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EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI AND PHOSPHATE FERTILIZATION ON WHEAT YIELD AND ROOT SURFACE FUNGI

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The current study is designed to evaluate the effect of arbuscular mycorrhizal fungi (AMF) and phosphorus fertilizers (superphosphate & rock phosphate) on growth, productivity and nutrient uptake of wheat plants (*Triticum aestivum* L.). Mycorrhizal inoculated wheat plants were significantly high in terms of growth, productivity parameters and nutrient uptake than non-inoculated plants at all phosphate fertilizers levels. It was observed that AMF root colonization was high in plants treated with low level of rock phosphate (25%), which decreased progressively with increasing fertility level of rock phosphate. The yield components of mycorrhizal treated plants in the presence of superphosphate and rock phosphate levels significantly rose as compared with non-mycorrhizal treated plants. The results revealed that AMF with low levels (25% & 50%) of phosphate fertilizers had a high significant effect on the three nutrient elements including nitrogen, phosphorus and potassium in the seeds and shoots of wheat compared with non-mycorrhizal treated plants. A total of 8 species belonging to 3 genera were isolated from 10 soils grown with wheat plants treated with different levels of superphosphate and rock phosphate and inoculated with or without mycorrhizal fungi. Meanwhile, 13 species representing 6 genera were isolated from 10 wheat root samples. As a conclusion, cultivation of wheat plant in the presence of mycorrhiza with low levels of fertilizers improved the growth, nutrient uptake and productivity values of wheat plants.

Keywords: Arbuscular mycorrhizal fungi, Wheat, Yield components, Nutrient uptake, Super phosphate, Rock phosphate

INTRODUCTION

Wheat (*Triticum aestivum* L.) represents about 37% of caloric consumption in the Middle East and North Africa (MENA) region. Food and Agriculture Organization's latest forecast for 2016 world wheat production stands at 724 million tonnes, 10.1 million tonnes or 1.4 percent lower than the 2015 record **(FAO, 2016).** Wheat is the most important grain crop in Egypt and grains are, in turn, the most important crop group. Wheat represents almost 10 percent of the total value of agricultural production and about 20 percent of all agricultural imports. The world's largest wheat importer in Egypt is seen to import 11.5 million tonnes **(FAO, 2016)**. Where it is one of the major sources of energy, protein and fiber in human diet, staple food for the world population and hence the most important cereal crop globally (**Arzani and Ashraf, 2017)**.

Phosphorus (P) is one of the major nutrient elements which limit agricultural production in the word. It is added to the soil in form of fertilizers, apart from which is utilized by plants the rest will rapidly be converted into insoluble complex in the soil **(Vassilev and Vassileva, 2003; Nosratabad *et al.*, 2017).** The average P-content in soils is about 0.05% (w/w) but only 0.1% of the total P is available to plants **(Zou *et al.*, 1992)**. This leads to the need of frequent application of fertilizers, but its use on a regular basis has become a costly affair and also environmentally undesirable **(Reddy *et al.*, 2002)**. Addition of P fertilizer to agriculture soil can make the soil environment more favorable for microbial growth (**Chu *et al.*, 2007**; **Iovieno *et al.*, 2009**). Although the application of P is required to achieve high wheat yields (**MacDonald *et al.*, 2011; Gemenet *et al.*, 2015**). Excessive amount of P fertilization to attain high crop yields can lead to excess levels of P in croplands (**Wang *et al.*, 2016**) and then to reduced environmental quality and food security (**Cordell *et al.*, 2009**).

Therefore, plants have developed mechanisms, such as symbiotic relations with soil fungi, to increase their access to soil phosphate. Probably the most important symbiotic mechanism is the formation of mycorrhizae, mutualistic symbiotic associations between plant roots and specific soil fungi. Arbuscular mycorrhizal (AM) fungi are the most widespread type of mycorrhiza formed by the majority of crop plant species and more than 70% of all terrestrial plants **(Brundrett 2002).** Mycorrhizal fungi play an important role in P uptake and growth of many cereals, legumes, and other crop plants **(Smith and Read, 2008).** The elongation of the extra radical hyphae of AM fungi into soil increases the surface area for the uptake of P, which is often depleted in rhizosphere soil solution. Generally, P acquisition

by crop plants is accomplished by extending roots into soil **(Schnepf *et al.*, 2008)**. This process of enhancing P absorption by plants is particularly important in highly weathered and fine textured, where great proportions of applied P fertilizer are not available to plants due to strong fixation of P on iron and aluminum oxides **(Jama *et al.*, 1997; Bunemann *et al.*, 2004).** Arbuscular mycorrhizal fungi comprise a key functional group of microbial soil that can greatly increase crop productivity and ecosystem sustainability. Addition of mycorrhizal fungi to soil is extensively recognized as an environment-friendly agronomic measure in the practice of organic agriculture to enhance wheat production (**Zhu *et al.*, 2017**). Mycorrhiza was shown to have a beneficial effect on plants of which acting as a bio-fertilizer, bio-stimulant and bio-protective agent (**Hart and Trevors., 2005; Gianinazzi *et al.*, 2010; Rouphael *et al.*, 2015**). Additionally, mycorrhizal fungi act as a plant health promoter (**Whipps, 2004**) and may be utilized to improve crop yield and nutritional properties of the plants (**Rozpądek *et al.*, 2016; Abu-Elsaoud *et al.*, 2017**). **Smith and Read** (**2008**) reported that AM not only improve plant growth but they have also 'non-nutritional'effects in stabilizing soil aggregates, in preventing erosion, and in alleviating plant stress caused by biotic and abiotic factors.

This study was undertaken to assess (1) The influences of AMF communities and colonization in wheat, (2) The influences of P application on the development of AMF colonization, (3) Importance of AMF in growth, yield components and nutrient uptake of wheat plants and (4) Effect of AMF on root surfaces fungi was also assessed.

MATERIALS AND METHODS

Mycorrhizal inoculum

Native arbuscular mycorrhizal species were isolated from rhizosphere soil samples of wheat plants collected from different agriculture fields in Assiut Governorate. Spores of mycorrhizal fungi were isolated from soil by Wet Sieving and Decanting Method **(Gerdemann and Nicolson, 1963)**. The most abundant mycorrhizal species were selected and morphologically identified according to the descriptions of reference cultures from the International Culture Collection of Arbuscular Mycorrhizal Fungi (INVAM) (at http://invam.caf.wvu.edu) and by consulting the protocols available at the AMF-phylogeny website (www.lrz.de/schüßler/amphylo). The genera and families presented in this study follow the consensus classification of **Schüβler and Walker (2010)** and **Redecker *et al.* (2013)**.

Mycorrhizal spores were propagated on wheat as host plant on sterilized clay-sand mixture (50% soil and 50% sand). Plants were cultured in the greenhouse for 3 months. The roots colonized by AM fungi were checked during the culture, and the presence of spores was confirmed by sieving. One hundred grams of soil containing mixture of mycorrhizal spores, extraradical hyphae and roots fragments were applied as mixture culture inoculum. Sterilized soil was used for control treatments.

