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Effects of Dual Inoculation with Irradiated or Non-Irradiated *Streptomyces alfalfa* Strain XY25 and *Mesorhizobium ciceri* on Yield and Plant Performance of Chickpea

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CHickpea (*Cicer arietinum* L.) is considered a high nutritional value legume crop, due to its high content of total carbohydrates, crude protein, nutrients, and vitamins. Therefore, it is interest and requirement to the use of new microbial genera for biological fertilization for improving the nutritional value, and productivity of chickpea crops. The dual biofertilizers *Mesorhizobium ciceri* with irradiated *Streptomyces alfalfae* strain XY25, andwith non-irradiated *S. alfalfae* strain XY25 with the addition of activation nitrogen fertilizer were investigated compared to full dose of chemical fertilization.Moreover, the relation betweenirradiated *S. alfalfae* strain XY25 and non-irradiated *S. alfalfae* strain XY25 with the addition of full NPK chemical fertilizers, in the winter of 2019/2020 was studied. The experiment design was a complete randomized block (RCPD) in triplicate. The results showed that the dual biofertilizers *Mesorhizobium ciceri*with irradiated and non-irradiated *S. alfalfae* strain XY25 had positive effect onmicrobial soil enzymes,dehydrogenase, phosphatase and nitrogenase.Also, theyincreased the plant's content of phytohormone, micro-elements (Zn, Fe and Mn), phosphorus, potassium, total carbohydrates, and crude protein. All previous improvements due to dual biofertilizers were positively reflected on seed production and the dry weight of 100 seeds.

Keywords: Micro-nutrients; NPK; crude protein; soil enzymes; phytohormones; chickpea yield.

1. Introduction

Chickpea (*Cicer arietinum* L.) has recently been considered an economical legume crop because of its high nutritional value. It can grow in arid and semi-arid soils with low water tension, and can provide nutritious food for the growing world population. The annual production of chickpeas on the global market has increased to more than 2.3 million tons, and the main exporters are Canada, Australia, and Argentina (Merga and Haji, 2019). Globally first legume plant produced is a common bean (*Phaseolus vulgaris L.*), followed by the field pea (*Pisum sativum L.*), and the world's third largest intensive edible legume is the chickpea (*Cicer arietinum L.*). Chickpeas are the most produced legume in South Asia and are grown in more than 50 countries; Asia, 89.7%, Africa, 4.3%, America 2.9%, Oceania 2.6%, and Europe 0.4% (Gaur *et al*., 2010 and Merga and Haji, 2019).Egyptian chickpea seeds have a protein content of 20-25% and a carbohydrate content of 51-62%. As well, the seeds are used in the Malana or immature green stage or boiled and cooked as a snack in the form of mature dried seeds (Madkour and Nassar, 2015 and El-Said *et al*. 2021). It contains fiber, starch, vitamins, carbohydrates, unsaturated fatty acids, lipids, and minerals, which may represent the amount per 100g seeds, such as 4.1 mg Zn, 5 mg Fe, 138 mg Mg, 160 mg Ca, and 334 - 446 Kcal per 100 g, therefore, the dried seed powder of chickpeas can be used as a supplement in biscuits, bread and weaning food mixtures (Petterson *et al*., 1997, Alajaji and El-Adawy, 2006, Wood and Grusak, 2006,

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Jukanti *et al*., 2012, Ray *et al*., 2014 and Kahraman *et al*., 2017). According to Aparna *et al.* (2014), Bidyarani *et al*. (2016) and Plett *et al.* (2021) chickpea as a legume can obtain a significant proportion of its nitrogen requirement symbiotically by nitrogen fixation, may represent 60-80% of its nitrogen requirements, when grown in association with efficient and compatible *Rhizobium* strains, with starter chemicals nitrogen fertilization, such as a urea range between 50 - 75 kg ha^{-1} could be useful in improving the growth, development and the yield of inoculated chickpea plants. Additionally, the use of dual inoculations with different activities combined with symbiotic nitrogen-fixing agents has the greatest impact on soil biological activity. For example, co-inoculation between beneficial rhizospheric *Streptomyces* sp. as plant growth-promoting bacteria, and *Mesorhizobium ciceri* (chickpea nitrogen fixer nodulating rhizobia) promotes shoot dry weight, root surface area, nodulation process, and nitrogen fixation by increasing nitrogenase activity that increases flower number and seed number or crop productivity. The reason is the positive correlation between the production of indole acetic acid (IAA), siderophores, phosphorus, and zinc solubilization, and the number of seeds. So, some speciesof *Streptomyces* are used as an adjuvant or Co-inoculum with *M. ciceri* (Vijayabharathi *et al*., 2018).

This is in addition to many studies on the role of irradiation, whether for plant seeds, such as bean seeds, in increasing the plant's efficiency in absorbing elements especially under sand soil conditions, which is reflected in the productivity of plants, or the use of irradiated microbial strains as a biological fertilizer for the same purpose(Farid *et al*., 2021).

Therefore, this study aims to evaluate the effects of irradiated and non-irradiated *Streptomyces alfalfae* strain XY25 separately and/or combined with *Mesorhizobium ceceri*on soil microbial enzymes, micronutrients, some chickpea hormones, some enzyme activities, nutrients, for improving the growth and yield of chickpea.

2. Materials and Methods

Materials of study Surface soil samples (0-30 cm) were collected from the experimental farm of Soils and Water Research Department (SWRD), Nuclear Research Center (NRC), Egyptian Atomic Energy Authority (EAEA), Abou-Zaable, Egypt and vegetables farm, Faculty of Agriculture, Benha University from during the winter season of 2019/2020. These samples were thoroughly mixed, air-dried, crushed, and then sieved through a 2-mm sieve. Chemical and physical characteristics of the collected sample were determined according to the standard methods outlined by Klute (1986) and Sparks *et al.* (1996), respectively. The results obtained are summarized in Table 1.

TABLE 1. Main properties of soil of the experimental field

Parameter	Particle size distribution, %			Textural class (USDA)	pH*	EC* dS m^{-1}	OM* g kg^{-1}	$CaCO_3$, g kg^{-1}
	Clay	Silt	Sand					
Clayey soil value	87.1	10.9	2	Clay	8.3	0.42	1.99	0.00
Sandy soil Value	10.6	7.1	82.3	Sand	7.7	0.3	0.493	0.00

*OM: Organic matter content, pH: was determined in soil:water suspension (1:5), EC: was determined in soil paste extract

2.1. Chickpea seeds

The chickpea seed (*Cicer arietinum L.*) variety Giza195 was provided by the Field Crops Res. Inst., Legume Crop Dept., Agric. Res. Cent, Giza, Egypt.

2.2. Microorganisms

The specific*Mesorhizobiumciceri*(ICARDA 36) applied as nodulating inoculum was kindly provided by Biofertilizers Production Unit, Agricultural Microbiology Department, Soil, Water and Environ. Res. Inst., ARC, Giza, Egypt. *Streptomycesalfalfae* strain XY25 NR 147713.1 has been isolated from Siwa oasis in Egypt, genetically identified, and its biochemical activity before and after irradiation treatment has been studied (Weesa, S.E., 2021 unpublished data).

