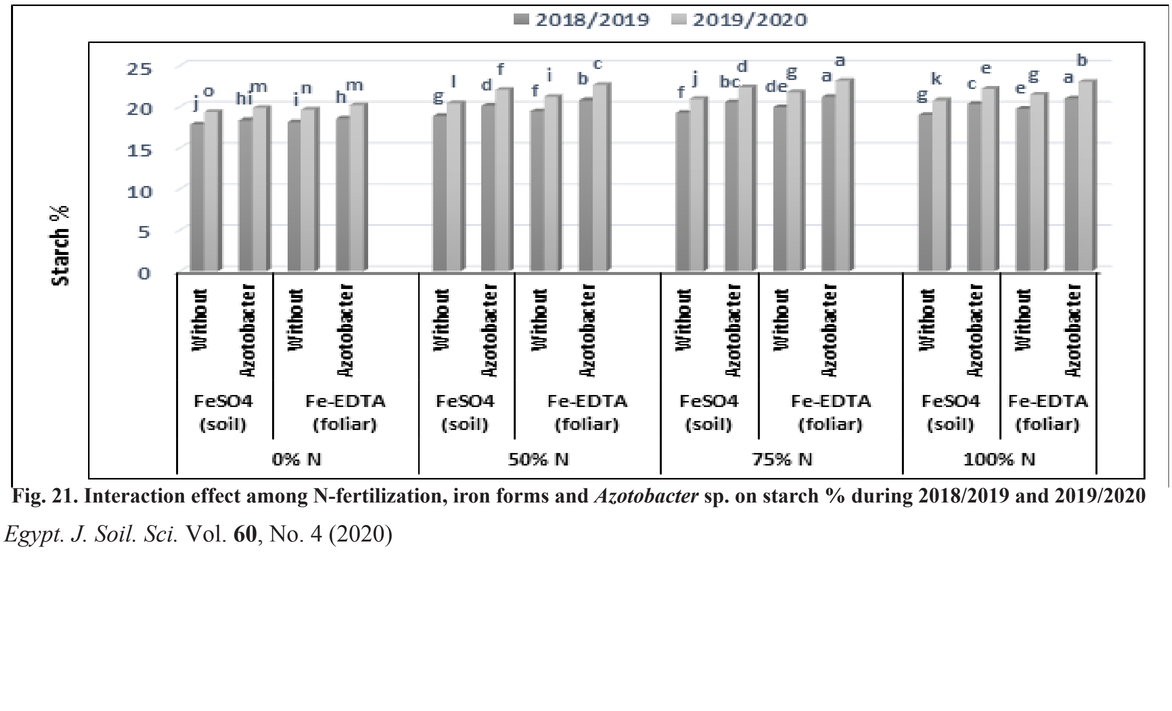

Fig. 21. Interaction effect among N-fertilization, iron forms and *Azotobacter* sp. on starch % during 2018/2019 and 2019/2020

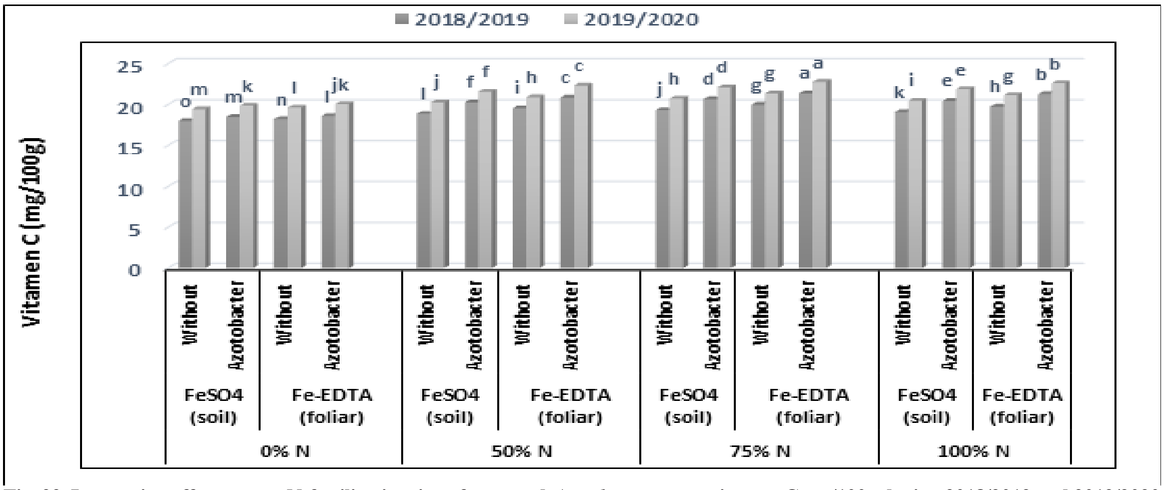

Fig. 22. Interaction effect among N-fertilization, iron forms and *Azotobacter* sp. on vitamen C mg/100g during 2018/2019 and 2019/2020