Plant material and fertilizers

Wheat seeds of the experimental plant cultivar (Suds 1) were taken from Department of Crops, Agriculture Research Center (ARC). The treatments were allocated to the recommended dose super phosphate (480 kg.P_1 ha^{-1}) as chemical fertilizer and rock phosphate (480 kg.P_2 ha^{-1}) as natural fertilizer. These fertilizers were added to soil before sowing of seeds.

Experimental set up and design

A representative soil sample was collected from the experimental site at the depth of 0.0-30 cm before planting. The soil and sand samples were air-dried, and sieved with 2 mm sieve to remove any debris from the samples. Some chemical and physical properties of experimental soil before planting were determined according to **Jackson (1973)**.

Plastic circular pots (20 cm height x 26 cm width) were filled with 5 kg mixture of loam and sand soil (2: 1, w/w). Treatments were as follows: non-inoculated plants **(NM);** plants fertilized with superphosphate fertilizer **(NM +100% P_1);** plants fertilized with rock phosphate fertilizer **(NM +100% P_2);** plants inoculated with AM fungi **(M)**; plants inoculated with AM fungi and treated with different superphosphate concentrations **(M + 25%, 50% and 75 % P_1)**; plants inoculated with AM fungi and treated with different concentration of rock phosphate fertilizer **(M +25%, 50% and 75 % P_2).** All treatments were installed in five replicates for each treatment, with the total of 50 pots.

Seeds were surface sterilized by ethanol (70%; 30 s), NaOCl (5%; 3 min) and washed 5 times with sterilized water. Five seeds were planted. Growing plants were carefully watered as needed with tap water to maintain soil moisture near field capacity. Mineral fertilizers were added for all treatments at sowing time expect the non-inoculated plants. Urea (46%N) and potassium sulfate (50% K_2SO_4) were respectively used as sources of nitrogen and potassium.

Determination of mycorrhizal colonization

At vegetative and harvesting stages of wheat plants, fresh root samples were used for assessment of mycorrhizal colonization and line intersect method was used to evaluate the percentage of colonization **(Giovannetti and Mosse, 1980)** for each treatment after staining with trypan blue **(Phillips and Hayman, 1970).**

Mycorrhizal colonization (%) = (Total number of root segments colonized / Total number of root segments studied) × 100.

Fresh and dry weight of plants and yield components

Five plants were randomly taken for each treatment and washed with tap water to remove any soil particles. Shoots were separated from roots. The dry weights of plant parts (roots and shoots) were recorded after drying in a forced air oven at 65 °C for 48h.

Yield components were determined as follows: plant height (shoots and roots), number of spikes per plant, number of seeds per spike, length of spikes per plant and 100 grain weight (g) (seed index) per treatment.

Plant tissue analysis (seeds and shoots):

Dried plant samples were accurately weight and placed in a beaker for subsequent digestion. The wet ashing method using a mixture of sulphuric acid and hydrogen peroxide was followed **(Black *et al.*, 1965)**. For total nitrogen in plant was determined using micro Kjeldahl method, total phosphorus in plant was determined spectrometrically using the colorstannous phosphomolybdic acid method in a sulphuric acid system and total potassium in plant was determined by the flame photometer method **(Jackson, 1973)**.

Isolation of fungi from wheat roots:

Rhizosphere fungi

It was carried out according to **Timonin (1940)** and **Moubasher and Abdel-Hafez (1986)** as follows:

Blocks of soil containing plant roots were cut out and gently crushed, with a little tearing of roots as possible. The roots were uprooted and gently shaken to separate and collect superfluous soil. Ten gram of collected soil particles adhering to roots were transferred into conical flask containing 100 ml of sterilized distilled water. After thoroughly shaking, desired dilution (1/1000) was prepared. One ml of the final soil suspension was transferred to each

sterilized Petri dish, 12-15 ml of melted and cooled PDA medium were poured. For each treatment, five plates were incubated at 28ºC ±1 for 7 days during which the developing fungi were counted, examined morphologically for identification and calculated as colony forming unit "CFU" per g dry soil.

Rhizoplane fungi

Samples of roots were then subjected to a series of washings in sterile distilled water and then dried between two sterilized filter papers. Roots were then cut under aseptic conditions into equal segments (one cm each). The segments (five segments per plate) were inserted on the surface of the PDA medium in Petri-dishes **(Abdel-Hafez *et al*. 1990)**. Five plates per each treatment were incubated at 28ºC±1 for 7 days. The developing fungi were counted and calculated per 25 root segments.

Statistical analysis

The data were subjected to one-way ANOVA using the SPSS 19.0 software program. Means and standard deviations were calculated for five replicates. Means were compared by the Duncan's multiple range tests and statistical significance was determined at 5% level. GraphPad Prism 7 software program was used.

RESULTS AND DISCUSSION

The results of physico-chemical properties of experimental soil were shown in **Table 1**. The texture grade of the soil under investigation was sandy loam. The electrical conductivity (EC) was ١.٥٧ $dS.m^{-1}$. Ca^{++} value was 10.2 $mmol_c.L^{-1}$, while Na^{+} content was 23.3 $mmol_c.L^{-1}$. The mean values of available nitrogen, phosphorus and iron were 220, 1.21 and 12.30 (as $mg.kg^{-1}$ dry soil), respectively.

Mycorrhizal symbiosis is a key component in helping plants survive under adverse environmental conditions **(Sheng *et al*., 2008)**. Our results showed that AMF formed mycorrhizal symbiosis in wheat plants under different levels of phosphorus fertilizers (superphosphate and rock phosphate). Some parameters of wheat plants such as root colonization, fresh and dry weights, yield components and nutrient uptake were found to be improved by the tested AMF species under P fertilizers.

Table 1: Physico-chemical properties of experimental soil before planting.

Soil Properties	Values
Particle size distribution	
Clay %	17.6
Silt %	25.3
Smooth Sand %	19.5
Rough Sand %	37.6
Texture grade	Sandy Loam
pH (1:2.5 soil water suspension)	8.12
EC (dS m^{-1})	١.٥٧
Soluble cations ($mmol_c$ L^{-1})	
Ca^{++}	10.2
Mg^{++}	6.48
Na^{+}	23.3
K^{+}	0.52
Soluble anions ($mmol_c$ L^{-1})	
HCO_3^{-}	2.5
Cl	34.5
$SO_4^{=}$	3.5
Available Macronutrients (mg kg^{-1} soil)	
N	220
P	1.21
K	137
Available Micronutrients (mg kg^{-1} soil)	
Cu	3.48
Fe	12.3
Mn	13.37
Zn	1.61

Mycorrhizal colonization:

Six morphotypes of mycorrhizal fungi belonging to the families Aculosopraceae, Glomeraceae and Gigasporaceae were commonly isolated from soil of wheat plants. The population consists of ***Acaulospora bireticulata*** F.M. Rothwell & Trappe, ***Funneliformis coronatum*** (Giovann.) C. Walker & A. Schüßler comb. nov., ***Funneliformis mosseae*** (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler comb. nov. ***Glomus spinuliferum*** Sieverd. & Oehl, in Oehl, Wiemken & Sieverding,

Gigaspora gigantea (Nicol. & Gerd.) Gerd. & Trappe, ***Scutellospora armeniaca*** Blaszk **(Figure 1A-F)**. These species were used as mycorrhizal inoculum in the present work.

