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Effect of inoculating mycorrhizal fungi and *Rhizobium* on broad bean (*Vicia faba* L.) growth at different phosphorus levels in calcareous soil.

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Abstract

The experiment was carried out, under plastic house at the college of Agricultural Engineering Sciences, University of Sulaimani, Bakrajo, during 2018-2019 to determine influence of inoculation with arbuscular mycorrhizal fungi (AMF) and *Rhizobium leguminosarum* at different phosphors levels (0, 40, 80, 120 and 160 kgPha^{-1}) on broad bean (*Vicia faba* L.) growth and nutrient uptake. The experiment was performed in a factorial experiment with completed randomized design (CRD) in a silty clay soil, with three replications for each treatment. After ten weeks of growth, the plants were harvested to determine plant growth (root colonization, shoot dry weight, root dry weight, nodule number, and shoot nutrient content N, P, K, Fe and Mo The results showed that inoculated broad bean plants with mycorrhiza or the bacterium *Rhizobium leguminosarum* increased plant growth and nutrient uptake compared with non-inoculated plant. Inoculated soil with AMF increased root colonization, shoot dry weight, root dry weight, and nodule number the highest value was (65%, 15.09gpot^{-1}, 7.72gpot^{-1} and 128.67 nodule pot^{-1}) respectively and the highest value for shoot N, P, K, Fe and Mo nutrients were (15.68gkg^{-1}, 4.38gkg^{-1}, 17.72 gkg^{-1}, 184.00µgg^{-1} and 0.83 µgg^{-1}) respectively recorded at highest P level (160 kgPha^{-1}). But when the soil inoculated with the *Rhizobium leguminosarum*, the effect was increased plant growth, (root colonization ,shoot dry weight, root dry weight and nodule number) The highest value was(26.67% , 15, 60gpot^{-1}, 8.03gpot^{-1} and 191.33nodule pot^{-1}) recorded at highest P level, and inoculation with *R. leguminosarum* was increased significantly shoot N, P, K, Fe and Mo contents, the highest value were (20.35gkg^{-1}, 3.72 gkg^{-1},16.78 gkg^{-1}, 175.33 µgg^{-1} and 0.80 µgg^{-1})respectively recorded at highest P level (160 kgPha^{-1}).

Introduction

Nitrogen (N) and phosphorus(P) are among the most limiting nutrients for plant growth and production [1] and they are required for plant growth in most soil of the world, nitrogen and phosphorus are the major important among all plant nutrients, they are playing crucial role in plant growth [2]. The plant growth in temperate ecosystems is limited by nitrogen and phosphorus [3]. Deficiency of nitrogen and phosphorus in plant causes reduction in growth and yield. The high cost of chemical fertilizers is a major expenditure for farmers with high energy cost and problem by increasing ecosystem pollution [4]. There are many ways to increase available nitrogen, phosphorus and other nutrients in the soil and to minimize water, air and soil pollution the most important way is the symbiotic between microorganisms and legume plants the symbiotic association between certain plants and microorganisms which play an important role in soil fertility and improve their growth, and mineral nutrition [5]. The symbiotic association between mucorrhizal fungi, legume plants and Nitrogen -fixing, *Rhizobium* bacteria are the two most commonly symbioses, microbes use photosynthetic products of the host plant, and they supply it with phosphorus and nitrogen and other nutrients representing limiting factor in the soil and increase plant growth, nutrition and soil fertility [6]. Broad bean (*Vicia faba* L.*)* is an important crop for protein, human and animal consumption of the word and it is a good source of nutrients such (K, Ca, Mg, Cu and Zn) different vitamins, amino acid, protein, and carbohydrate [7] and it improve soil fertility due to fixing atmospheric nitrogen. There are problems in Iraqi–Kurdistan region that most of the soil contain a high amount of $CaCO_3$, which fix the available and precipitate it as tri-calcium phosphate, in which soluble nitrogen sources leach readily from root zone or loosed by denitrification or volatilization in which render the nitrogen availability and deficiency of available nitrogen. Since there are no detailed studies carried out on using mycorrhizal fungi and *Rhizobium leguminosarum* as bio fertilizers for broad bean plant especially in Kurdistan region. It is therefore necessary to pay attention to such studies in local area.

Materials and Methods

This experiment was carried out at the Natural Resources Department and plastic house of the Department of Horticulture, College of Agricultural Engineering Sciences, University of Sulaimani in Bakrajo, during 2018-2019 to study the effect of Arbuscular mycorhhizal Fungi (*Glomus mosseae*) and *Rhizobium leguminosarum* on broad bean *(Vicia faba L.*) plant growth at different phosphorus levels in calcareous soil (0, 40, 80, 120 and 160 $kgPh^{-1}$). KH_2PO_4 was used in this study as a source of phosphorus. The soil used was silty clay belong to (Vertisols order). The soil was collected from research field experiment at college of Agricultural Engineering Sciences in Bakrajo at the depth from (20-40 cm), and simply the soil is low in phosphorus content. The soil samples were air dried, ground to pass through a 4mm aperture sieve. Table (1) shows some physical and chemical properties of the studied and then six kg of unsterilized soil was transferred in to sterilized plastic pots (25 cm) diameter by (30 cm) depth. The five levels of phosphorus (0, 40, 80, 120 and 160 $kgPha^{-1}$) in the form KH_2PO_4 was added to all. Soil potted inoculated, and non-inoculated. The inoculated pots with arbuscular mycorrhiza fungi (*G. mosseae*) were achieved by mixing (180 g) inoculant consisting of spore, hyphae fragment and piece of mycorrhiza roots with the soil of each pots, non-inoculated soil pots were mixed with the same quantity of soil. The sterilized broad bean seeds were soaked in a suspension of bacteria *Rhizobium leguminosarum* for (30 min) Arabic gum (16%) was added as on adhesive agent prior to inoculation. Then five broad bean seeds were planted in each pots treatment, soil pots were also inoculated and non-inoculated control with (5 ml) of a suspension of The Bacterium *Rhizobium leguminosarum* which was uniformly added directly in to the planting holes in each pot (5 cm) diameter by (5 cm) depth and then seeds were covered with (5 cm) thick soil. The pots were arranged in plastic house benches in a completed randomized design (CRD) with three replicates per treatment. After germination, the seedlings were thinned to three per pot, the plant were grown under natural light in the plastic house. The pots were watered to maintain 70% of field capacity by regular weighting of pots, during the study period. After(10 weeks)of growth, the plants were harvested and the shoots were removed from the roots, after nodules were removed from the root washed, and counted, the fine roots were separated from thick roots, washed and cut (1-2) cm length pieces, then the degree of AMF colonization was determined after shoot and root dried at 70 °C for 72 hours and weighted, and the shoot dry weight ground to pass through a(0.5 mm) sieve and some of macro (N, P, K) and micro (Fe, Mo) nutrient content were determined. The data were statically analyzed by using XLSTAT version 12 software.

Table1: Some important physical and chemical properties of the soil used in the experiment.