2.2.1. Preparation of biofertilizers inocula

The spore stage of growth with density 10^8 spore ml^{-1} were subjected to increasing doses of gamma-irradiation 5, 10, 15 and 20 KGy inside the irradiation chamber of a gamma cell 220 equipment, Cyclotron project, Nuclear Research Center, Atomic Energy Authority. The source of irradiation was cobalt-60 (^{60}Co MC20, Russia). The average dose rate of this gamma radiation source was 600.515 Gy/h at the time of the experiment (Choi *et al*., 2012 and Sakr*et al*., 2013). The irradiated spores were plated on ISP-4 agar plates and incubated for 7 days at 28°C. The inoculum of selected irradiated and un-irradiated*Streptomycealfalfae* strain XY25 was grown in ISP-4 agar medium at 28°C for two weeks in three triplicate until the spores count density was 1-2x10^8 spore ml^{-1} (Manteca and Sanchez, 2009). Whereas the *Mesorhizobiumciceri*was grown in yeast-extract-mannitol broth for 7 days at 28°C until the cell density became 30x10^6cfu ml^{-1} and used as freshly prepared inoculum in the further experiments.

2.3. Experimental design

This experiment was carried in two locations, location 1 (clayey soil) vegetables farm, Faculty of Agriculture, Benha University, under flood irrigation every 3 weeks and location 2 (sandy soil) Soil and Water Research Department, Nuclear Research Center, Atomic Energy Authority, Abou-Zaable, Egypt (AEAE), with the drip irrigation system, the irrigation rate was 9 m^3 h^{-1} once weekly (Ibrahim *et al*., 2018).

The field experiment was carried out from November 2019 1st to May 2020 1st. The seed required for sowing is 40 kg fed.$^{-1}$. Before cultivation, the irradiated, un-irradiated *S. alfalfae* strain XY25 spore suspension and *M. ciceri* cell suspension, individually, were transferred to 30% glucose solution, then the seeds were soaked for 30 min. with inocula based on treatments. Whilst in un-inoculated treatments, seeds were treated with uninoculated medium. The inocula were added at the 25th and 55th days after the seeds sowing date (Gopalakrishnan *et al.*, 2015a). The chemical fertilization program was carried out according to the recommendations of the chickpea Egyptian Agricultural leaflet (Ministry of Agriculture, 2010). The phosphate fertilizer was applied at a rate of 200 kg fed^{-1} (equal to 476 kg ha^{-1}) was used as super phosphate 15% P_2O_5 and is thoroughly mixed with the soil during the preparation process before cultivation. Potassium fertilizer was added at a rate of 50 Kg fed^{-1} (equal to 119 kg ha^{-1}) and used as potassium sulfate... Recommended nitrogen dose; 30.75 Kg fed^{-1} (equal to 73.2 kg ha^{-1}) used as ammonium sulfate (20.5% N)

Moreover, the foliar application of Zn as $ZnSO_4.7H_2O$ was done at a rate of 2.1 kg fed^{-1} (equal to 5 kg ha^{-1}) according to Hidoto*et al*., (2017). The iron-EDTA was applied at a rate of 200 g fed^{-1} (equal to 476 g ha^{-1}), in addition to other trace elements applied in the pre-flowering and pod formation stages according to Nandan *et al*., (2018). The experiment was contained six treatments included: Un-inoculated + NPK, non-irra. *S. alfalfae* + NPK, irra. *S. alfalfae* + NPK, *M. ciceri* + ½N + PK, *M. ciceri* + non-irra. *S. alfalfae*+ ½N + PK, and *M. ciceri* + irra. *S. alfalfae*+ ½N + PK. The treatments were distributed in a randomized complete block design (RCBD), with three replicates (Table 2).

TABLE 2. Field experiment treatments for clayey and sandy soil

Full N dose (30.75 kg fed.$^{-1}$) + PK (recommended)			Half N dose (15.37 kg fed.$^{-1}$) + PK (recommended)		
Without bio-inoculation	With irradiated *S.alfalfae*strain XY25	With non-irraadiated*S. alfalfae* strain XY25	+ *M. ciceri*	With *M. ciceri*+ irradiated *S.alfalfae*strain XY25	With *M. ciceri*+ non-irradiated *S.alfalfae*strain XY25

2.4. Microbiological enzyme activities in rhizosphere samples

Dehydrogenase, alkaline phosphatase and nitrogenase activity in rhizosphere samples were estimated after 30, 60, 90 and 120 days according to (Kumar *et al.*, 2013 andJanuszek *et al*., 2014), Tabatabai (1994), and Silvester (1983), respectively.

2.5. Determination of some phytohormones activities of fresh chickpea leaves

2.5.1. Indol Acetic Acid (IAA) content

According to Morgan and Durham 1983, at 45 days from seeding, IAA is extracted from plant leaves through a methanol-water solvent. One milliliter of the extraction was mixed with 2 ml of Salkowski reagent and incubated in the dark for 30 min. (Glickmann and Dessaux 1995). The appearance of a pink color indicates the production of IAA. Optical density was read at 535 nm. The level of IAA produced is estimated according to IAA standards (Chaiharn and Lumyong, 2011).

2.5.2. Gibberellin content

At 45 days from seeding, sample of 1 g fresh-weight plant material was frozen and ground in an ice-bath with 10 ml methanol/H_2O solution (4:1) in a mortar, and then centrifuged (4°C at 4900 rpm for 20 min). After centrifugation, the supernatant was filtered, and a total of 1.2 ml of plant extracts were collected. The sample is used for colorimetric determination with GA_3 as a reference (Candau *et al*. 1991 and Assunção *et al*., 2009). Mix 0.2 ml of the extracted supernatant with 0.2-ml ethanol (96%, v/v) and 2 ml of a cooled mixture of equal volumes of sulfuric acid and 96% ethanol. After the mixture was incubated at 48°C for 30 min, the fluorescence emission at 464 nm was measured using a JenWay 6305 spectrophotometer (Reyes *et al*., 1992).

2.5.3. Cytokinin content

Chlorophyll was extracted from 1 g fresh-weight young leave sample (at 45 days from seeding) with cold acetone, the volume was adjusted to 10 ml and centrifuged at 2500 × *g* for 10 min. The chlorophyll content is determined by measuring their absorbance at 665 nm (Assunção *et al*., 2009). Calculate the amount of cytokinin based on the chlorophyll content.

2.6. Determination of plant growth characteristics

After 90 days of sowing, 5 plants were randomly selected from each plot to evaluate the growth characteristics, i.e., stem length (cm), number of branches/plants, number of leaves/plant, number of flowers/branch and dry weight of shoot (g).

2.7. Micro-elements content determination

The micronutrients Zn, Mn and Fe content in soil after 60^{th} and 90^{th} days from sowing and plant samples after 90^{th} days from sowing and in obtained seeds were digested by wet digestion read by Buck Scientific Atomic Absorption Spectroscopy (Paul *et al*., 2014).

2.8. Total nitrogen and crude protein content in chickpea seeds

The mature pods were harvested at the appropriate maturity stage, and then the seeds are then dried at 30° C for 24 hr. Nitrogen content in dry seeds was estimated using the method of Kjeldahl according to AOAC (2005). Calculate the percentage of total crude protein by multiplying the total nitrogen value of the seed by 6.25.

2.9. Total carbohydrate determination in chickpea seed

To extract carbohydrate from the seeds, a sample of 0.05 g of air dried powdered seeds were shaken, in a screw cap tube, in 10 ml of concentrated $H_2SO_4$for 1 hour. Transfer 1 ml of previous solution to a volumetric flask and complete to 25 ml. The extract was filtered and used to determine total carbohydrates, where 1 ml of the solution was quantitatively transferred into a test tube and treated with 1 ml aqueous phenol solution (5%) followed by 5 ml concentrated sulfuric acid (98%), and then the pink colour development was observed at A_{490nm}. The blank experiment was carried out using 1 ml of distilled water instead of carbohydrate solution (Herbert *et al.*, 1971 and Masuko *et al*., 2005). A pink colour was observed at A_{490nm}. Total soluble carbohydrates calculated based on a standard solution known glucose content(Smith *et al*., 1956).