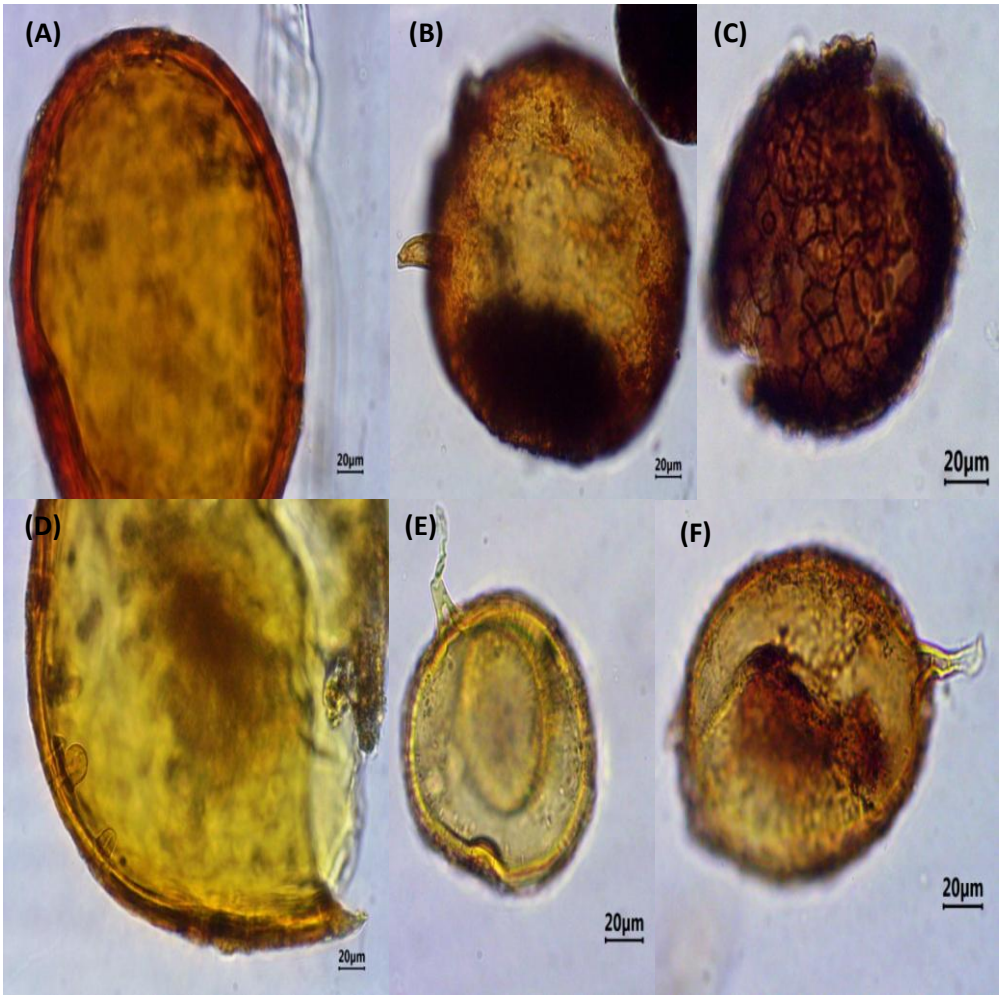

Figure 1: Spores of (A) *Scutellospora armeniaca* Blaszk.; (B) *Funneliformis coronatum* (Giovann.) C. Walker & A. Schüßler comb. nov; (C) *Acaulospora bireticulata* F.M. Rothwell & Trappe; (D) *Gigaspora gigantea* (Nicol. & Gerd.) Gerd.& Trappe; (E) *Glomus spinuliferum* Sieverd. & Oehl, in Oehl, Wiemken & Sieverding; (F) *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler comb. nov.

Arbuscular mycorrhizal fungi were found in the roots of all inoculated wheat plants but they were missed from the non-inoculated plants. There were large differences between colonization patterns in wheat roots **(Figure 2).** Roots colonization was strongly influenced by phosphorus fertilizers. At the harvest stage, the incidence of mycorrhizal fungi in roots was up to 92%, and the fungal structures noted were hypha, arbuscules and vesicles. There were significant differences between treatments in terms of hyphal colonization, which ranged between 56-82% **(Figure 2).** The percentage of vesicles was high and affected by fertilizers, of which the highest value was recorded in mycorrhizal wheat plants treated with high level of super phosphate (75% P_1). Meanwhile, the highest value of abuscular colonization was detected in plants amended with high level of rock phosphate (75% P_2) as shown in **Figure (2),** where the lowest value was recorded in wheat roots treated with low level of rock phosphate (25% P_2) and moderate level of super phosphate (50% P_1). The colonized roots in plants inoculated with AM fungi **(M)** were occupied by hypha, arbuscules and vesicles **(Figure 3A-E).**

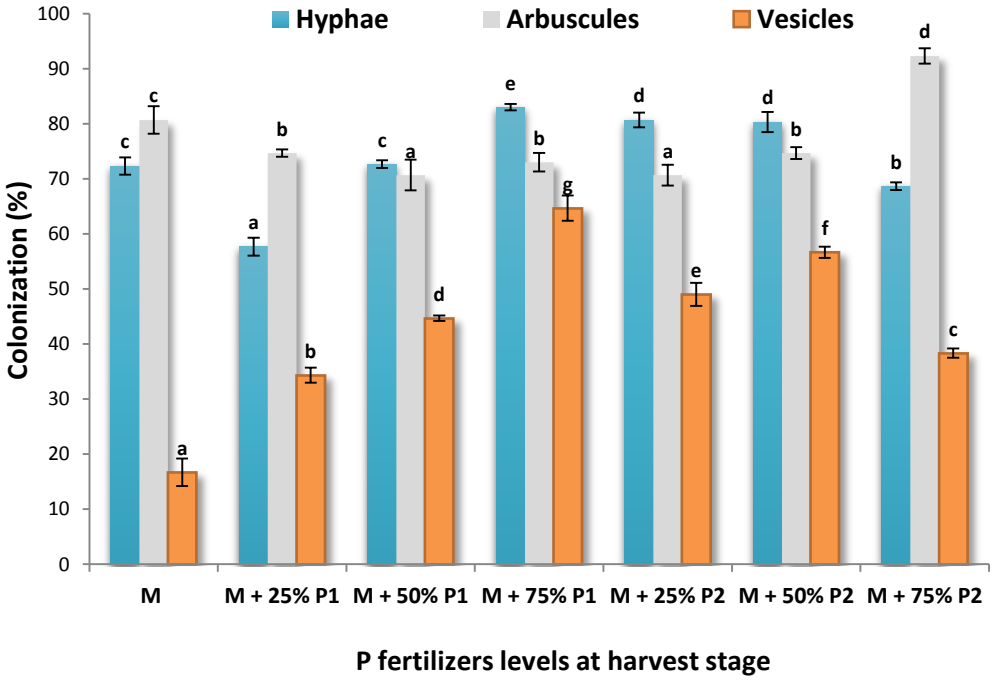

Figure 2: Effect of different levels of superphosphate (P_1) and rock phosphate (P_2) fertilizers on the percentage of wheat roots colonization at harvest stage, Mean ± SD (n=5).