Soil properties	*The value*
Sand (gkg^{-1})	*42.60*
Silt (gkg^{-1})	*484.20*
clay (gkg^{-1})	*473.20*
Texture	*Silty clay*
pH	*7.39*
ECe $ds.m^{-1}$ at 25 °C	*0.32*
Organic Matter (gkg^{-1})	*9.50*
Calcium Carbonate (gkg^{-1})	*255*
Total Nitrogen (gkg^{-1})	*0.11*
Available phosphorus (gkg^{-1})	*2.90*
Soluble K^+($mmol.l^{-1}$)	*0.40*

Results and Discussion

Root Colonization

The results reveals and clearly showed that the inoculation of broad bean plant with AMF caused significant increase in the percentage of root colonization, and significantly decreased by increasing the rate of P application compared to non-inoculated plants (Figure 1). these results are compatible with the result which found by [8] and [9]. The result indicated that the highest percentage of root colonization achieved (65%) in treatment inoculated with AMF at medium phosphorus level (40 $kgPha^{-1}$) and the lowest value was (22.67%) observed at highest phosphorus level (160 $kgPha^{-1}$). There was low mycorrhizal colonization detected in the root of non-inoculated plants with AMF, the highest value for root colonization was (21.33%) recorded at low P level (40 $kgPha^{-1}$) while the lowest value for root colonization was (6%) recorded at highest phosphorus level (160 $kgPph^{-1}$) and the percentage of root colonization decreased by increasing P levels. The raising of root colonization in inoculated plant with AMF at low amount of added chemical P fertilizers, return to the mycorrhizal fungi in which able to increase the root infection significantly [10] or may be due to increasing the ability of spore to germination and help AMF to infected roots. [11]. The decreasing of root colonization by AMF by increasing phosphorus levels may be due to inhibition of hyphal growth which subsequently decrease the spread of mycorrhiza colonization and plant root become resist to colonization high phosphorus concentration in the soil solution hampering root colonization and inhibition of spore germination which reflected the adverse of AMF activity [12] or may be due to when the phosphorus increase in the soil and plant tissue led to increase of phospholipids in root cell membrane and decrease of root exudation of soluble amino acids and reducing sugar from the root depends upon the phospholipid level in root and associated changes in permeability properties of root membranes and decrease of root exudation and decrease infection rate [13] . when the soil inoculated with Rhizobium leguminosarum, the root colonization non-significantly increased at different phosphorus levels compared with a non-inoculated plants. The highest value for root colonization was (26.67%) which recorded at low phosphorus level (40 $kgPha^{-1}$), which the lowest value for root colonization was (6%) recorded in highest phosphorous level (160 $kgPha^{-1}$) this result was compatible with [14]. The increasing of root colonization in broad bean plants which inoculated with Rhizobium leguminosarum in low P level may be due to the ability of Rhizobium to fix atmospheric nitrogen which supply nitrogen to the plant and increasing carbohydratic production due to the increasing photosynthesis rate which increasing in root colonize of AMF [3]. But increasing the rate of P application cause decrease in the root colonization this may be due to inhibition hamper and or tolerance of AMF activity to colonization at high phosphorus level [15] and decrease in mycorrhizal infection the root cell that are less able to infect.

Figure 2: shows the coefficient of determination R^2 for root colonization at different P levels, the maximum value was obtained by inoculation with mycorrhiza treatment which was 0.8268 while the minimum value recorded in *Rhizobium leguminosarum* was 0.665.

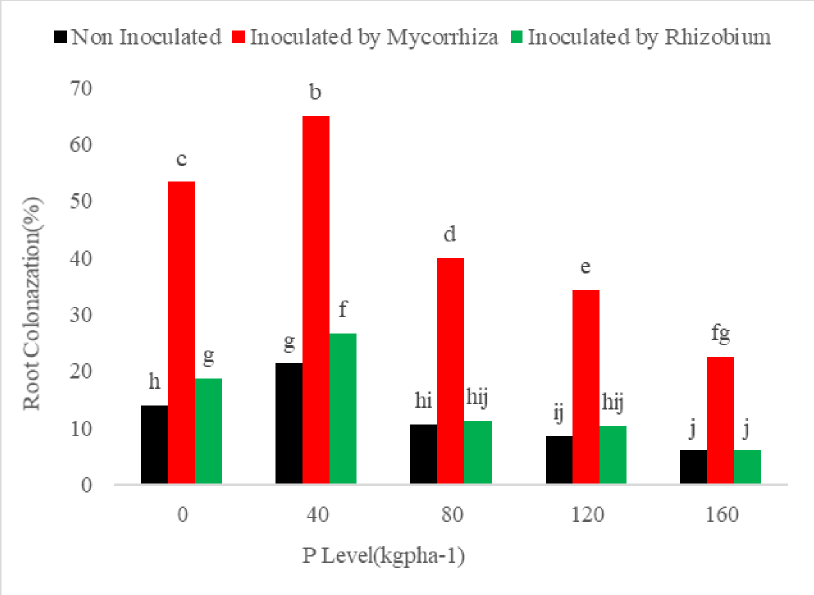

Figure-1 Effect of mycorrhiza, Rhizobium inoculated and non-inoculated soil on broad bean root colonization at different phosphorus levels

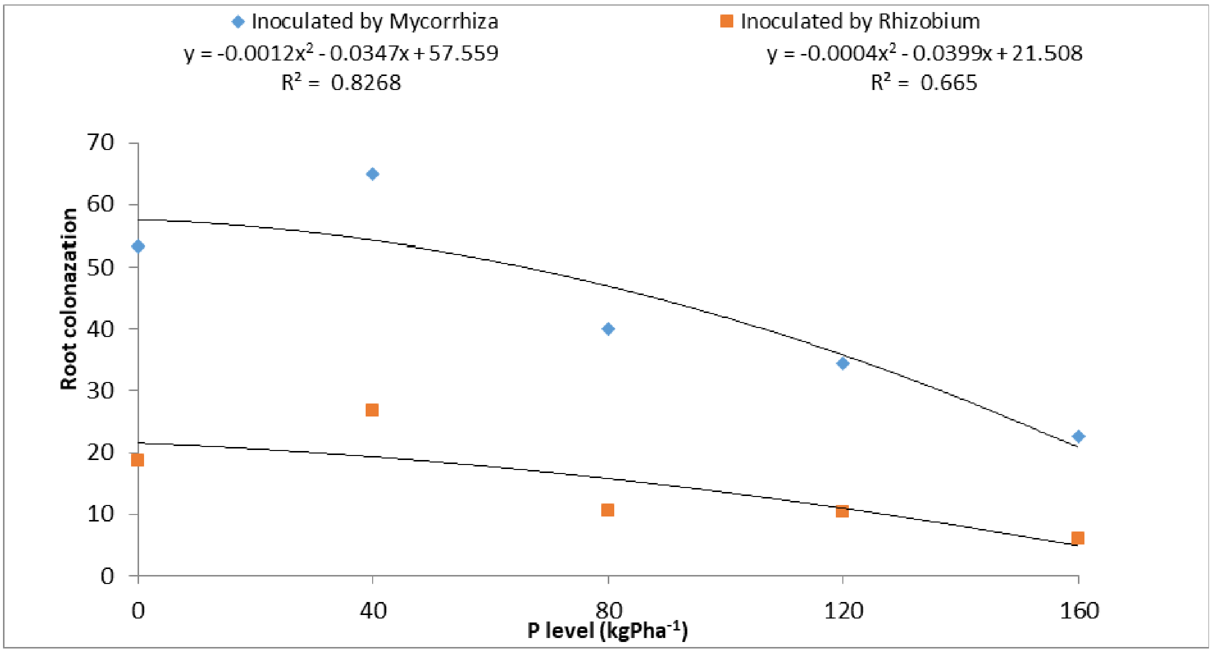

Figure-2: The relation between Root colonization and phosphorus levels of broad bean inoculated with mycorrhiza and *Rhizobium*.