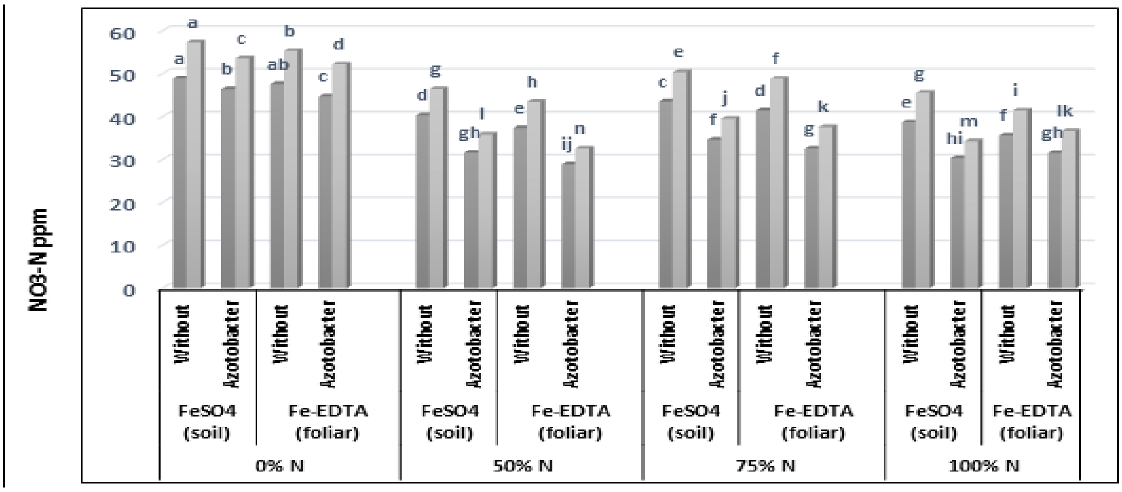

Fig 23. Interaction effect among N-fertilization, iron forms and *Azotobacter* sp. on NO3-N ppm during 2018/2019 and 2019/2020

Discussion

The observed increment of vegetative growth parameters in this study might be connected with nitrogen fertilization to the fact that higher nitrogen concentration encourage the carbohydrates assimilation and protein, which in turn promote cell division, cell elongation and formation of more tissues that resulted in improve vegetative growth of the plant (Anabousi et al. 1997) and also indirectly affect tissue formation and consequently height of the plant. These results corroborate the findings ofHemada and Awadall 2012; Getie et al. 2015; Ghosh 2017; Kumar et al., 2017; Pandey et al. 2018. The increase in chlorophyll content may be attributed to the satisfactory effects of N-fertilization on the chlorophyll content and its prominent part in the chlorophyll molecule or chlorophyll pigment synthesis in the plant tissues, these results corroborate the findings of (Baddour, 2014; Sriom et al., 2017 and Pushpalatha et al. 2017). In addition, the highest mean values of N, P, K % and Fe mg.kg^{-1} recorded with 75% N- fertilization from N-fertilization may be return to the elements availability for plant and improving root growth, hence increasing the absorbing area of root, these results were consistent with finding ofBaddour, 2014; Xing et al. 2016and Sriom et al., 2017. Furthermore, increase in yield and its components may be due to the essential part of nitrogen in cell division process and the biosynthesis of protein could explain the beneficial effect of the proper rate of N on all mineral and organic nutrients movement to meet the superior increment in growth and development of tuber. Also, addition of N fertilizer might be ascribed to its essential part in increasing organic compounds (protein, chlorophylls, enzymes, hormones mucleic acids and vitamins). These compounds divert to the tuber which in turn increased yield (Hemada and Awadall 2012), the significant relationships were reported by Hemada and Awadall 2012; Getie et al. 2015; Xing et al. 2016; Ghosh 2017; Kumar et al., 2017; Pandey et al. 2018.

The results of this study indicate that foliar application by Fe-EDTA increased vegetative growth parameters obtained in this study,this result is also in conformity with the findings of (Moinuddin et al. 2017; Hamad and Tantawy 2018 and Singh & Singh, 2019). Moreover, increasing in chemical constitute as chlorophyll content, N, P, K% and Fe ppm, may be attributed to the benefit use as foliar spraying, also, may be due to the speed absorption by leave tissues, consequently the vigour plant was resulted. The present results are in conformity with the findings of Sanaz and Arash (2015). In the same experiment found an increase because of both forma application of iron and the highest was observed with using Fe-EDTA as indicated by Awad et al. (2010). Therefore, its found that the using of nutrient containing with Fe specially in foliar way which are the main component of Chlorophyll help increasing chlorophyll content in potato plant. because of, iron is necessary for the biosynthesis of chlorophyll and cytochrome, that it increases the biosynthesis of materials and growth. Increasing in yield as a result of iron fertilization may be due to that foliar spraying, which may be a reason to the speed absorption by leaves tissues, consequently the vigour plant was resulted. Also, yield addition of iron which would have improve photosynthesis and other metabolic activities which leads to increment in cell elongation and cell division and ultimately increase in size of tuber parameters. The present results are in conformity with the findings of (Moinuddin et al. 2017; Singh and Singh 2019).