It was also observed that phosphorus fertilizers had a strong effect on AMF development and colonization increased with the decrease of P concentrations. Plants treated with lower P concentration showed higher mycorrhizal root colonization as compared to high P concentration at vegetative stage. This might be due to that high soil phosphate level determines the reduction of hyphal growth and spore production of arbuscular mycorrhizal fungi. These findings support the earlier observations of **Guillemin *et al.* (1995)** who demonstrated that in P-sufficient soils mycorrhizal infections were reduced by phosphate fertilization, meanwhile in P- deficient soil fungal infection is not modified. Moreover, **Hu *et al.* (2009)** found that high P may be detrimental to mycorrhizal colonization and limit the phosphorus uptake. According to **Stottlemyer *et al.* (2008)**, the source of mycorrhizal inoculum can be the aerial spores since they used sterilized soil and reported that AM spore number and AM colonization were observed in uninoculated plants which imply that contamination might have occurred due to aerial mycorrhizal spores at some stage of the experiment.

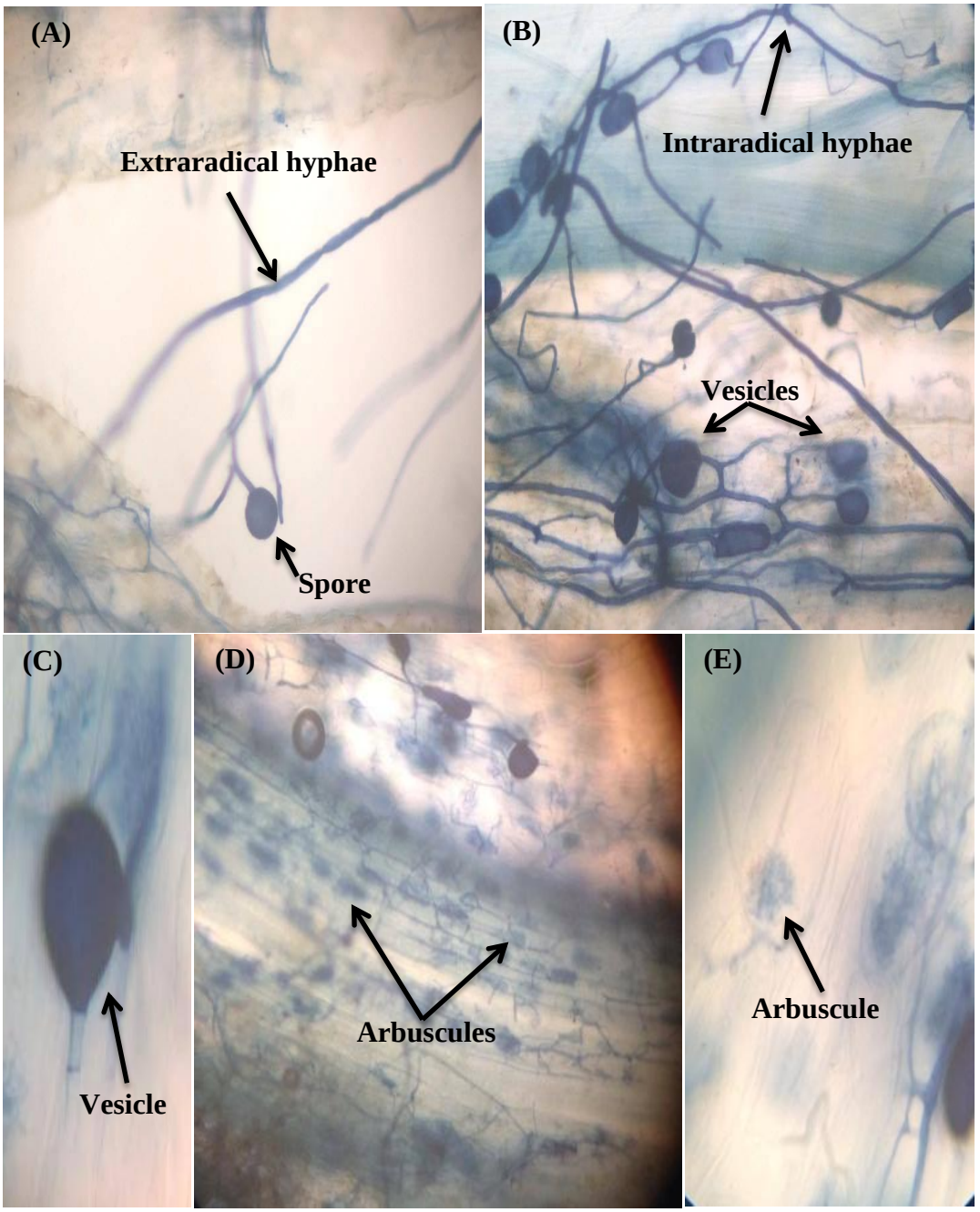

Figure 3 (A-E): Root colonization patterns of wheat plants at harvest stage inoculated with mycorrhizal fungi.

Plant fresh and dry weights:

At harvest stage the data in **Figure (4 A-D)** showed that in mycorrhizal inoculated plants, shoot growth significantly increased after treatment with superphosphate and rock phosphate. As well, single inoculation of AM fungi also increased shoots fresh and dry weights. Root growth was not significantly increased in all treatments. Also, there was an increase in fresh and dry weights of shoots and roots in mycorrhizal wheat plants treated with 25% super phosphate and 50% rock phosphate.