Shoot Dry Weight

The obtained results demonstrated that inoculation broad bean plants with AMF significantly increased shoot dry weight at different phosphors levels compared to non-inoculated plants. However, the best value for shoot dry weight was (15.09 $gpot^{-1}$) observed in (160 $kgPha^{-1)}$ and the lowest value was (11.83 $gpot^{-1}$) which recorded at (zero $kgPha^{-1}$) and the shoot dry weight increased with increasing P supply figure 3 This result was compatible with the result which found by [16], [33] and [8]. The increasing shoot dry weight for broad bean plants inoculated with AMF may be due to the role of AMF in stimulation the absorption of elevated root surface area for absorbing more macro and micro nutrients and greater rate of photosynthesis which increase more plant growth and raised shoot dry weight [17]. The result of the present study clearly indicates that the inoculation broad bean plants with *Rhizobium leguminosarum* was highly beneficial for enhancing plant growth and significantly increase shoot dry weight for broad bean at different phosphors levels. The maximum value for shoot dry weight was (15.60 $gpot^{-1}$) recorded at (160 $kgPha^{-1}$) while the minimum value was (12.45

gpot^{-1}) recorded at (zero kgPh^{-1}) this result compatible with the result which found by [18]. The increasing shoot dry weight due to inoculated plant with *Rhizobium leguminosarum* may be due to suggesting a promising way for enhancing the growth of legume plant [1], or may be due to the important of *Rhizobium leguminosarum* in increasing nodulation and Nitrogen-fixation which supply nitrogen to plant and increase the ability of plants to uptake more nutrients and raised plant shoot biomass [19].
Figure: 4 shows the coefficient of determination R^2 for shoot dry weight at different P levels, the maximum value was obtained by inoculation with *Rhizobium leguminosarum* was 0.9944 while the minimum value recorded in mycorrhiza treatment was 0.9669.

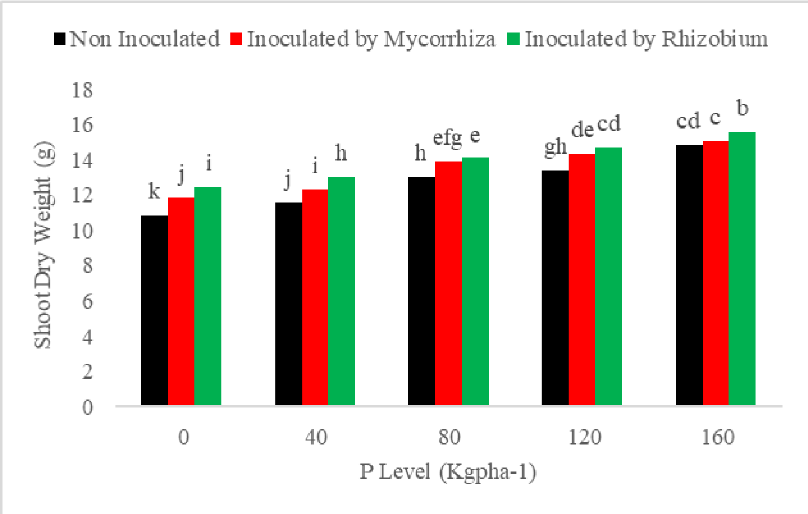

Figure-3: Effect of mycorrhiza, Rhizobium inoculated and non-inoculated on broad bean shoot dry weight (g) at different phosphors levels.

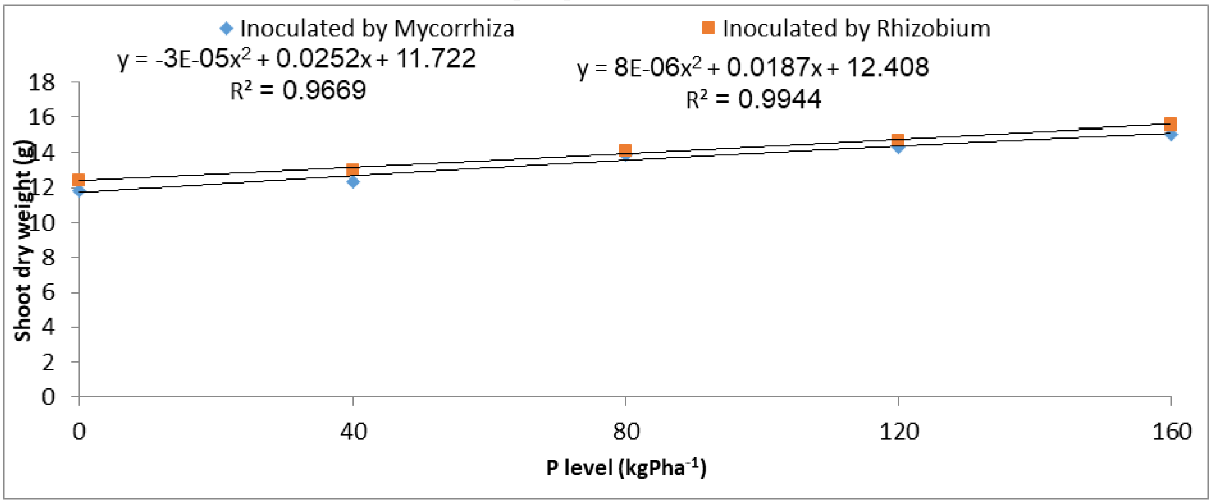

Figure-4: The relation between shoot dry weight and phosphorus levels for plants inoculated with mycorrhiza, Rhizobium on broad bean plant.

Root Dry Weight

The result in (Figure: 5) found that the root dry weight in broad bean plant were higher in inoculated plants with AMF than non-inoculated plant at different phosphors supply and increased by increasing phosphors levels. the maximum value for root dry weight in mycorrhizal plants was (7.72 gpot^{-1})recorded at highest P level (160 kgPha^{-1}), while the lowest value for root dry weight was(5.20 gpot^{-1}) recorded at level (zero kgPha^{-1}).this result compatible with the result which found by [9], the increasing of root dry weight in inoculated plants with AMF may be due to the important of mycorrhiza, that extended plant root system, with higher root system, root surface, root length, root diameter, root density and more root branching which help plants to uptake more nutrients and the plant become more active in growth and high root biomass with increase root dry weight [20], or may be due to absorption of phosphors faster by roots than non-mycorrhiza plants and grater surface area of mycorrhiza plants which help plants for more uptake nutrients resulted to increase root biomass [21]. The result in (Figure: 5) shows that where the soil inoculated with *Rhizobium, leguminosarum* the root dry weight of broad bean plants elevated compared with non-inoculated plants and root dry weight increase by increasing phosphors levels. The maximum value for root dry weight was (8.03 $gpot^{-1}$) was recorded at highest phosphors level ($160 kgPha^{-1}$), while the lowest value (5.37 $gpot^{-1}$) was recorded at lowest phosphors level (zero $kgPha^{-1}$). This result compatible with the result of [8], the increasing of root dry weight in plant which inoculated with *Rhizobium leguminosarum* may be due to the important of *Rhizobium leguminosarum* to improve plant growth due to forms nodules and fixing nitrogen which supply nitrogen to the plant which important for photosynthetic rate and increasing plant growth [22], or may be due to improving root and shoot biomass. Also the result show that root dry weight increase by increasing phosphors levels, the maximum value was (7.47 $gpot^{-1}$) recorded at highest phosphor (160 $kgPha^{-1}$) and the lowest value was (4.69 $gpot^{-1}$) recorded at (zero $kgPha^{-1}$). The increasing root dry weight by increasing phosphors levels may be due to the ability of root to uptake more available phosphors from the soil which help to increasing plant growth, and root biomass [23].

Figure: 6 shows the coefficient of determination R^2 for root dry weight at different P levels, the maximum value was obtained by inoculation with *Rhizobium leguminosarum* was 0.9983 while the minimum value recorded in mycorrhiza treatment was 0.9552.