2.10. Yield and yield components

Five chickpea plants from each plot were randomly harvested at maturity stage (150 days) from sowing to estimate the number of seeds/pods, dry weight of pods (g), the number of pods/plants, average weight of 100 seeds (g) and seed yield (kg fed^{-1}).

2.11. Statistical analysis

Statistical analysis was conducted according to Stein *et al.* (1997). The differences between the mean values of various treatments were compared with Tukey's Honest Significant Difference test (Nanda *et al.*, 2021).

3. Results and Discussion

3.1. Microbial enzyme activities in rhizosphere samples

3.1.1. Dehydrogenase activity (DHA)

Dehydrogenase activity (DHA) is periodically assessed 30, 60, 90, and 120 days after sowing, as a guide for the respiration rate and total microbial activity in the soil. Data in Fig. (1) emphasized that the maximum DHA activity (µgTPF/g dry soil/day) was observed in clayey soil on the 60^{th} day after sowing, while, in sandy soil was observed on the 90^{th} day. DHA activity increased rapidly in clayey soil and gradually decreased. In sandy soil, the DHA activity was gradually increased, to reach the highest value on the 90^{th} day, and then decreased rapidly.

In the clayey soil, there is no significant difference between the dual biofertilizers; *M. ciceri,* irra. *S. alfalfae* + ½N +PKtreatment (13.05), dual bio-fertilizers; *M. ciceri,* non-irra. *S. alfalfae* + ½N +PKtreatment (12.73) and irra. *S. alfalfae* + NPK treatment (12.51). Whilst, in sandy soil, the results showed that dual bio-fertilizers; *M. ciceri,* irra. *S. alfalfae* + ½N +PKtreatment had the highest DHA activity on the 90^{th} day after sowing. The lowest results were obtained in clayey and sandy soil conditions after 30 days with un-inoculated NPK and non-irra. *S. alfalfae* + NPK treatments. This result is in consistent with those obtained by Aparna *et al*., (2014) and Abdel-Rahman *et al* (2021 a, b), who said that soil biological condition improved after the use of nitrogen fixing agents and acid phosphate solubilizing bacteria, as an indicator of soil microbial health, and agreed with Bidyarania*et al*., (2016), who found when the dual bio-fertilizer Anabaena &*M. ciceri* formula is used, the activity is highest in terms of soil dehydrogenase activity.

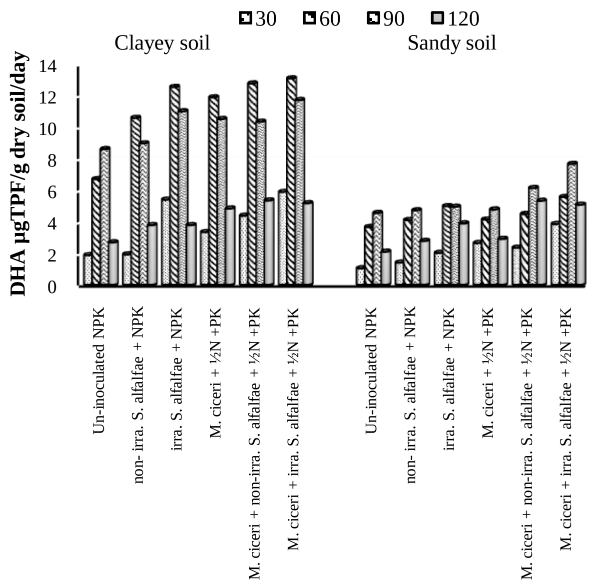

Fig.1. Periodical changes of dehydrogenase activity in chickpea rhizosphere

3.1.2. Alkaline phosphatase activity

Phosphatase activity (PHA) is estimated periodically at 30, 60, 90, and 120 days after sowing, as a guide for the availability of phosphorus in the soil. Data in Fig. (2) emphasized that on the 30th day after sowing, the two soil types gave the lowest PHA values. Whereas, the highest phosphatase activity (µg p-nitrophenol g^{-1} dm^{-1} h^{-1}) in clayey and sandy soils was after 90 days. The highest PHA activity was observed in clayey soil with dual bio-fertilizer; *M. ciceri*, irra. *S. alfalfae* + ½N +PKtreatment (69.53), followed by *M. ciceri*+ non-irra. *S. alfalfae* + ½N +PK and *M. ciceri*+ ½N +PK and were 64.67, 62.22, respectively.

In sandy soil, the phosphatase activity was the highest, with dual bio-fertilizer; *M. ciceri*, irra. *S. alfalfae* + ½N +PKtreatment (55.31). On the 120th days after sowing, the treatment *M. ciceri*+ non-irra. *S. alfalfae* + ½N +PK treatment (55.28) showed no significant difference.

Aparna *et al*., (2014) research can explain this result. In the plant vegetative and flowering stages, nitrogen fixers' bacteria and phosphate solubilizing bacterial inoculation significantly affect phosphatase activity. Because phosphatase is mainly related to the dry matter of plant roots. Kumar *et al*., (2021) showed that the activity of soil microbial enzymes in the root zone of plants is directly related to and responds to rhizo-deposition, which is the main source of soil microbial carbon and nutrients, especially to drought conditions as roots move through the dry soil and to sustain healthy root-soil association. Wen *et al.*, (2021) research on 20 chickpea genotypes showed that the more available P in the soil, the higher the content of root sheath carboxylates, and the influence of chickpea growth on soil nutrient accumulation and dilution.

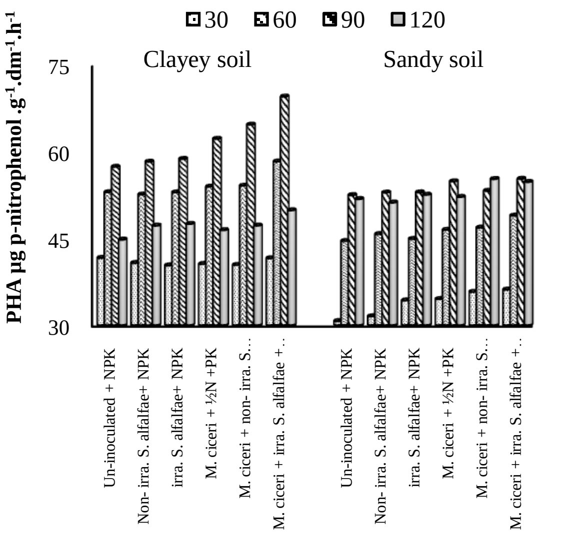

Fig. 2. Periodical changes of phosphatase activity in chickpea rhizosphere

3.1.3. Nitrogenase activity (N_2-ase)

The nitrogenase activity (N_2-ase) in the chickpea rhizosphere area was periodically estimated at 30, 60, 90, and 120 days after sowing, as a guide for the effect of the irradiated and non-irradiated *Streptomyces alfalfae* strain XY25 as co-biofertilizer on free living nitrogen-fixers activity. The quantitative measurement of N_2-ase activity was Nmole C_2H_4/ g dry soil/h, as measured by the acetylene reduction assay (ARA) method.

Data in Fig. (3) showed that the N2-ase activity of clayey soil was higher than that of sandy soil, this may be attributed by the organic carbon (OC) retention, water and nutrient holding capacity of sandy soil are low (Riaz and Marschner, 2020).