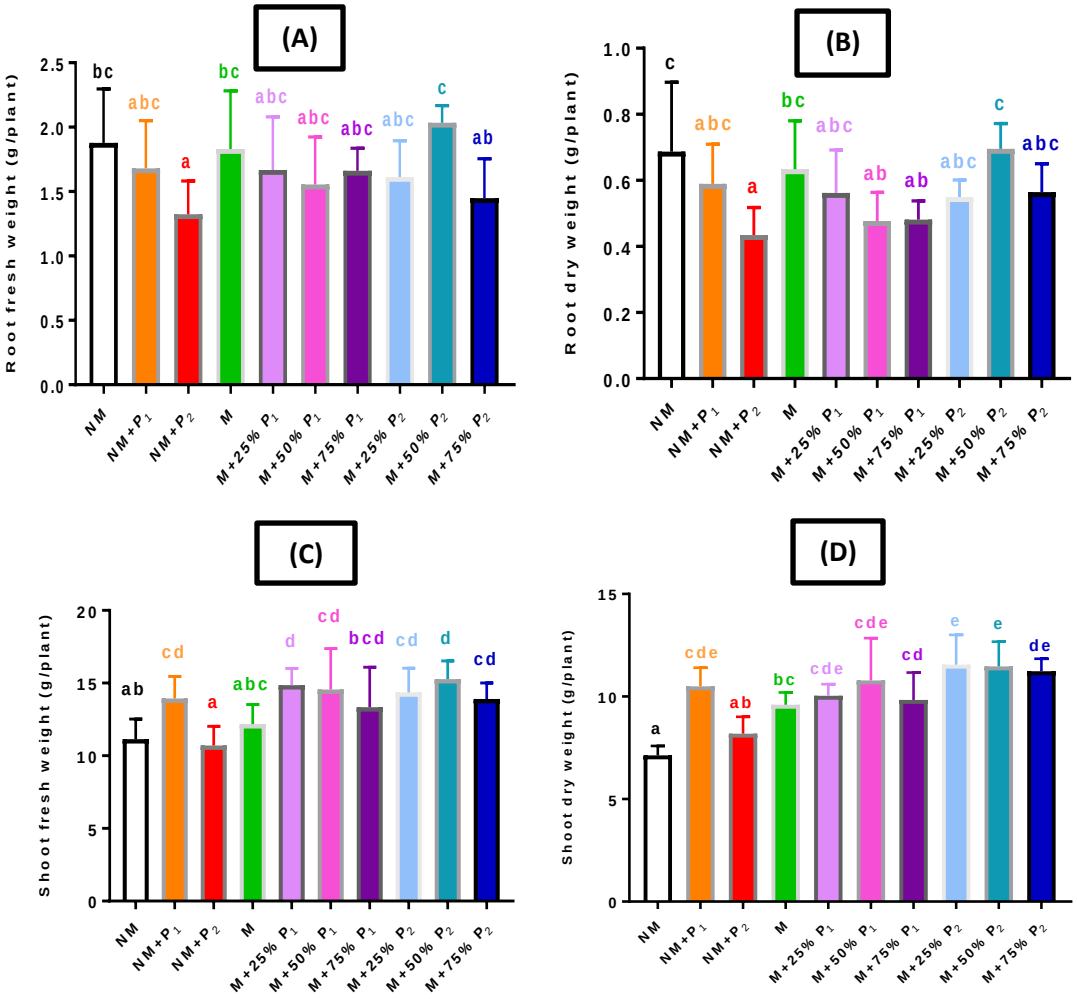

Figure 4 (A-D): Fresh and dry weights (g/plant) of roots **(A, B)** and shoots **(C, D)** of wheat plants at harvest stage grown under different levels of superphosphate (P_1) and rock phosphate (P_2) and inoculated with or without mycorrhizal fungi (M), Mean ± SD (n=5).

Yield components

Yield and its component consider the main target for the activity of plants. So the data recorded in **Table (2)** show that, mycorrhizal inoculation and P fertilizers generally caused a marked increase in wheat yield in terms of plant height, number of spike per plant, spike length, number of seeds per spike and weight of 100 seeds in comparison to the control plants. Mycorrhizal wheat plants attained maximum plant height of 109.8 cm at 50% rock phosphate level and minimum plant height of 96.8 cm was recorded from non-mycorrhizal wheat plants at superphosphate level. Furthermore, plant height of mycorrhizal wheat plants in the presence of superphosphate and rock phosphate treatments was significantly higher than non-mycorrhizal plants. Spike length of all treated plants was significantly increased with AM fungi, especially at superphosphate and rock phosphate levels. The highest values of spike length (11.51, 11.33 and 11.14 cm) were obtained from non-mycorrhizal rock phosphate plants, mycorrhizal wheat plants treated with 25% rock phosphate and mycorrhizal wheat plants treated with 50% superphosphate, respectively.

Data in **Table (2)** also showed that, the number of spikes / plant at all treatments was significantly increased with mycorrhizal inoculation. The highest number was obtained from non-mycorrhizal and mycorrhizal plants treated with different superphosphate and rock phosphate levels compared with control. The number of grains per spike of wheat plants treated with superphosphate and rock phosphate levels in the presence or absence AM fungi was not significantly changed as compared with control plants. The highest values of biological yield (number of grains in all spikes per plant) (4.81 and 4.62 g/plant) were obtained from mycorrhizal wheat plants treated with 25% and 50% rock phosphate respectively and the minimum value of biological yield (3.29 g/plant) was recorded from non-mycorrhizal plants treated with rock phosphate. The results showed a significant ($P<0.05$) reduction in seed index of non-mycorrhizal plants. The seed index of mycorrhizal plants in the presence of superphosphate and rock phosphate levels was significantly rose as compared with non-mycorrhizal plants. The highest significant value of seed index was obtained from mycorrhizal plants treated with 25% superphosphate, while non-mycorrhizal plants grown under rock phosphate conditions recorded the lowest as compared with control plants.

In this study mycorrhizal inoculation and P fertilizers caused a marked effect on the wheat grain yield per plant and its components as compared with the untreated plants. With regard to AM the improved yield and yield components in wheat plants reported here demonstrate the potential of

mycorrhizal inoculation to increase all yield components. This increase in yield components can be due to the effects of mycorrhiza fungus on absorbing various nutriments such as nitrogen, calcium, potassium, copper, zinc, sulphur and especially better P nutrition **(Sharifi *et al.*, 2007).** As mentioned by **Ambrosini *et al.* (2015)** mycorrhiza fungus increases plant growth and affects devoting and transferring nutriments between stem and root so that dry weight of shoot is increased by increasing absorption of nutriment and their transfer.

Also, Plants and fungi interact naturalistically, while the plant receives mineral nutrients and water through the fungus, the fungus is supplied with carbohydrates by its host **(Smith and Read, 1997).** It was proposed that AM fungi play an important role in improving yield **(Kanwal *et al.*, 2012),** because it is known that mycorrhizal roots acquire P more efficiently than non-mycorrhizal roots especially at low soil fertility levels. However, high available P may be detrimental to mycorrhizal colonization and may limit their benefits in agrosystems **(Hu *et al.*, 2013).**

Nutrient uptake (N, P and K)

Plant nutrient (N, P, and K) uptake was influenced by mycorrhizal fungi and P fertilizers at harvest stage as denoted in **Table (3)**. Concerning the total N-content, the obtained data showed significant increasing in N-uptake of both seeds and shoots compared to control treatment, ranged between 0.078 to 0.127 mg/plant and 0.038 to 0.068 mg/plant, respectively. The obtained results indicated that using mycorrhizal fungi combined with low concentration of rock phosphate (25%) gave the highest value of N in seeds. The nitrogen content of shoot achieved high value with mycorrhizal wheat plants under high level of super phosphate (75%). On the other hands, the lowest value of nitrogen in seeds and shoots was observed in non-mycorrhizal wheat plants under 100% rock phosphate compared to control treatment. These results indicated that the using of mycorrhizal fungi combined with low levels from phosphorus fertilizers gave significant increases in N-uptake of seeds and shoots of wheat compared to non-mycorrhizal wheat plants and control treatment.