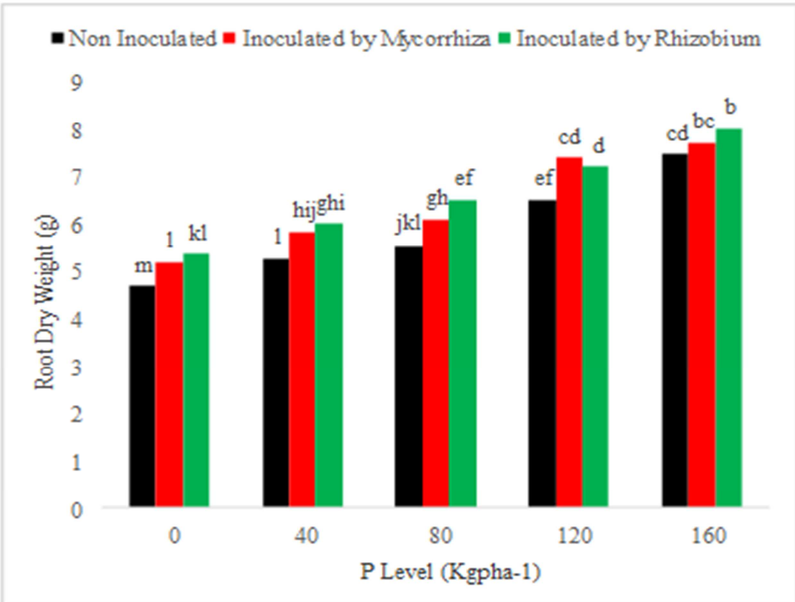

Figure-5: Effect of mycorrhiza, Rhizobium inoculated and non-inoculated on broad bean root dry weight (g) at different phosphors levels.

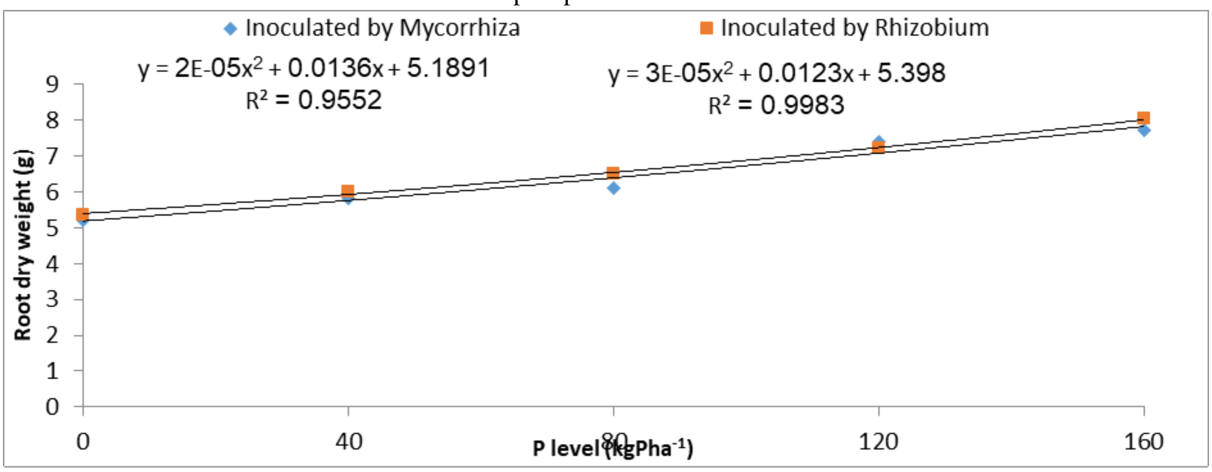

Figure-6: The relation between root dry weight and phosphorus levels for plants inoculated with mycorrhiza, or Rhizobium on broad bean plant.

Nodules Number

The results show that mycorrhizal plants had higher nodules number at different phosphors levels compared to non-inoculated plants, this result was compatible with the result which found by [8] and [24] Figure 7 the maximum value for nodules number was (128.67 nodule pot^{-1}) recorded at the highest P level (160 kgPha^{-1}) and the lowest value was (69.33 nodule pot $^{-1}$) recorded at (zero kgPha^{-1}). The elevated nodules number for inoculated plant with mycorrhiza may be due to the role of mycorrhiza in stimulating absorption of phosphorous which enhanced plant growth resulted in raised forming nodule number and, mycorrhiza elevated the ability of plant root to uptake more P which enhanced nodulation and Nitrogen-fixation [6] and [25]. The obtained results demonstrated that inoculation broad bean plants with *Rhizobium leguminosarum* significantly increase number of nodules at different phosphors levels and the number of nodules increased by increasing the rate of phosphors, this result was compatible with the result [18]. In non–inoculated plant with AMF and *R. leguminosarum* the nodules number increased by increases the supply of phosphorus levels, the highest value for nodule number was (109.67 nodules pot$^{-1)}$ recorded at (160 kgPha^{-1}) and the lowest nodule number was (59.67 nodules pot^{-1}) recorded at (zero kgPha^{-1}). Elevated nodule number for non-inoculated by increasing the rate of phosphors may be due to the important of phosphorus which improve root biomass and raised the capacity of roots to uptake more available phosphors which help plant to form nodules [26].

Figure: 8 shows the coefficient of determination R^2 for nodules number pot^{-1} at different P levels, the maximum value was obtained by mycorrhiza inoculation was 0.9982 while the minimum value recorded in *Rhizobium leguminosarum* treatment was 0.9713.

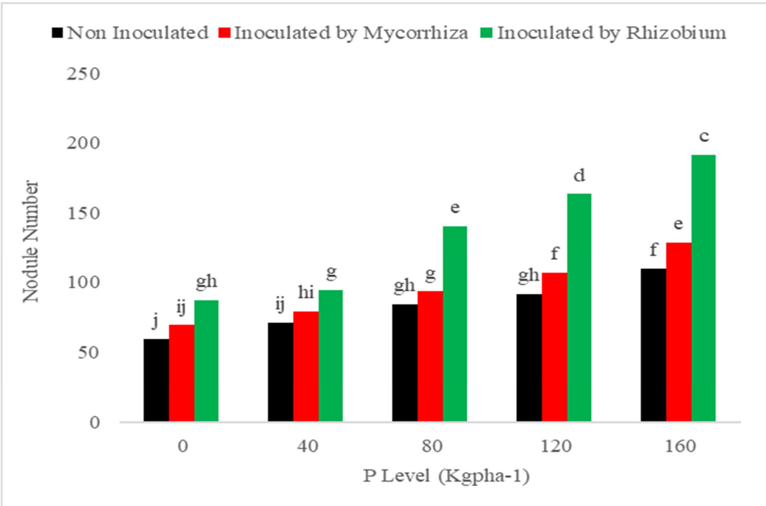

Figure-7: Effect of inoculated and non-inoculated soil with mycorrhiza and Rhizobium on broad bean nodule numbers at different phosphorus levels.