In clayey soil, the N_2-ase activity increased rapidly, and then slowly decreased. The most effective treatment(486.86)after 60 days of sowing was observed with the dual inoculation *M. ciceri*+ irra. *S. alfalfae* + ½N + PK, succeeded by the treatment *M. ciceri* + non-irra. *S. alfalfae* + ½N + PK (411.47). Where, the lowest value was obtained from the un-inoculated NPK treatment on the 120th day of sowing. However, in sandy soil, N_2-ase activity started from a low level, then gradually increased during the chickpea growing season, and was still active for the 120 days. The highest N_2-ase activity was detected after 90 days of seeding (257.83) using the dual inoculation *M. ciceri*+ irra. *S. alfalfae* + ½N + PK, followed by the treatment *M. ciceri*+ ½N + PK (228.09). While, the lowest value of all treatments was obtained on the 30th day of sowing.

The results showed the relationship between *M. ciceri*and *S. alfalfae* treatments, whether irradiated or non-irradiated promotes microbial activity around the rhizosphere of plants, and sometimes a single treatment of *S. alfalfae* can also have a positive effect. It is consistent with those obtained by Dahal*et al.*, (2017) who said that the abilities of some genera belonging to four phyla such as *Actinomycetes, Bacteroidetes, Proteobacteria* and *Firmicutes* can increase the activity of atmospheric nitrogen-fixing bacteria. Additionally, Alok *et al*. (2020), found that the investigation of Bt-chickpea root and nodule endophytic bacterial community structure showed that in the four phyla detected, Actinobacteria accounted for 0.17% of the phyla Proteobacteria, Cyanobacteria, Firmicutes, and Actinobacteria. According to Abadi *et al*. (2020) who found that the attendance rate of these four phyla is affected by the host plant. Similarly, Correa and Anzola (2016) demonstrated different activities of Actinobacteria, such as p-solubilization, siderophores production, and nitrogen fixation.

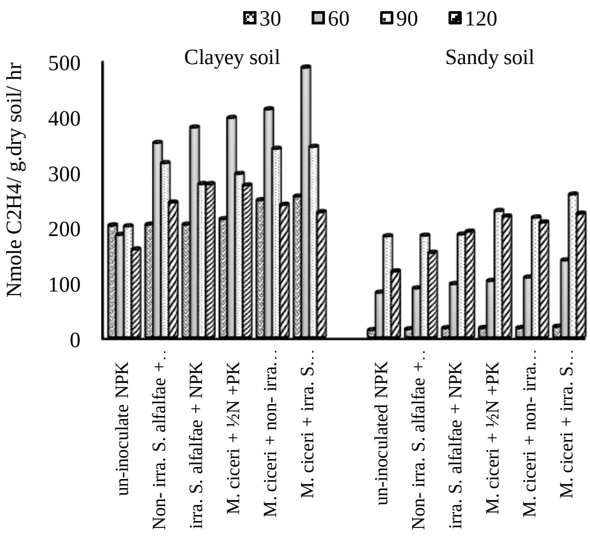

Fig. 3. Periodical changes of nitrogenase activity (nmole C_2H_4/ g.dry soil/ hr) in chickpea rhizosphere

In this experiment, soil parameters such as soil available nitrogen, phosphorus and potassium are positively correlated with plant uptake of nitrogen, phosphorus and potassium, dehydrogenase activity and phosphatase activity. These results are consistent with Gupta *et al*. (2021) who observed a positive and significant correlation between the soil and plant parameters, which might be due to the incorporation of phosphate-solubilizing bacteria that increase the availability of nutrients to crop, through improvements in root nutrient acquisition and absorptive capacity, thus enhancing the soil and plant nutrient status. It was also reported by many workers that phosphatase activity is in direct correlation with the activity of phosphate-solubilizing bacteria in soil that improved nutrients obtained from soil (i.e. C, N, and P) through increased activities of alkaline phosphatase, invertase and dehydrogenase, which lead to plant growth promotion.

3.2. Determination of some phytohormones activities of fresh chickpea leaves

Data recorded in Table 3 showed that the content of some phytohormones e.g. IAA, gibberellin and cytokinin in fresh plant leaves. The obtained IAA data, compared with the control, showed that the IAA results of chickpea plants grown in clayey soil was higher than those grown in sandy soil. The highest content value under the clayey soil conditions was observed with the dual inoculation; *M. ciceri*, non-irra. *S. alfalfae* + ½N +PKand dual inoculation; *M. ciceri*, irra. *S. alfalfae* + ½N +PK. In the same effect, treatment *M. ciceri*, non-irra. *S. alfalfae* + ½N +PK had the enhancement effect on the IAA value under sandy soil conditions. Compared with the un-inoculated NPK (control), the content of IAA in plants tissues of clayey soil increased by 10.2%, and that of sandy soil increased by 16.9%.

The role of auxin IAA is a well-known plant stimulant, which can increase the root volume, surface area and diameter of numerous plants. Regulate growth and development processes (cell division, elongation, tissue differentiation and apical dominance), responsible for light, gravity and pathogen responses (Fu *et al.*, 2015). Many reports have provided an increase in IAA production in plants treated with microbial inoculants. In addition, it also directly or indirectly protects plants from stress, such as promoting heavy metal toleration. For example, Pb, Al and Zn also play an important role in mitigating hostile effects caused by salt stress (Egamberdieva *et al*., 2017).

Despite the differences in soil types and growing conditions, gibberellin results for chickpea leaves showed convergence. The highest content value of gibberellin under clayey soil conditions was recorded with both dual bio-inoculation; *M. ciceri*, irra. *S. alfalfae* + ½N +PK and *M. ciceri*+ non - irra. *S. alfalfae* + ½N +PK treatments. However, in sandy soil conditions, the highest gibberellin content was obtained with dual bio-inoculum; *M. ciceri*, irra. *S. alfalfae* + ½N +PK treatment.

According to Syed *et al.* (2002) and Khan and Mazid (2018) who showed that the result obtained is that the use of bio-fertilization produces plant hormones more efficiently than external treatment, but this fact depends on the plant variety. The role of plant hormones is studied and explained by (Khan and Mazid, 2018). It has long been established that plant hormones (auxin, cytokinins gibberellins and ethylene) are involved in controlling developmental actions, such as cell division, elongation, and protein synthesis. This hormone can be stored in the plant in a reversible conjugate form, which means that the plant will release it as needed.

Finally, the obtained chickpea leaves cytokinin data showed a certain degree of convergence under all treatments. Although the soil type is different, the highest value obtained is related to the dual bio-inoculation; *M. ciceri,* non-irra. *S. alfalfae* + ½N +PK treatment is carried out in clayey and sandy soil.

Cytokinin is one of the main substances in plant tissues. According to Liu *et al.* (2018), the role of cytokinin inside plant included stress resistance in plants, such as heat, cold, salt and drought resistance, besides, it is a role as plant growth promoting substance, encourage cell division, plant development and amino acids, inorganic salts and hormones redistribute. Simultaneously, Khandal *et al.*, (2020) studied the effects of different types and levels of cytokinins, and their effects on increasing the number of lateral roots and root biomass besides, the agronomic trait improvement in an edible legume crop. Additionally, Zarrin *et al*., (2005) compared the effects of kinetin, IAA and abscisic acid on the growth, yield parameters and N-fixation of chickpeas under natural conditions, whether in a single form or as integrated form, The results of kinetin establish to be most efficacious in increasing growth parameters, especially, roots, shoot length, biomass, grain yield, N-fixation specifically at the flowering stage and total N-fixed per plant.