Concerning to the concentration of phosphorus, data in **Table (3)** showed almost similar trend mentioned in nitrogen content of seeds and shoots. The data revealed that significantly increased in P content of both seeds and shoots in mycorrhizal wheat plants under high level of superphosphate (75%) compared to other treatments and control. P content in shoots was non-significantly between treatments. The application of mycorrhizal fungi combined with low concentration of P fertilizers gave values higher than

non-mycorrhizal wheat plants treatment with P fertilizers and control. Seeds and shoots values fluctuated between 0.007 to 0.012 mg/plant and 0.005 to 0.014 mg/plant, respectively. Data of K content (**Table 3**) behave similar trend obtained in total N and P contents. Its values in seeds and shoots ranged between 0.020 to 0.032 mg/plant and 0.181 to 0.299 mg/plant, respectively. The highest value of potassium content in seeds and shoots was in mycorrhizal plants under low level of rock phosphate (25%). The lowest value shown of potassium content in seeds and shoots was recorded in non-mycorrhizal wheat plants with 100% rock phosphate compared to other treatments and control plants.

The results showed that AMF increased the nutrient uptake of N, P and K in seeds and shoots with low levels fertilizers. With the continued rise in phosphorus fertilizer levels, the nutrient uptake of these elements was decreased compared to non- mycorrhizal and control treatments **(Table 3).**

These results are in agreement with the finding of **Khalil *et al.* (1994),** who studied the mycorrhizal dependency of soybean and corn. They found that, soybean had a higher mycorrhizal dependency than maize when mycorrhizal and non-mycorrhizal plants were compared, the N, P, K, Ca and Mg uptake were significantly increased in mycorrhizal plants. Also, mycorrhizae of the host plant increases the absorption of food elements in soil. Unlike phosphorus, nitrogen is an important element. Studies showed that a mycorrhizae hosted plant increases the absorption of nitrogen **(Bolan, 1991).** Mycorrhiza hyphae have the ability of absolving the soil nitrogen and transferring it to the plant's root **(Bago *et al.*, 1996).** In the case of potassium absorption by mycorrhizae some researchers say that it has no effect and some are saying it is useful **(Barea *et al.*, 1992).**

Rhizosphere fungi from soil

Data in **Table (4)** revealed that 8 fungal species belonging to 3 genera were isolated from 10 soil samples in pots planted with wheat. The total counts of rhizosphere fungi were 1130×10^2 CFU per g dry soil. The results showed that soil grown with plants **NM+P2**, **M+25%P1**, **M+75%P1**, **M+50%P2** and **M+75%P2** were the richest in fungal population whereas **NM**, **NM+P1**, **M**, **M+50%P1** and **M+25%P2** were the poorest. *Aspergillus* was the most common genus accounting for 98.9% of total fungi. The counts of *Aspergillus ustus* and *A. flavus* represented 70.07% and 15.59% of the total *Aspergillus*, respectively). *Botrytrichum* and *Fusarium* were isolated in low and rare occurrence represented by one species namely, *B. piluliferum* and *F. oxysporum* yielding 0.5% and 0.4% of total fungi, respectively.

Table 2: Yield parameters of wheat plants grown under different levels of super phosphate and rock phosphate and inoculated with or without mycorrhizal fungi, Mean ± SD (n=5).

Treatments / Inoculation	Plant height (cm plant^{-1})	No. of spikes (plant^{-1})	Spike length (cm plant^{-1})	Number of grains (spike^{-1})	Grain yield (g spike^{-1})	Biological yield (g plant^{-1})	Seed index (g 100 seeds^{-1})
NM	100.7 ± 0.76^{ab}	2 ± 0.00^{a}	9.61 ± 0.79^{a}	35 ± 3.34^{b}	1.41 ± 0.11^{abc}	2.81 ± 0.22^{a}	3.72 ± 0.47^{a}
NM + P_1	96.8 ± 7.75^{a}	3 ± 0.00^{c}	11.51 ± 0.22^{bc}	34.13 ± 3.98^{a}	1.43 ± 0.13^{abc}	4.30 ± 0.39^{cde}	4.25 ± 0.03^{bc}
NM + P_2	108.9 ± 4.31^{cd}	2 ± 0.00^{a}	10.72 ± 0.28^{d}	40.1 ± 4.98^{ab}	1.65 ± 0.19^{c}	3.29 ± 0.37^{ab}	4.12 ± 0.06^{b}
M	98 ± 2.78^{a}	3 ± 0.00^{c}	10.71 ± 0.29^{bc}	27.53 ± 3.82^{b}	1.21 ± 0.07^{a}	3.64 ± 0.21^{bc}	4.49 ± 0.06^{de}
M + 25% P_1	108.1 ± 0.89^{cd}	3 ± 0.00^{c}	10.53 ± 0.26^{b}	27.27 ± 3.68^{a}	1.29 ± 0.12^{ab}	3.86 ± 0.37^{bc}	4.78 ± 0.06^{f}
M + 50% P_1	107.2 ± 1.52^{cd}	2.4 ± 0.55^{b}	11.14 ± 0.54^{bcd}	39.47 ± 6.59^{b}	1.67 ± 0.33^{c}	3.89 ± 0.46^{bcd}	4.28 ± 0.05^{bcd}
M + 75% P_1	104.3 ± 3.87^{bc}	2.6 ± 0.55^{b}	10.84 ± 0.32^{bc}	33.7 ± 3.78^{ab}	1.54 ± 0.15^{bc}	3.95 ± 0.56^{bcd}	4.56 ± 0.02^{e}
M + 25% P_2	108.7 ± 3.29^{cd}	3 ± 0.00^{c}	11.33 ± 0.64^{cd}	39 ± 8.65^{b}	1.60 ± 0.26^{c}	4.81 ± 0.78^{e}	4.19 ± 0.08^{bc}
M + 50% P_2	109.8 ± 1.92^{d}	3 ± 0.00^{c}	10.59 ± 0.40^{b}	35.07 ± 5.65^{b}	1.54 ± 0.24^{bc}	4.62 ± 0.71^{e}	4.41 ± 0.04^{cde}
M + 75% P_2	104.9 ± 3.27^{bcd}	3 ± 0.00^{c}	10.91 ± 0.38^{bcd}	36 ± 3.10^{b}	1.51 ± 0.11^{bc}	4.54 ± 0.33^{de}	4.29 ± 0.11^{bcd}
***F* value**	**8.573****	**15****	**6.793****	**3.943****	**3.232****	**8.507****	**15.921****

NM - plants non inoculated with arbuscular mycorrhizal fungi, M - plants inoculated with arbuscular mycorrhizal fungi, P_1 - superphosphate fertilizer, P_2 - rock phosphate fertilizer. One way –ANOVA was performed for amendment treatment; Means for each parameter with different letters are significantly different from each other ($P< 0.05$) according to the Duncan test; NS not significant; * $P< 0.05$; ** $P< 0.01$. Mean ± SD (n=5)

Table 3: Nutrient uptake (g.plant^{-1}) by seeds and shoots of wheat plants grown under different levels of super phosphate and rock phosphate and inoculated with or without mycorrhizal fungi.