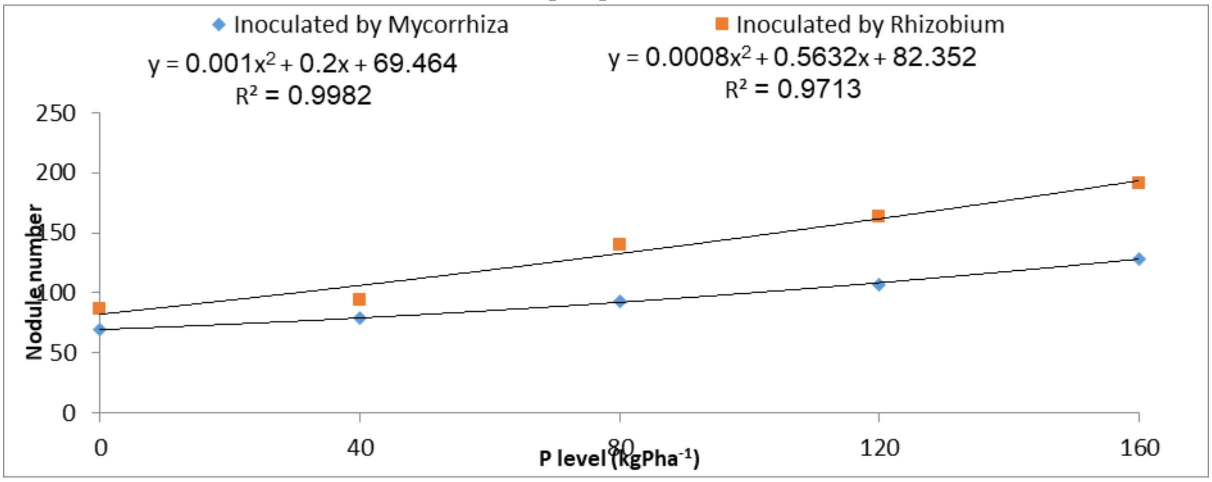

Figure-8: The relation between nodules number and phosphorus levels for plants inoculated with mycorrhiza, or *Rhizobium* on broad bean plant.

Shoot Nitrogen Contents)

The data in (Figure: 9) shows that when soil inoculated with AMF the nitrogen concentration in shoot of broad bean plants increased in all treated plants and increased by increasing phosphorus levels, compart with non-inoculated plants with AMF. The highest nitrogen content (15.68 gkg^{-1})was obtained at(160 $kgPha^{-1}$,) while the lowest value for nitrogen content was(12.02 gkg^{-1})recorded at lowest phosphorus level (zero $kgPha^{-1}$), however in non-inoculated plant with AMF the highest nitrogen content was (14.88 gkg^{-1})observed in (160 $kgPha^{-1}$)and the lowest nitrogen content was (10.75 gkg^{-1}) recorded at (zero $kgPha^{-1}$), this result was an agreement with [38], the increasing nitrogen concentration in broad bean which inoculated by AMF maybe due to the role of AMF that promoted biomass production and photosynthetic rates by increasing the rate of phosphorus to nitrogen accumulation due to AMF colonization that significantly elevated the activity of nitrogenase enzymes, nodulation and Nitrogen-fixation in legume plant, resulting increasing nitrogen content in plant [20] and [27] or may be due to the ability of mycorrhiza which increased nutrient uptake by hyphae such nitrogen in the soil and transfer to plant [3]. The result seems that the effect of inoculation plant with *Rhizobium leguminosarum* significantly effect on increasing shoot nitrogen content and it increased by increasing the rate of applied phosphorus compared with non-inoculated plants. The highest value of nitrogen content was (20.35gkg^{-1}) recorded at (160 $kgPha^{-1}$) while the lowest value was (13.08 gkg^{-1}) recorded at (zero $kgPh^{-1)}$ ^{the} increasing of nitrogen content in AMF plants may be due to the important of *R. Leguminosarum* which supply nitrogen to the plant during nodulation and nitrogen fixation and phosphorus is a major factor limiting the rate of Nitrogen-fixation.

Figure: 10 shows the coefficient of determination R^2 for shoot nitrogen content at different P levels, the maximum value was obtained by inoculation with *Rhizobium leguminosarum* which was 0.9927 while the minimum value recorded in mycorrhiza treatment was 0.9846.

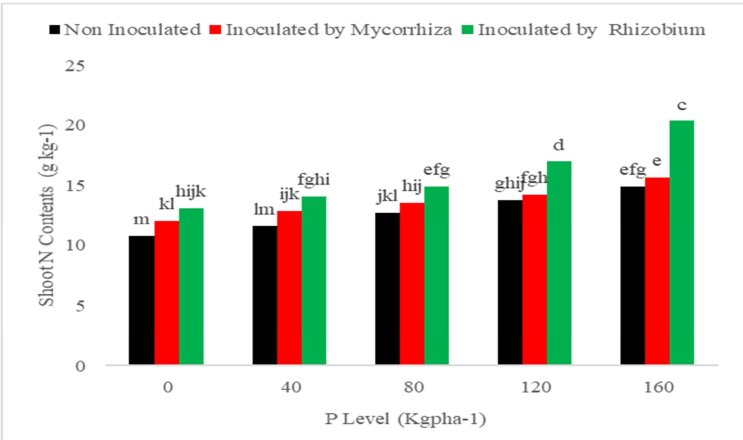

Figure-9: Effect of mycorrhiza, Rhizobium inoculated and non-inoculated on broad bean shoot nitrogen contents at different phosphors levels.

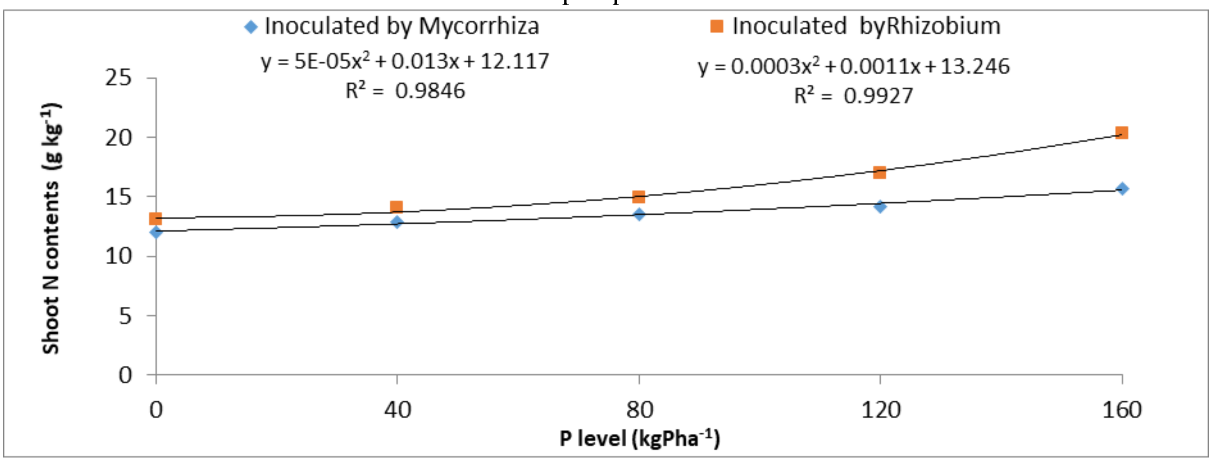

Figure-10: The relation between shoot nitrogen and phosphorus levels for plants inoculated with mycorrhiza, or Rhizobium on broad bean plant.