TABLE 3. Chickpea leaves content of indole acetic acid (IAA), cytokinin and gibberellin at 45 days

Treatments	IAA ($\mu g\ ml^{-1}$)		Gibberellin ($\mu g\ ml^{-1}$)		Cytokinin ($\mu g\ ml^{-1}$)	
	C.S	S.S	C.S	S.S	C.S	S.S
Un-inoculated + NPK	0.88cd	0.83d	28.24c	28.54d	1.97c	1.88b
Non-irra. *S. alfalfae* + NPK	0.86d	0.83d	40.28b	43.31b	2.14b	1.85b
irra. *S. alfalfae* + NPK	0.90bc	0.87c	41.27b	27.78d	1.96c	1.53c
M. ciceri + ½N + PK	0.92b	0.88c	43.54b	33.84c	2.16b	1.84b
M. ciceri+ non-irra. *S. alfalfae*+ ½N + PK	0.97a	0.97a	48.16a	44.45b	2.36a	2.14a
M. ciceri + irra. *S. alfalfae*+ ½N + PK	0.96a	0.91b	48.92a	48.24a	1.94c	1.88b

NPK:full dose (100%) of chemical fertilizers. A column followed by the same letters are not significantly differed at p= 0.05 when compared by Duncan test. C.S: Clayey soil, S.S: Sandy soil.

3.3. Determination of plant growth characteristics

The growth parameters on the clayey and sandy soil were measured on the 90th day (TABLE4).

The results of clayey soil emphasized that the harmony between the results of all plant growth characteristics and the results of non-irra. *S. alfalfae* + NPK treatment. However, the number of leaves/branch in the same treatment showed almost three folds that of un-inoculated NPK control. Dual bio-inoculum recorded the lowest values of the chickpeas growth characteristics, while the plant height showed a rapprochement between the results.

The sandy soil data points out that non-irra. *S. alfalfae* + NPK and *M. ciceri*+½N +PK treatments were associated with high or moderate chickpea growth characteristics. Besides, the result of dual bio-inoculum; *M. ciceri, irra. S. alfalfae* + ½N +PKtreatment was moderate for number of leaves/branch. While the flowering was delayed with the non-irra. *S. alfalfae* + NPK, irra. *S. alfalfae* + NPK, dual bio-fertilizer; *M. ciceri, non-irra. S. alfalfae* + ½N +PKand dual bio-fertilizer; *M. ciceri, irra. S. alfalfae* + ½N +PKtreatments.

These results are consistent with the results of Khan *et al.* (2020) who said that phosphorous and potassium mineral fertilizers significantly increase the high grain yield and nutrient uptake of chickpeas. Similarly, Seleiman and Abdelaal (2018) stated that the use of organic matter and either microelement or macroelement fertilizers has a direct effect on the leaves, branches, pods, flowers count, height, dry weight and production of chickpeas, but this response depends on chickpea variety. In addition, Bidyarani*et al.,* (2016) studied the combined use of Anabaena and *Rhizobium* inoculants, and separate Anabaena inoculum to improve the growth and yield of chickpea plants. It explains that microbial activity has a highly positive direct impact on grain yield through a positive correlation, and a direct and/or indirect impact through nutrient availability.

TABLE 4. Interaction between irradiated and non-irradiated *Streptomyces alfalfae*, mineral fertilization and *Mesorhizobium ciceri*on growth characteristics of chickpea

Treatments	Number of branches / plant		Number of leaves / branch		Number of flowers / branch		Plant Stem length (cm)		Dry weight of shoot (g)	
	C.S	S.S	C.S	S.S	C.S	S.S	C.S	S.S	C.S	S.S
Un-inoculated + NPK	21.67bc	13.67abc	201.33c	21.00b	18.00b	11.67b	41.83b	34.33b	17.46c	5.11b
Non-irra. *S. alfalfae* + NPK	43.67a	22.00a	740.00a	24.00b	39.00ab	0.00c	52.33ab	40.67a	30.39a	5.68a
irra. *S. alfalfae* + NPK	30.33ab	5.00c	383.00b	22.33b	43.67a	0.00c	56.33a	38.33ab	26.34b	3.85c
M. ciceri + ½N + PK	15.67c	15.67ab	206.67c	40.67a	13.67c	21.33a	43.50ab	37.00ab	11.25d	3.53c
M. ciceri+ non-irra. *S. alfalfae*+ ½N + PK	18.67c	8.67bc	157.33d	16.33c	14.67c	0.00c	40.50b	24.33c	15.54c	2.87d
M. ciceri + irra. *S. alfalfae*+ ½N + PK	27.67b	7.67bc	307.67bc	26.67ab	19.67b	0.00c	44.67ab	23.67c	17.97c	2.76d

Abbreviations as those stated for Table 1.C.S: Clayey soil, S.S: Sandy soil

3.4. Micro-elements composition in chickpea soil

Estimate the availability and the total of Fe, Mn and Zn in soil samples on the 60th and 90th days, respectively after sowing. The contents of these micronutrients in clayey and sandy soils were recorded in TABLE5.

3.4.1. Fe content

Effectiveness of dual inoculation; *M. ciceri,* irra. *S. alfalfae* + ½N+PK showed the highest content in clayey soil on the 60th day after sowing and was 27.87 mg g^{-1} compared to the un-inoculated full dose of NPK, it, which decreased less than two folds after one month. Moderate soil iron content is related to un-inoculated NPK and dual bio-inoculation; *M. ciceri,* non-irra. *S. alfalfae* + ½N +PKtreatments. The dual inoculation; *M. ciceri,* non-*irra. S. alfalfae* + ½N +PKtreatment after one month become the correlated with the highest total iron content in the soil. However, on the 60th and 90th days, the lowest available and total iron content in clayey soil were observed with *M. ciceri*+ ½N +PK.

The availability of iron in sandy soil indicates that the highest content of Fe occurs on the 60th day of sowing treated with *M. ciceri*+ ½N +PK. One month later, the iron content in the soil was still moderated value. However, the lowest content was observed for treatment dual inoculation; *M. ciceri, irra. S. alfalfae* + ½N +PK and dual inoculation; *M. ciceri,* non-irra. *S. alfalfae* + ½N +PK at 60and 90 days from sowing.

These results explained by Pooja and Sarawad (2019) who studied the Fe and Zn residues in the soil after the chickpeas were grown. They provide that the Fe and Zn residues are affected by the absorption rate of plants, the application method and dosage of Fe and Zn. Among them, the application of iron and zinc fertilizers can help increase the yield and element uptake of chickpea and can also improve the iron and zinc status of the soil after the crop is harvested. An excellent review was expressed by Verma *et al*. (2021) regarding the modulation of plant macro and micronutrient absorption systems and their availability in the root zone. They concluded that microorganisms can produce an array of signals that can modulate the expression of genes involved in nutrient uptake and transport. Many microorganisms' genera including endophytes and mycorrhiza possess the capability to modulate the uptake systems for various plant nutrients especially N, P, and Na (Zhang *et al*. 2009, Saia *et al*. 2015 and Liu *et al*. 2018). Zhang *et al*. (2009) showed that the activation of iron induced the transcriptional regulator (FIT) by *Bacillus subtilis* GB03 in *Arabidopsis* resulted in the upregulation of ferric chelate reductase (FRO) and iron-regulated transporter (IRT1), thereby increasing the iron absorption. Similar observations were also made by Zhou *et al*. (2016) inoculation of *Paenibacilluspolymyxa* BFKC01 in *Arabidopsis* plants, activation of Fe-deficiency-induced transcription factor (FIT) up-regulation of the divalent metal transporters FRO and IRT1.