The improvement in vegetative characteristics might be due to the ability of *Azotobacter* sp. to fix atmospheric N, which may share its role in increasing the percentage of mineral nutrient in soil. In addition, it increases the surface area of the root hairs followed by increment in average absorption of mineral nutrients (Farida et al., 2003). Similarly, Arteca (1996) reported that *Azotobacter* sp. had the ability to release some chemical compounds that may affect the improvement of plant growth. The results of this study are in harmony with results whose obatianed by (Awad et al. 2010; Abd El-Gwad and Salem 2013; Tabatabai et al. 2014 and Castellanos et al. 2020). The enhancing effect of using the biofertilizer as *Azotobacter* sp. in increment of chlorophyll might be attributed to supporting the growth of potato plants with available N and the role of nitrogen in increasing the leaf area which enhance the photosynthetic rate (Al-Moshileh, 2001; Abd El-Gwad and Salem (2013); Baddour, 2014 and Singh 2017). In addition, the increase in N% and all other treatments can be attributed to the positive part of the used *Azotobacter* sp. bacteria as a inoculum with a wide range of positive properties that promote plant growth, including its ability to stabilize the nitrogen and then increase the plant concentration of nitrogen. Also, increase in nutrient content and quality parameters may be due to the fact that the ability of the components of the local bio-fertilizer to increase soil elements uptake is linked to its ability to secrete some plant hormones such as cytokines, auxins and gibberellins, which have an essential effect in increasing the surface area of the roots by increasing the lengths of the main roots and their branches, increases nutrient absorption (Samurai, 2002). This result is consistent with the results of Baddour (2014); Fawy et al. (2016); Singh et al. (2017) and Sriom et al., (2017). The enhancing effect of using the bio-fertilizer as *Azotobacter* sp. in yield and its components might be attributed to supporting the vegetative growth of potato plants with available nitrogen and the part of nitrogen in increasing the leaf area which enhance the photosynthetic rate and ended with increasing formation of the carbohydrates and its translocation down to the tuber as described by (Al-Moshileh, 2001) on onion. The results of this study with the results are consistent of (Abd El-Gwad and Salem 2013; Tabatabai et al. 2014; Fawy et al. 2016 and Castellanos et al. 2020).

Conclusion

It can be inferred from the present findings that, optimum nitrogen application is essential to improve potato tuber yield. Although with the increase in nitrogen levels, vegetative parameters of crop growth increased with maximum values achieved on application of 100% N from recommended doses but application of 75% N from recommended doses proved to be superior for obtaining higher yield and yield attributing characters with addition of *Azotobactersp*. and iron foliar application Thus, application of optimum dose of 75% N from recommended doses in presence of iron and *Azotobactersp*. was observed to be superior in terms of yield, therefore, be economically recommended for cultivation of potato under the same conditions.

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التقوية الحيوية لنبات البطاطس بمستويات مختلفة من النيتروجين و اضافة صور الحديد في وجود وغياب الازوتوباكتر

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أجريت تجربه حقليه خلال موسمى النمو ٢٠١٨-٢٠١٩ و ٢٠١٩-٢٠٢٠ لدراسه تأثير مستويات النيتروجين المختلفه والرش بالحديد في وجود وعدم وجود بكتريا الازوتوباكتر على نبات البطاطس. صممت التجربة في قطاعات منشقه متعامده في ٣ مكررات تحتوى على ١٦ معامله قسمت الى ٤ مستويات تسميد نيتروجيني (صفر، ٥٠، ٧٥، ١٠٠٪ من الموصي به لنبات البطاطس) في قطع رئيسيه. صورتين لإضافه الحديد (أرضي، رش) في قطع منشقه في وجود و عدم وجود الازوتوباكتر كقطع تحت منشقه. أوضحت النتائج أن إضافه ٧٥٪ من الموصى سماد نيتروجيني سجل اعلى القيم المعنويه لصفات النمو الخضرى و بالتالى حسن بشكل كبير صفات المحصول المختلفه. و مع ذلك وجد أن الرش بالحديد المخلبى أدى الى زياده في النمو الخضري لنبات البطاطس و عدد الدرنات و متوسط وزن الدرنه و خاصة في وجود الازوتوباكتر. و بالتالى وجد أن استخدام ٧٥٪ من الموصى به سماد نيتروجيني + الرش بالحديد المخلبى في وجود بكتريا الازوتوباكتر مفيده اكثر و صديقه للبيئه و تعطى درنات ذات صفات جوده عالية.