Shoot Phosphorus contents

The shoot phosphorus content in broad bean plants are shown in (Figure: 11). The result reveal that the inoculation plant with AMF significantly influenced the amount of phosphorus content on broad bean plants as compared to the control treatment and phosphors content significantly increase by increasing the rate of phosphorus supply. This result was compatible with the result which found by [8] and [28]. The highest value for shoot phosphors content in mycorrhizal plants was (4,38 gkg^{-1}) recorded at highest p level (160 $kgPha^{-1}$), while the lowest value was (2.22 $gkg^{-1)}$ recorded at lowest level (zero $kgPha^{-1}$), but for non-inoculated plants with AMF the highest value for shoot P content was (2.87 gkg^{-1}) recorded at highest P level (160 $kgPha^{-1}$) and the lowest value was (1.13 gkg^{-1}) recorded at lowest level (zero $kgPha^{-1}$). The increasing of P contend in inoculated plants by AMF may be due to hyphal network that develops in the rhizosphere in which it absorbs inorganic P that is transferred to the host plant through intradical hyphae which extends beyond the P depletion zone around the root to absorb P from the area where the roots alone cannot reach and fast transfer of P by hyphae to plant [3] and [29], or may be due to the ability of AMF to improve plant growth, root development and nutrient absorption such P by plant [30], or may be due the ability of AMF which play a critical role in the absorb relatively immobile nutrient uptake especially P from the soil and transfer it in to the host plant [31]. The result show that inoculated soil with *Rhizobium leguminosarum* increase shoot phosphorus content in broad bean plant at different phosphorus levels and increase with increasing the rate of applied phosphorus compared with non-inoculated with *R. leguminosarum* this results agreement with the result which found by [18]. The highest value of shoot P content (3.72 gkg^{-1}) was recorded at highest P level (160 $kgPha^{-1}$) while the lowest value (160 gkg^{-1}) was recorded at lowest level (zero $kgpha^{-1}$). The increasing shoot phosphors content in broad bean plants which inoculated with *R. leguminosarum* may be due to the important of phosphors in the soil which enhance Nitrogen fixation, while nitrogen and phosphorus are two major important nutrient essentials for plant growth and high biomass and more uptake phosphors in the soil resulting more phosphors in plant [31].

Figure: 12 shows the coefficient of determination R^2 for shoot phosphor content at different P levels, the maximum value was obtained by inoculation with mycorrhiza was 0.9997 while the minimum value recorded in *Rhizobium leguminosarum* treatment was 0.9983.

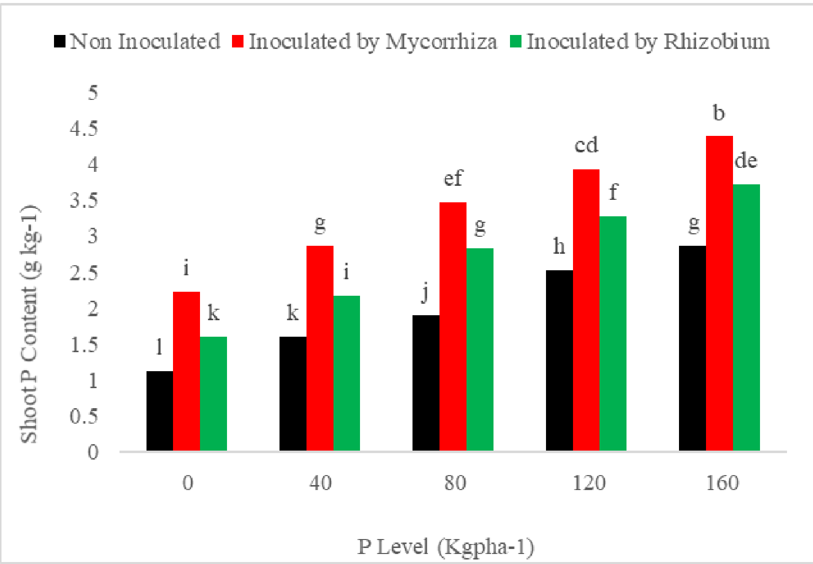

Figure-11: Effect of mycorrhiza, Rhizobium inoculated and non-inoculated on broad bean shoot phosphorus content at different phosphors levels.

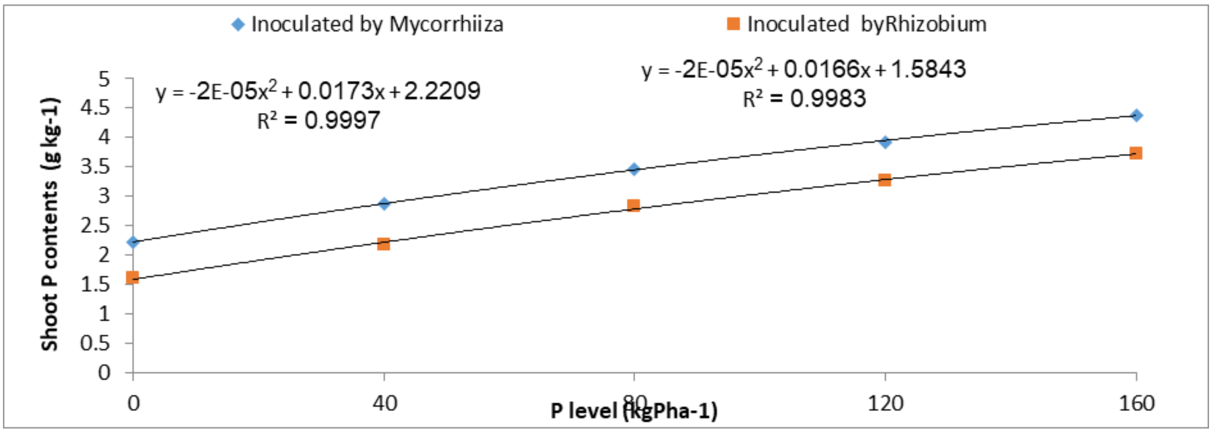

Figure-12: The relation between shoot phosphorus contents and phosphorus levels for plants inoculated with mycorrhiza, or Rhizobium on broad bean plant.

Shoot Potassium Contents

The data in (Figure: 13) shows that the amount of potassium in broad bean plants significantly increased due to inoculated plant with AMF at different P levels compared with non- inoculated treatment, and increased by increasing the rate of P supply, this result agreed with the result of [32] and [33]. The highest K content was (17.72 gkg^{-1}) obtained at (160 $kgPha^{-1}$), while the lowest value for K content (14.10 gkg^{-1}) recorded in control (zero $kgPha^{-1}$). Increased K content due to inoculated plant with AMF may be due to the positive effect of AMF on nutrient acquisition which increased plant growth and density of root plant that raised more uptake K by plant and elevated the concentration of K in mycorrhiza plant [34], or may be due to the ability of AMF that having a positive effect on K uptake by extra radical hypha and enhancement of K translocation to plant. The result indicates that inoculation plant with *Rhizobium leguminosarum* affected on increasing shoot K content in broad bean plant at different phosphorus levels non-significantly compared with non- inoculated plant with AMF at different P level. The increasing of shoot K content in inoculated plants with *R. leguminosarum* may be due to excretion of different organic acids by *R. leguminosarum* that increased available K in the soil solution which helped legume plants growth and improved nutrients uptake such K by plants and lead to increase the concentration of K in the plants [8].

Figure: 14 shows the coefficient of determination R^2 for shoot K content gkg^{-1} at different P levels, the maximum value was obtained by inoculated with AMF which was 0.9948 and minimum value was recorded by *R. leguminosarum* which was 0.9836.