3.4.2. Zn content

Data in TABLE5 showed that on the 60th day, the treatment of *M. ciceri*+ ½N +PK in the clayey soil increased the Zn availability rate by 76.3% compared with the un-inoculated control. At the same time, but under sandy soil conditions, the sametreatment slightly increased the availability of Zn by 17.9%.

On the 90th day, the dual inoculation; *M. ciceri,* irra. *S. alfalfae* + ½N +PKtreatment improved the total Zn content in both clayey and sandy soils. In clayey soil, the heightening ratio is 68.3%, and in sandy soil conditions it is 90.5%.

However, the reduction of total Zn in clayey soil is related to *M. ciceri*+ ½N +PK and dual inoculation; *M. ciceri*, non-irra. *S. alfalfae* + ½N +PKtreatments by ratio 11%. At the same time, the decreasing ratio 26.3% in the total Zn content of sandy soil is related to irra. *S. alfalfae* + NPKtreatment.

Explanation of the Zn dispersion results may be due to the antagonistic effect of P and Zn in the soil. Increasing the dose of phosphorus reduce the absorption of zinc, and increasing the dose of zinc also reduce the content and absorption of phosphorus (Balai*et al.*, 2017 and Mian*et al.*, 2021). Kuldeep*et al.*, (2018) pointed out that Zn plays a key role in plant cell growth, differentiation and metabolism, leading to efficient growth and extensive root systems, leading to increased growth parameters. Regarding the availability of Zn in sandy soil, Kushwaha *et al.* (2021) studied the effect of zinc solubilizing *Bacillus sp*. (ZSB) inoculation of zinc (Zn) availability (mg kg^{-1}) in the soil with and without $ZnSO_4$ in a pot experiment and they announced that the soils amended with $ZnSO_4$ supplementation showed higher (1284 ± 30 mg kg^{-1}) Zn contents compared to other soils (soils treated with recommended NPK, or those inoculated with specific strains only). As observed in plants, their soils inoculated with ZSB had shown significantly higher content of Zn (1104±29 mg kg^{-1}) than soil without ZSB inoculation (690±20 mg kg^{-1}). They attributed the Zn availability in the soil to the substantial decline in pH observed in the soil inoculated with ZSB strain compared to the un-inoculated control, which may be due to the production of organic acid (Chen *et al*., 2006 and Goteti *et al*., 2013) lead to acidification of the medium. It has been reported that the higher the acid production, the higher the available zinc content in the medium (Desai *et al*., 2012 and Goteti*et al.*, 2013). The solubilization of Zn is highly dependent on the pH of the medium. The organic acid production is a key mechanism for converting the insoluble form of Zn into a soluble form (Fasim*et al*., 2002 and Ku *et al*., 2019).

3.4.3. Mn content

On the 60th day after sowing, the enhancement in the availability of Mn in the clayey soil was increased by nearly four hundredfold compared with the un-inoculated NPK treatment was related to non-irra. *S. alfalfae* + NPK treatment, which is the lowest available Mn content in the soil. One month later, the total content of Mn in the soil showed the convergence of all un-inoculated NPK, non-irra. *S. alfalfae* + NPK and irra. *S. alfalfae* + NPK treatments. In addition, the other treatment represent moderate total Mn content in the soil.

In sandy soil, on the 60th day after sowing, the maximum available Mn was related to the treatment *M. ciceri*+ ½N +PK. On the 90th day, this treatment showed moderate total Mn in the soil and the highest total Mn content in soil was observed with un-inoculated NPK treatment. Reversibly, compared with the un-inoculated control, the non-irra. *S. alfalfae* + NPK treatment showed a decrease in total Mn content. This phenomenon was correlated with the presence of chickpea plants where it absorbance more Mn from soil when un-bioinoculated, which induced depletion in available remained in soil (Weesa, S.E., 2021 unpublished data).

Finally, there is a direct relationship between the *M. ciceri*+ ½N +PK treatment and the highest Fe, Zn and Mn content in sandy soil on day 60th after sowing. Similarly, on the 90th day after sowing, the same treatment provided good total soil Fe, Zn and Mn content.

The comparison of clayey and sandy soils (Table 3) indicated that when Fe, Zn and Mn content are considered, there is no big significant difference between them. In other turn, these micronutrients accumulate in chickpea plants grown on sandy soil higher than those grown on clayey one (Weesa, S.E., 2021 unpublished data). The higher uptake of such microelements by plants affected by either individual or dual inoculation tends to left a little or minimized the available form remaining in the soil after harvest.

The difference in the data obtained may be the result of soil pH, which may be maintained at a neutral and slightly alkaline pH through bio-inoculum treatment. The study of Pradeep *et al.* (2020) pointed out that neutral and slightly alkaline pH is the ideal pH range for the availability of Fe, Zn and Mn for plant growth. This range is between 5.5 and 8.9, which exceeds the pH 9 microelements deficiency symptoms that appear on plants. Also, Khan *et al.* (2020) pointed to the effect of soil organic matter composition on the microelement availability.

Manganese is a very abundant element found in the earth's crust and in seawater (Hebbern*et al*., 2009). Schmidt *et al*. (2016) clarified the role of root exudates and soil microflora in Mn availability, specifically H^+ and low-molecular-weight organic acids. Sadeghzadeh and Rengel (2011) and Xie*et al*., (2019) clarified the complex exchange of soil elements. The P/Fe gain in both shoots and roots is reversible relationship, Phosphorus acquisition promoted under the deficiency of iron, and conversely, P deficiency significantly increases the Fe availability in plants. Also, Fe deficiency leads to an accumulation of Zn, while too much Zn causes physiological Fe deficiency. The reason of that is physiological interactions similarity between P and Fe nutrition, and between Fe and Zn nutrition. Amaresan*et al.* (2018) explained the antagonistic relationship between the P/Fe as both are substrate to chelating siderophore-like molecules secreted by *Streptomyces* spp.

The levels of plant-available micronutrients in soils controlled by a few soil properties such as pH, cations and cation exchange capacity (CEC), soil texture, soil organic matter content and $CaCO_3$, all play an essential role in nutrient availability and

3.5. Microelements content in chickpea leaves and seeds under the clayey and sandy soil.

The content of these micronutrients were recorded in Table 6.

The Fe content of leaves under the clayey soil had a certain response to the bio-inoculum treatment. The optimal Fe leave content was observed with the treatment of *M. ciceri* + ½N +PK and dual bioionculation; *M. ciceri*, irra. *S. alfalfae* + ½N +PK. Similarly, the optimal leave content of Zn and Mn. The highest Fe, Zn and Mn in seeds were obtained with the dual bioinoculation; *M. ciceri*, irra. *S. alfalfae* + ½N +PK treatment. Also, the treatment dual biofertilization; *M. ciceri*, non-irra. *S. alfalfae* + ½N +PK had the highest Zn seed content.

These results matched the high siderophores production and zinc solubility of the *S. alfalfae* either before or after irradiation (Weesa, S.E., 2021 unpublished data).

elemental toxicity. In addition, the main clay minerals, seasonal changes in soil drainage and climatic conditions, as well as crop types and their micronutrients requirements (Ragheb*et al*., 2017). Similarly, the availability of micronutrients in the soil is affected by many factors, including soil fertility, cropping practices, nutrient loss, farmyard manure types, application methods and amounts, soil pH, nitrogen and phosphorus chemical fertilizer types and amount. The interrelationship between the soil minerals and elements (Shiwakoti *et al.,* 2019).