Plant parameters/ Treatments	Uptake of N (g.plant^{-1})		Uptake of P (g.plant^{-1})		Uptake of K (g.plant^{-1})	
	Seeds	Shoots	Seeds	Shoots	Seeds	Shoots
NM	0.046 ± 0.002^{a}	0.022 ± 0.005^{a}	0.009 ± 0.002^{abc}	0.006 ± 0.002^{ab}	0.019 ± 0.001^{a}	0.130 ± 0.007^{a}
NM + P_1	0.110 ± 0.011^{cd}	0.060 ± 0.009^{cd}	0.011 ± 0.001^{bc}	0.005 ± 0.000^{a}	0.028 ± 0.005^{bc}	0.245 ± 0.009^{cd}
NM + P_2	0.078 ± 0.003^{b}	0.038 ± 0.007^{b}	0.009 ± 0.001^{abc}	0.007 ± 0.002^{ab}	0.02 ± 0.001^{a}	0.181 ± 0.020^{b}
M	0.094 ± 0.008^{bc}	0.060 ± 0.008^{cd}	0.009 ± 0.001^{abc}	0.007 ± 0.002^{ab}	0.021 ± 0.002^{a}	0.232 ± 0.014^{bc}
M + 25% P_1	0.099 ± 0.009^{c}	0.052 ± 0.010^{bcd}	0.009 ± 0.002^{abc}	0.010 ± 0.011^{ab}	0.024 ± 0.003^{ab}	0.259 ± 0.019^{cd}
M + 50% P_1	0.110 ± 0.022^{cd}	0.062 ± 0.014^{cd}	0.012 ± 0.005^{c}	0.011 ± 0.003^{ab}	0.029 ± 0.005^{bc}	0.277 ± 0.054^{cd}
M + 75% P_1	0.104 ± 0.004^{c}	0.068 ± 0.011^{d}	0.012 ± 0.001^{c}	0.014 ± 0.004^{b}	0.028 ± 0.001^{bc}	0.263 ± 0.025^{cd}
M + 25% P_2	0.127 ± 0.018^{d}	0.060 ± 0.004^{cd}	0.008 ± 0.001^{abc}	0.009 ± 0.002^{ab}	0.032 ± 0.004^{c}	0.299 ± 0.027^{d}
M + 50% P_2	0.112 ± 0.007^{cd}	0.063 ± 0.007^{cd}	0.008 ± 0.002^{ab}	0.010 ± 0.003^{ab}	0.029 ± 0.003^{bc}	0.290 ± 0.058^{d}
M + 75% P_2	0.109 ± 0.011^{cd}	0.051 ± 0.011^{bc}	0.007 ± 0.002^{a}	0.011 ± 0.004^{ab}	0.028 ± 0.003^{bc}	0.281 ± 0.015^{cd}
F value	**12.511^{**}**	**6.987^{**}**	**2.285^{ns}**	**1.249^{ns}**	**5.554^{**}**	**9.373^{**}**

NM - plants non inoculated with arbuscular mycorrhizal fungi, M - plants inoculated with arbuscular mycorrhizal fungi, P_1 - superphosphate fertilizer, P_2 - rock phosphate fertilizer. One way –ANOVA was performed for amendment treatment; Means for each parameter with different letters are significantly different from each other ($P< 0.05$) according to the Duncan test; NS not significant; * $P< 0.05$; ** $P< 0.01$, Mean ± SD (n=5).

Rhizoplane fungi from soil

Thirteen species representing 6 genera were isolated from10 wheat root samples collected from the pots experiment. The total count of rhizoplane fungi was 335 CFU per 25 fresh root segments **(Table 5).** The results demonstrated that samples collected from **NM+P2** comprised the highest fungal population than other treatments giving rise to 14.03% of the total fungi, while the lowest count was found in **NM** and **NM+P1** (4.18% of total fungi for each). *Aspergillus* occupied the first place in frequency of occurrence and was recovered from all treatments (10 out of 10 samples) yielding 29.6% of total fungi. *Fusarium* was the second dominant genus being isolated from 9 out of 10 samples yielding 31% of total fungi. *Fusarium oxysporum* (87.5% of the total *Fusarium*) was the most common species and was isolated in high frequency of occurrence. The genus *Cochliobolus* was isolated from 6 samples and was represented by two species namely, *C. lunatus* and *C. spicifer* yielding 0.6% and 12.2% of total fungi, respectively. *Alternaria* and *Setosphaeria* were isolated in moderate occurrence represented by one species namely, *A. alternate* and *S. rostrata* yielding 4.8% and 16.4% of total fungi, respectively. *Rhizopus* (represented by *R. stolonifer*) was isolated in low occurrence yielding 0.9% of total fungi.

The first report attempted to specifically study the interaction of pathogenic fungus and a species of AM fungus was that of **Safir (1968).** Plants colonized by AMF differ from non-mycorrhizal plants in rhizosphere microbial community, resulted in alterations in root respiration rate, quality and quantity of the exudates **(Marschner *et al*., 2001).** The role of AMF in improving plant nutrition and their interactions with other soil biota has been investigated with reference to the host plant growth **(Tahat *et al.*, 2010)**, this is in agreement with the present results showed that the highest count of fungal population recorded in mycorrhizal soil whereas the lowest population recorded in control soil or non-inoculated soil fertilized with superphosphate and the highest rhizoplane fungi population recorded also from plants inoculated with mycorrhizal fungi.

CONCLUSION

To summarize, the present study suggested that the combined application of AMF (***A. bireticulata***, ***F. coronatum, F. mosseae, G. spinuliferum***, ***G. gigantea*** and ***S. armeniaca***) and low levels of phosphorus fertilizers of wheat plants is more effective than single treatment where, there was a significant improvement in plant growth, biomass and yield components. Furthermore, nutrient content of wheat can be increased as a result of mycorrhizal treatments. The results also indicated that use high P concentration reduce the beneficial mycorrhizal effect on plant growth, yield production and nutrient uptake. Wherefore, treatment with high levels of P fertilizers is not recommended because they reduce the beneficial mycorrhizal of plant.

Table 4: Total counts (TC×10^3) (CFU per g dry soil), number of cases of isolation (NCI, out of 10 treatments) and occurrence remarks (OR) of rhizosphere fungi isolated from 10 soils grown with wheat plants treated with different levels of super phosphate (P_1) and rock phosphate (P_2) and inoculated with or without mycorrhizal fungi (M).