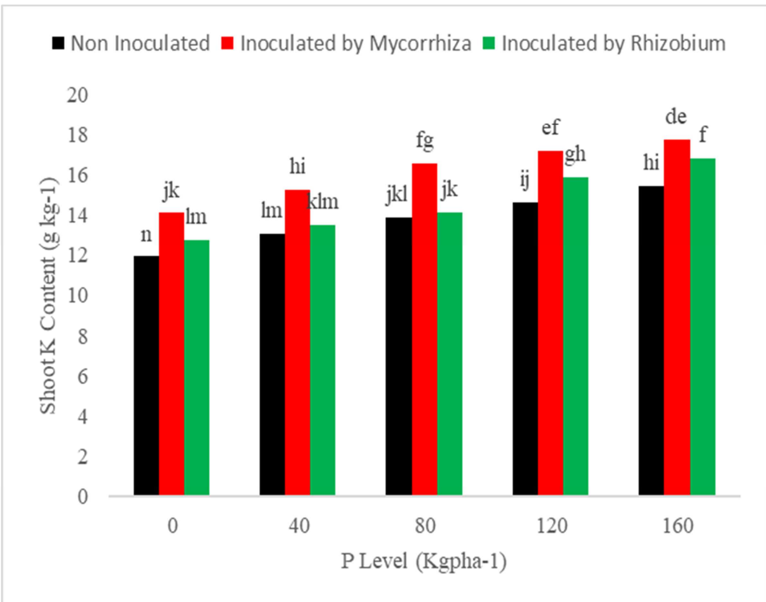

Figure-13: Effect of mycorrhiza, Rhizobium inoculated and non-inoculated on broad bean Shoot Potassium content at different phosphors levels.

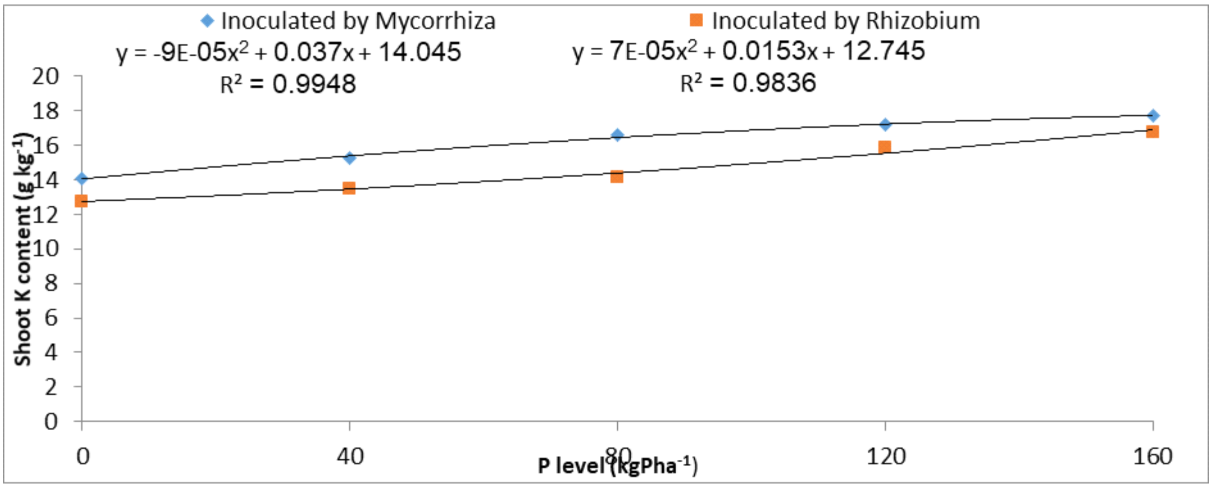

Figure-14: The relation between Shoot Potassium contents and phosphorus levels for plants inoculated with mycorrhiza, or Rhizobium on broad bean plant.

Shoot Iron Contents

The results in (Figure: 15) demonstrate that the Fe content in shoot broad bean plant inoculated with AMF significantly affected on increasing total of Fe content compared to control treatment at different phosphorus levels. This result was compatible with the result which found by [8] which found inoculated broad bean plant with AMF increase shoot Fe content. The maximum value for Fe content was (184.00 µgg^{-1}) recorded at heights P level (160 kgPha^{-1}), while the lowest value was (147.33 µgg$^{-1)}$ recorded at control treatment (zero kgPha^{-1}). In non- inoculated plant the maximum Fe content was(138.33µgg^{-1})recorded at highest P level (160 kgPha^{-1}), while the lowest was (96.67µgg^{-1}) recorded at lowest P level (zero kgPha^{-1}).The increasing of Fe content in inoculated plant with AMF may be due to the ability of AMF to improve plant growth and nutrient uptake such Fe, and ability of hypha to take up and transport of Fe to the host plant due to enhancement of total root surface, and by hyphae growth which absorb more Fe to plant, resulting in increasing plant Fe content [3], [8] and [35]. The result in the (Figure: 13) show that when the soil inoculated with *Rhizobium leguminosarum* the shoot Fe content increased by increasing P level compared to non-inoculated plants, the result an agreement with result which found by [18]. The highest value for inoculated plant with *R. leguminosarum* was (175.33µg g^{-1}) recorded at highest P level (160 kgPha^{-1}), while the lowest value was (134.67µgg^{-1}) recorded at lowest P level (zero kgPha$^{-1)}$). The increasing Fe content in plant which inoculated with *R. leguminosarum* may be due to the ability of *R. leguminosarum* for more Fe up take, because Fe is important for the structure of nitrogenase enzyme [36] which is necessary for nitrogen fixation.

Figure: 16 shows the coefficient of determination R^2 for shoot Fe content µgg^{-1} at different P level, the maximum value was obtained by inoculated with mycorrhiza which was 0.9906 and the minimum value recorded by inoculated with *Rhizobium leguminosarum* which was 0.9887.

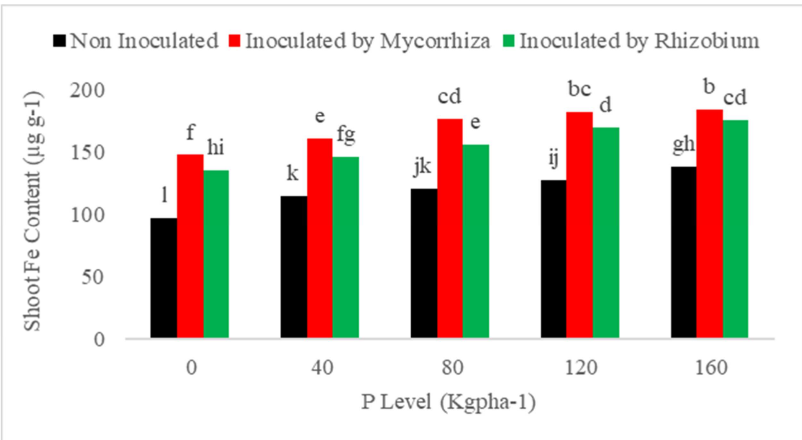

Figure-15: Effect of mycorrhiza, Rhizobium inoculated and non-inoculated on broad bean Shoot iron content at different phosphors levels.