Generally, the low content of Fe, Zn, and Mn in seeds are related to the un-inoculated NPK treatment. However, the lowest values of micronutrients in leaves show a distribution that is affected by soil type, fertilization treatment, and plant growth characteristics.

As well, Kahraman*et al*., (2017) studied the mineral content of 100 chickpea seeds per gram; each 100 grams of seeds contains Zn (4.1 mg), Fe (5 mg), Mg (138 mg), Ca (160 mg) and 334 - 446 Kcal. According to this study, the results of Zn we obtained in clayey soil conditions, the range is slightly lesser (2.82 - 3.05 mg $100g^{-1}$ seed), also the sandy soil data are in the same range (3.14 – 3.79 mg $100g^{-1}$ seed). Alternatively, our treatment under clayey soil conditions increased the Fe content in the seeds to 5.59 – 25.66 mg 100 g^{-1} seeds and 7.24 – 12.79 mg $100g^{-1}$ seeds under sandy soil conditions.

TABLE 5. Interaction of irradiated and non-irradiated *Streptomyces, Mesorhizobiumciceri* and mineral fertilization and its effect on microelements (zinc, iron and manganese) content (mg kg^{-1}) in clayey and sandy soil after chickpea plants

Treatments	Micronutrients available in chickpea soil 60 days (mg kg^{-1})						Micronutrients total in chickpea soil 90 days (mg kg^{-1})					
	Clayey soil			**Sandy soil**			**Clayey soil**			**Sandy soil**		
	Fe	**Zn**	**Mn**	**Fe**	**Zn**	**Mn**	**Fe**	**Zn**	**Mn**	**Fe**	**Zn**	**Mn**
Un-inoculated + NPK	23.96ab	1.77c	2.59e	34.68b	0.95b	1.16b	12.18ab	1.45b	1.31a	13.63b	0.95bc	2.10a
Non-irra. *S. alfalfae* + NPK	20.75b	2.29b	12.15a	37.46ab	0.93b	0.64c	8.56bc	1.36bc	1.28a	24.06a	0.92bc	1.60bc
irra. *S. alfalfae* + NPK	22.11b	0.79d	4.14d	33.04b	0.92b	0.43d	6.27cd	1.46b	1.34a	6.95d	0.70d	1.66b
M. ciceri + ½N + PK	13.43c	3.12a	8.16b	40.51a	1.12a	1.50a	3.51d	1.29c	1.12b	18.90ab	1.15b	1.88ab
M. ciceri+ non-irra. *S. alfalfae*+ ½N + PK	23.86ab	2.21bc	6.19c	31.85bc	0.99b	1.41ab	15.85a	1.29c	1.13b	6.31e	0.86c	1.69b
M. ciceri + irra. *S. alfalfae*+ ½N + PK	27.87a	2.31b	2.89de	28.64c	0.93b	1.27b	7.82c	2.44a	1.24ab	9.87c	1.81a	1.85ab

Abbreviations as those stated for Table 1.

Table 6. Interaction between irradiated and non-irradiated *Streptomyces alfalfae*, mineral fertilization and *Mesorhizobium ciceri*on micronutrients content of chickpea leaves and seeds in clayey and sandy soil

Treatments	Micronutrients content in chickpea plant Clayey soil						Micronutrients content in chickpea plant Sandy soil					
	Leaves (mg kg^{-1})			**Seeds (mg $100g^{-1}$)**			**Leaves (mg kg^{-1})**			**Seeds (mg $100g^{-1}$)**		
	Fe	**Zn**	**Mn**	**Fe**	**Zn**	**Mn**	**Fe**	**Zn**	**Mn**	**Fe**	**Zn**	**Mn**
Un-inoculated + NPK	7.49bc	0.95d	0.42c	5.59c	2.89b	0.69c	15.69cd	1.64bc	4.18ab	7.24d	3.18cd	0.61b
Non-irra. *S. alfalfae* +NPK	4.88c	1.08c	0.99ab	10.38bc	2.93ab	0.72b	16.92cd	1.82abc	4.18ab	10.58ab	3.14cd	0.71ab
irra. *S. alfalfae* + NPK	10.94b	1.08c	0.98ab	11.44b	2.82b	0.73ab	21.31bc	1.35c	3.09bc	8.01cd	3.43bc	0.72ab

M. ciceri + ½N + PK	16.97a	1.09c	1.03ab	9.77bc	2.98ab	0.72ab	31.74a	2.30ab	1.64c	9.14bcd	3.78a	0.75a
M. ciceri+ non-irra. *S. alfalfae*+ ½N + PK	12.56b	1.26b	0.99ab	6.71bc	3.05a	0.69c	25.18ab	2.05abc	2.15c	12.79a	3.49b	0.71ab
M. ciceri + irra. *S. alfalfae*+ ½N + PK	17.57a	2.06a	1.12a	25.66a	3.03a	0.90a	29.78a	2.44a	5.30a	12.73a	3.79a	0.75a

Abbreviations as those stated for Table 1.

3.6. Chickpeas seed evaluation

Data in TABLE 7 evaluated the nitrogen, phosphorus and potassium content, and crude protein and carbohydrate (%) in the seeds. Although the improved conditions and soil properties are different, the maximum carbohydrates, nitrogen seed uptake and crude protein percentage were related to dual bio-fertilization *M. ciceri*, irra. *S. alfalfae* +½N + PKtreatment under the two soils, followed by dual bio-fertilization; *M. ciceri*, non-irra.*S. alfalfae* +½N + PK treatment. Similarly, under the two soil conditions, the lowest value was related to the un-inoculated NPK treatment. The results of the other treatments vary depending on the type of soil.

However, in clayey soil the maximum phosphorus and potassium content in seeds was observed with un-inoculated NPK control, while, in sandy soil the optimal phosphorus and potassium content was obtained with irra. *S. alfalfae* +NPK treatment. Besides, the maximum potassium content of seeds under sandy soil conditions was related to non-irra. *S. alfalfae* +NPK. These results are consistent with the views of Pegoraro *et al.* (2018) who found when chickpea varieties treated with 150 kg ha^{-1} P_2O_5, showed higher content of soil P. The explanation is that phosphorus reduced the soil pH value and increases the P content, but the soil chemical properties do not change, indicating that the mobility of P is low and the acidification capacity of superphosphate in alkaline soils also is low.

The positive effects of *M. ciceri* in combination with irradiated or non- irradiated *S. alfalfae* on seed carbohydrate, nitrogen, and protein content % may be due to the high gibberellin plant content (Weesa, S.E., 2021 unpublished data). This result is in the same line with Khan and Mazid, (2018), who said that the major role of gibberellins, as a plant growth hormone, participates in protein synthesis and may be stored in plants as a reversible conjugate form, which means that plants can be released as needed. Likewise, according to Palta*et al*., 2005 who study the relationship between the increase in seed protein content and the increase in nitrogen availability during seed filling.

Referring to the study of Wallace *et al.* (2016) and Jukanti*et al.* (2012), the reason for the high carbohydrate content in chickpeas is that it contains all monosaccharides, disaccharides, and oligosaccharides in specific proportions. According to Uddin *et al.* (2014) showed the use of biological fertilizers has a direct effect on the carbohydrate content of chickpeas, especially those that have phosphate solubilization activity compared with nitrogen fixers.