Treatments / Fungal species	Total count (TC)										Total		
	NM	NM+P_1	NM+P_2	M	M+25%P_1	M+50%P_1	M+75%P_1	M+25%P_2	M+50%P_2	M+75%P_2	T.C	NCI	OR
Aspergillus	**1.2**	**8.2**	**27.8**	**1.2**	**10.8**	**9.2**	**13.8**	**9.6**	**18.8**	**11**	**111.6**		H
A. flavus Link	0.2	4.4	5.2	0.4	4.8	0.2	1	0.2	0.4	0.6	17.4	10	H
A.fumigatus Fresenius	0.8	3.2	1.2	0.2	0.6	0.2	2	0.2	0.8	0	9.2	9	H
A. niger van Tieghem	0	0.6	2.6	0	0	0.8	0	0.4	1	0.2	5.6	6	M
A. sydowii (Bainier & Sartory) Thom & Church	0	0	0	0	0	0	0	0.4	0	0	0.4	1	R
A. terreus Thom	0	0	0	0	0	0.4	0.4	0	0	0	0.8	2	L
A. ustus (Bain.) Thom and Church	0.2	0	18.8	0.6	5.4	7.6	10.4	8.4	16.6	10.2	78.2	9	H
Botrytrichum piluliferum	0	0	0.4	0	0	0	0.2	0	0	0	0.6	2	L
Fusarium oxysporum Schlechtendal	0	0	0	0	0	0	0	0	0.4	0	0.4	1	R
Sterile mycelia	0	0	0.4	0	0	0	0	0	0	0	0.4	1	R
Total counts	**1.2**	**8.2**	**28.6**	**1.2**	**10.8**	**9.2**	**14**	**9.6**	**19.2**	**11**	**113**		
No. of genera	1	1	2	1	1	1	2	1	2	1	3		
No. of species	3	3	5	3	3	5	5	5	5	3	8		

H = High occurrence; more than 7 treatments out of 10 treatments, M = Moderate occurrence; between 5-7 treatments, L = Low occurrence; between 2-4 treatments, R = Rare occurrence; less than 2 treatments.

Table 5: Total counts (TC) (CFU per 25 fresh root segments), number of cases of isolation (NCI, out of 10 treatments) and occurrence remarks (OR) of rhizoplane fungi isolated from roots of wheat plants grown under different levels of super phosphate (P_1) and rock phosphate (P_2) and inoculated with or without mycorrhizal fungi (M).

Treatments / Fungal species	Total count (TC)										Total		
	NM	NM+P_1	NM+P_2	M	M+25%P_1	M+50%P_1	M+75%P_1	M+25%P_2	M+50%P_2	M+75%P_2	T.C	NCI	OR
Alternaria alternate (Fr.) Keissler	1	1	0	4	0	5	0	5	0	0	16	5	M
Aspergillus	**7**	**3**	**10**	**16**	**4**	**12**	**14**	**2**	**19**	**12**	**99**		H
A. flavus Link	1	0	0	0	0	1	1	0	6	2	11	5	M
A. fumigatus Fresenius	0	0	0	8	0	0	0	0	0	0	8	1	R
A. nidulans (Eidam) Vuillemin	1	0	0	1	0	0	0	0	0	1	3	3	L
A. niger van Tieghem	0	0	6	5	4	1	0	0	0	1	17	5	M
A. ustus (Bain.) Thom and Church	5	3	4	2	0	10	13	2	13	8	60	9	H
Cochliobolus	**3**	**7**	**17**	**13**	**11**	**2**	**0**	**0**	**0**	**0**	53		M
C. lunatus	2	0	0	0	0	0	0	0	0	0	2	1	R
C. spicifer Nelson	1	7	17	13	11	2	0	0	0	0	51	6	M
Fusarium Link	**0**	**2**	**18**	**1**	**17**	**3**	**16**	**9**	**17**	**21**	**104**		H
F. oxysporium Schlechtendal	0	1	18	1	10	1	13	9	17	21	91	9	H
F. smitectum	0	0	0	0	7	2	0	0	0	0	9	2	L
F. solani (Martius) Saccardo	0	1	0	0	0	0	3	0	0	0	4	2	L
Rhizopus stolonifer (Ehrenberg) Vuillemin	1	0	2	0	0	0	0	0	0	0	3	2	L
Setosphaeria rostrata	1	0	0	0	0	10	3	20	10	11	55	6	M
Sterile mycelia	1	1	0	0	3	0	0	0	0	0	5	3	L
Total counts	**14**	**14**	**47**	**34**	**35**	**32**	**33**	**36**	**46**	**44**	**335**		
No. of genera	5	4	4	4	3	5	3	4	3	3	6		
No. of species	8	5	5	7	4	8	5	4	4	6	13		

H = High occurrence; more than 7 treatments out of 10 treatments, M = Moderate occurrence; between 5-7 treatments, L = Low occurrence; between 2-4 treatments, R = Rare occurrence; less than 2 treatments.

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تأثير الميكوريزا الشجيرية والتسميد الفوسفاتى على إنتاجية القمح والفطريات السطحية للجذور

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صُممت الدراسة الحالية لمعرفة تأثير فطريات الميكوريزا والأسمدة الفوسفاتية (سوبر فوسفات & الفوسفات الصخرى) على نمو وانتاج وامتصاص العناصر لنبات القمح. أجريت التجربة في تصميم يتكون من ١٠ معاملات مع خمسة مكررات لكل معاملة. تضمنت التجربة ثلاث معاملات من السوبر فوسفات (P1) وثلاث معاملات من الفوسفات الصخرى (P2) (٢٥%، ٥٠% ، ٧٥% من معدل الأسمدة الكاملة الموصى بها). كانت نباتات القمح الملقحة بالميكوريزا في جميع معاملات الأسمدة P أعلى بشكل كبير من حيث النمو ، ومعدلات الإنتاجية ، واستيعاب العناصر من النباتات غير الملقحة. لوحظ أن تواجد الميكوريزا فى جذور القمح كان أعلى في النباتات المعاملة بتركيز منخفض من الفوسفات الصخرى (٢٥٪ P2) ، والذي ينخفض تدريجيًا مع زيادة تركيز التسميد P2. وقد ارتفعت انتاجية المحصول الملقح بالميكوريزا بشكل كبير في وجود التركيزات المختلفة من السوبر فوسفات والفوسفات الصخرى مقارنة مع النباتات الغير ملقحة بالميكوريزا. وأظهرت نتائج هذا البحث ايضا أن الميكوريزا مع التركيزات المنخفضة (٢٥٪ و ٥٠٪) من الأسمدة الفوسفاتية كان لها تأثير معنوي كبير على العناصر الغذائية الثلاثة النيتروجين والفوسفور والبوتاسيوم (NPK) وتمثل ذلك فى البذور والمجموع الخضرى للقمح مقارنة مع النباتات الغير معاملة . تم عزل ٨ أنواع فطرية تنتمي إلى ٣ أجناس من عينات تربة نباتات القمح والمعاملة مع مستويات مختلفة من السوبر فوسفات والفوسفات الصخرى والملقحة مع أو بدون الفطريات الميكوريزا وفي الوقت نفسه ، تم عزل ١٣ نوعًا يمثل ٦ أجناس من ١٠ عينات لجذور القمح. وختاما ، فإن زراعة نبات القمح في وجود الميكوريزا مع تركيزات منخفضة من الأسمدة الفوسفاتية قد حسنت من نمو نبات القمح وامتصاص عناصر النيتروجين والفوسفور والبوتاسيوم ومعدل إنتاجيته.