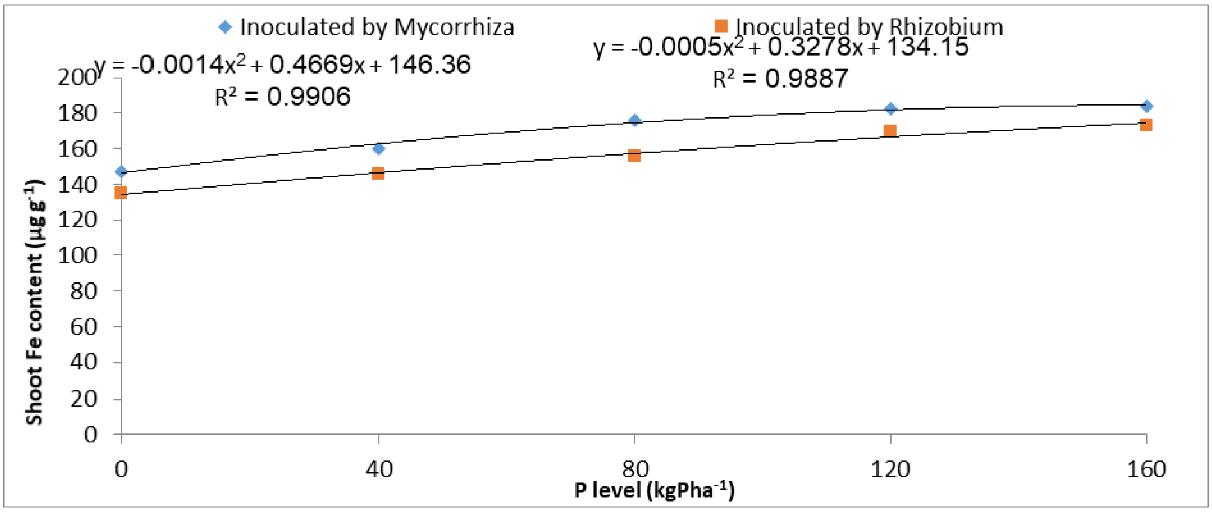

Figure-16: The relation between shoot Iron contents and phosphorus levels for plants inoculated with mycorrhiza, or Rhizobium on broad bean plant.

Shoot Molybdenum Contents

Figure: 17 illustrate that inoculated broad bean plant by AMF increased shoot Mo content significantly at different P levels compared with non- inoculated plants with AMF. This result was a compatible with the result which found by [37]. The highest value for shoot Mo content in inoculated plants was (0.94 µgg^{-1}) recorded at medium P levels (80 kgPha^{-1}), while the lowest value was (0.64 µgg^{-1}) recorded at control treatment (zero kgPha^{-1}). Also in non-inoculated plant with AMF the highest concentration of Mo in shoot plant was (0.64 µgg^{-1}) recorded at (160 kgPha^{-1}) while the lowest value was (0.32 µgg^{-1}0 recorded at (zero kgPha^{-1}). The increasing of Mo in shoot plants inoculated with AMF may be due to the hyphae of mycorrhiza can actively uptake Mo in the soil outer the Mo duplication zone, and transport to the root plant or may be due to the phosphorus which increase Mo uptake and improve Mo translocation from root to shoots, and AMF increase available phosphors may enhance more available Mo release to soil solution which absorbed by plant [37]. The result in (Figure: 17) show that when the soil inoculated with *R. leguminosarum* the concentration of Mo in shoot plant increased at different P levels compared with non- inoculated plants, the maximum value for Mo in inoculated plants with *R. leguminosarum* was (0.85 µgg^{-1}) recorded at (120 kgPha^{-1}) while the lowest value was (0.68 µgg^{-1}) recorded at (zero kgPha^{-1}). The increasing Mo content in shoot broad bean plant which inoculated with *R. leguminosarum* may be due to the important of Mo in the structure of nitrogenase enzyme which is necessary for nitrogen fixation in *Rhizobium* which convert Nitrogen element to ammonium in plants, or may be due to excretion of different organic acids to the soil by *Rhizobium leguminosarum* which increase availability of Mo, hence increasing the concentration of Mo in the soil ,result in increase the uptake of Mo by plant which increase the amount of Mo in shoot plant [36].

Figure: 18 shows the coefficient of determination R^2 for shoot Mo content µgg^{-1} at different P level, the maximum value was obtained by inoculated with *Rhizobium leguminosarum* which was 0.9129 and the minimum value recorded by inoculated with mycorrhiza which was 0.9043.

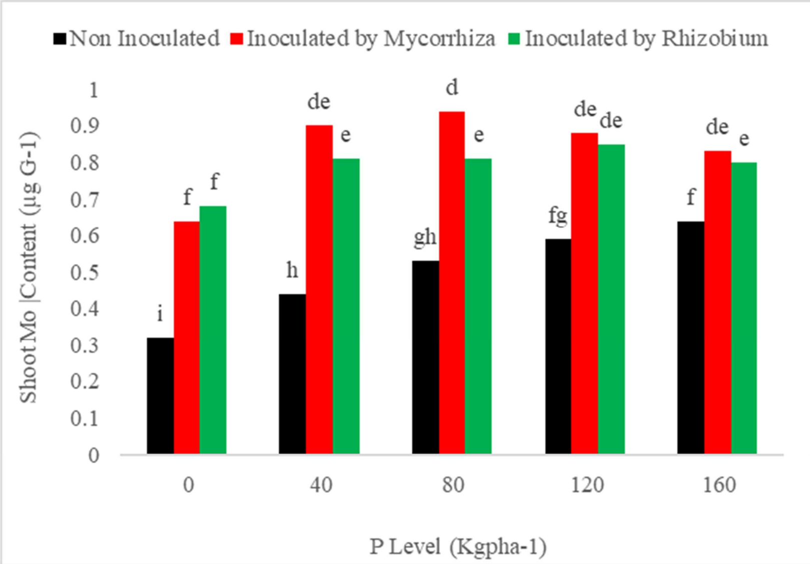

Figure-17: Effect of mycorrhiza, Rhizobium inoculated and non-inoculated on broad bean shoot molybdenum content at different phosphors levels.

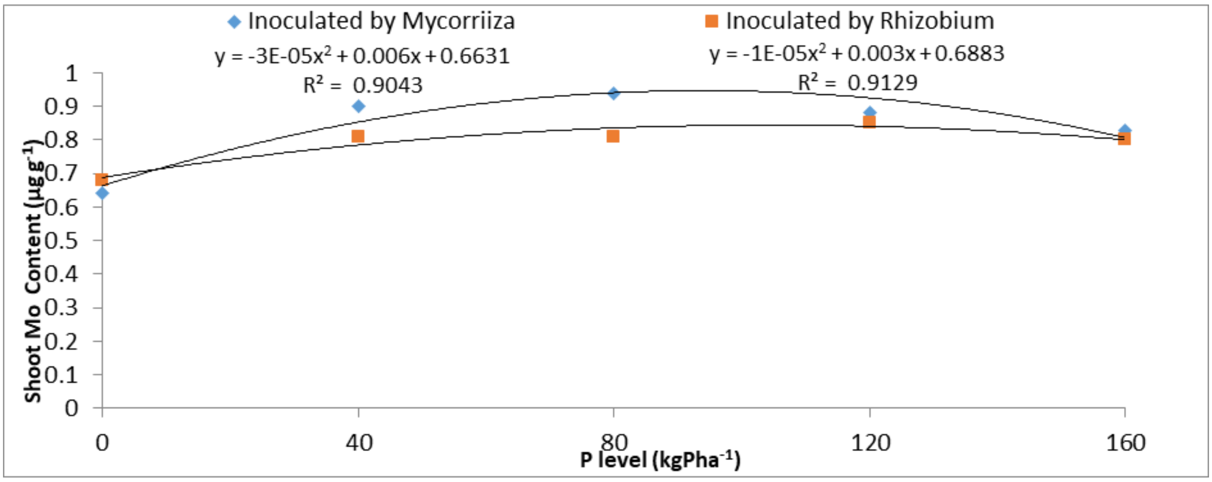

Figure-18: The relation between shoot molybdenum and phosphorus levels for plants inoculated with mycorrhiza, or Rhizobium on broad bean plant.

Conclusion

Inoculation of broad bean plant with mycorrhiza fungi, or *Rhizobium leguminosarum* with phosphorus improved plant growth and nutrient uptake in calcareous soil. In low soil phosphorus concentration. The activity of AMF was more than high phosphorus concentration. Increasing soil phosphorus levels caused the increase in nodulation and Nitrogen –fixation in broad bean plant.

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