Additionally, Aallam*et al.* (2021) studied the use of *Streptomyces* species that efficiently solubilize phosphate such as *S. bellus* and *S. enissocaesilis* strategies of the acidification of the medium and excretion of siderophores to solubilize phosphate form either organic or inorganic sources.

Goud *et al.* (2014) and Jadeja *et al.* (2019) explained that the uptake of potassium and phosphorus by seeds during the growing season is directly related to the application of chemical fertilizers. However, the research of Htwe*et al.*, (2019) provides when *Streptomyces griseoflavus*is co-inoculated with the nodulating forming bacteria *Bradyrhizobium japonicum* and *B. elkanii*in beans and soybean plants, growth parameters, nodulation process and NPK uptake affected positively and have a direct impact on yield production.

Yield of chickpeas

Data in Table 8 proved the straw, seed yield (kg Fed^{-1}) and dry weight (g) of 100 seeds.

Under the clayey soil, the highest values of seed yield and 100 seed weight were obtained with *M. ciceri*+½N +PK and dual bio-fertilization; *M. ciceri*, irra. *S. alfalfae* + ½N +PK, followed by dual bio-fertilization; *M. ciceri*, non-irra. *S. alfalfae* + ½N +PK. the lowest records were observed with un-inoculated +NPK treatment. However, the yield of straw with un-inoculated NPK and irra. *S. alfalfae* + NPK treatments was the highest, followed by non-irra. *S. alfalfae* + NPK treatment.

TABLE 7. Interaction between irradiated and non-irradiated *Streptomyces alfalfae* mineral fertilization and *Mesorhizobiumciceri* on nitrogen, phosphorus, potassium, crude protein, and carbohydrate content of chickpea seed.

Treatments	Seeds composition under Clayey soil conditions	Seeds composition under Sandy soil conditions

	Carbohydrate (%)	N content (%)	Crude protein (%)	P content (%)	K content (%)	Carbohydrate (%)	N content (%)	Crude protein (%)	P content (%)	K content (%)
Un-inoculated + NPK	34.18d	2.90c	18.13c	3.17a	2.34a	20.06d	2.42c	15.13c	3.17bc	0.91b
Non-irra. *S. alfalfae* + NPK	76.76b	3.72b	23.25b	2.76ab	1.33bc	28.97c	2.48c	15.5c	3.47b	1.69a
irra. *S. alfalfae* + NPK	73.93b	3.05c	19.06c	3.07a	1.48b	25.50cd	2.66c	16.63c	4.85a	1.69a
M. ciceri + ½N + PK	61.77c	3.80ab	23.75ab	2.90ab	1.07cd	45.26b	3.13b	19.56b	3.40b	0.78bc
M. ciceri+ non-irra. *S. alfalfae*+ ½N + PK	87.62a	3.95ab	24.69ab	2.82ab	1.16bcd	81.32a	3.24b	20.25b	2.97d	0.67c
M. ciceri + irra. *S. alfalfae*+ ½N + PK	93.48a	4.03a	25.19a	2.59b	0.75d	75.89a	3.97a	24.81a	3.15cd	0.75bc

Abbreviations as those stated for **Table 1**.

However, under sandy soil conditions, the seed yield and 100 seed weight are divided into 5 levels, the maximum treatment level was related to dual bio-fertilization; *M. ciceri,* non-irra. *S. alfalfae* + ½N +PKand *M. ciceri,* irra. *S. alfalfae* + ½N +PKtreatment, followed by the treatment *M. ciceri* + ½N +PK. The third level associated with irra. *S. alfalfae* + NPK, the fourth level associated with non-irra. *S. alfalfae* + NPK, and the lowest value was obtained under un-inoculated + NPK treatment. Conversely, the highest straw yield is related to theirra. *S. alfalfae* + NPK treatment.

It can be calculated that the use of more than one of the soil bacterial genera with different activities enriches the activity of the rhizosphere of plants, which is reflected in the growth and productivity of the plant. These results are consistent with those obtained by Vijayabharathi*et al*., (2018), which reported that *Streptomyces* species as growth-promoting inocula added to the rhizosphere of chickpea plants increased growth and yield components.Toumatia*et al*., (2016) and Boubekri*et al*. (2021) attributed to the ability of *Streptomyces* sp. to promote the growth of wheat seedlings have multiple of activities, such as organic acids releasing, IAA, GA3, siderophores secretion and ammonia production to promote this growth. It exhibits a coarse root architecture and the highest performance of wheat shoot and root growth.

Kumari (2017) studied the use of slow phosphate solubilizing bacteria as a co-inoculum for nitrogen-fixing bacteria. His results indicated that this co-inoculation enhanced the plant's extensive root system, improved the nodulation process and the efficiency of nitrogen fixation. Finally, it successfully corrected the crop yield. In the investigation of Bidyarani*et al.,* (2016), the relationship between various traits and bio-fertilizer-based planting system effects the grain yield of chickpeas. These traits suggest that the key to chickpea enhancing the yield of chickpea is nitrogen fixation and soil available nitrogen.

Similarly, the yield of chickpea is affected by the type of soil used, plant verity, irrigation methods and time (Ahmed *et al.* 2013 and Fayed *et al.,* 2021). In this way, we can explain the difference in yield between the two soil types.

TABLE8. Interaction between irradiated and non-irradiated *Streptomyces alfalfae*, mineral fertilization and *Mesorhizobiumciceri*on chickpeas production.

Treatments	Clayey soil			Sandy soil		
	Dry weight 100 seed (g)	Seed production kg Fed^{-1}	Straw production kg Fed^{-1}	Dry weight 100 seed (g)	Seed production kg Fed^{-1}	Straw production kg Fed^{-1}
Un-inoculated + NPK	32.79bc	601.73bc	1905.08a	31.24c	218.63c	1492.50cd
Non-irra. *S. alfalfae* + NPK	34.10b	625.65b	1865.18ab	33.02abc	231.15bc	1635.90ab
irra. *S. alfalfae* + NPK	34.20b	627.6b	1893.23a	34.53abc	241.73abc	1928.33a
M. ciceri + ½N + PK	38.47a	705.98a	1756.58b	35.46ab	248.25ab	1658.93ab
M. ciceri+ non-irra. *S. alfalfae*+ ½N + PK	37.79ab	693.38ab	1710.15bc	36.98a	258.83a	1516.43bc
M. ciceri + irra. *S. alfalfae*+ ½N + PK	38.11a	699.30a	1699.58c	36.51a	255.53a	1345.73d

Abbreviations as those stated for **Table 1**.

4. Conclusion

The dual inoculation of Co-relation between irradiated *Streptomyces alfalfae* strain XY25 and *Mesorhizobium ciceri* in soil treated with starter nitrogen dose (50% of recommended rate) resulted in enhancement the soil enzymes activities in the study soils,phytohormones content in chickpea tissues, and maximizes the plant's content of Zn, Fe and Mn, seeds nutritional values of carbohydrates, crude protein NPK content, as well as seed yields and dry weight of 100 seeds in the tested soils. It seems that the irradiated *S. alfalfae* is active as an activator or enhancer for *M. ciceri*. The growth parameters of chickpeas in clayey soil were significantly higher than those detected in sandy soil, which may be partly because the nutrient content in clayey soil is higher than in sandy soil. Finally, compared with individual biological inoculum, there is a correlation between *S. alfalfae* strain XY25 and *M. ciceri* (dual inoculum) in different soils, impacting soil microbial enzyme activity, micronutrients, and plant nutritional value.

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