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Response of Pearl Millet to Fertilization by Mineral Phosphorus, Humic Acid and Mycorrhiza Under Calcareous Soils Conditions

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Fertilizers are a costly input but continue to make a significant contribution to the production of additional food. To maximize benefits and minimize nutrient loss from fertilizer, it must be used in the right amount, source and combination at the right time using the suitable method. The experiment was carried out during the 2018/2019 summer season in Mariout, Desert Research Center Agricultural Experiment Station, southwest of Alexandria, Egypt to study the effect of the inoculation with Mycorrhizal (MF) fungi, Humic Acid (HA) applied to soil at levels (0, 8 and 16 kg fed^{-1}) and phosphorous foliar application as phosphoric acid (H_3PO_4 72.5% P_2O_5) at rates of (0, 0.75 and 1.5 ml/ l) on the yield, yield components and chemical composition of pearl millet (*Pennisetumgalaucum* L.). Results revealed that inoculation of pearl millet by Mycorrhizae, combined with application humic acid (16 kg/fed) and phosphoric acid (1.5ml/l) was the most effective treatment in improving fresh and dry forage yield which recorded (80.9 and 21.3 ton/fed), respectively through the whole season and recorded the highest protein content which was 24.3% for the 3rd cut compared with other cuts. The inoculation of pearl millet by Mycorrhizae, combined with application humic acid (16 kg/fed) was the most effective treatment in improving dry matter and starch 31.1and 23.7% respectively, as the mean for three cuts. Also, this treatment recorded the highest values of nitrogen as concentration and uptake 2.65% and 184.7 kg N/fed as soon as, phosphorus concentration and uptake values 0.46% and 32.9 kg P_2O_5 /fed respectively, as the mean for three cuts in the dry forage of pearl millet.Regarding yield, there was an increase in forage yield and nutrient uptake, especially P and Nand total antioxidants. Increasing rates of humic and phosphoric acid addition with mycorrhizae treatments increased total antioxidant activity (TAA) of leaf. TAA recorded the highest in the H_2 and P_2 with inoculation about 796, 663 and 425 (µg AAE/mg ext.) in 1st, 2nd and 3rd cuts respectively.These results indicated a marked improvement in the nutrient availability status of calcareous.

Keywords: Calcareous soil, Pearl millet, Mycorrhizae, Humic acid, phosphorus fertilizer, Total Antioxidant Activity and Salt Stress

1. Introduction

Today in Egypt, with the continues occurrence of green forage shortage during summer seasons, increasing the productivity of some promising annual forage types is getting interest. Growth and yield of Pearl millet (*Pennisetumglaucum* L.) can be enriched through improving nutrient status of plant grown in calcareous soils

Calcareous soils exist in large areas particularly in semi-arid regions. In Egypt, these soils are generally characterized by low fertility levels and easily ammonia volatilization due to their high content of calcium carbonate and alkaline pH. In addition, the availability of most nutritional elements is considerably low especially phosphorus and micronutrients. Concerning the problem of phosphate fixation in calcareous soils, it can be dealt by using inorganic-phosphate-solubilizing bacteria (IPSB) which can dissolve the fixed phosphate in soil,Vazquez et al. (2000).

Pearl millet is an important and widely cultivated staple crop in many African countries. It provides a dual purpose annual summer crop utilized for human food and livestock feeding. The crop is well knowable by forage producers especially in arid and

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semi-arid areas for its heat and drought tolerance, vigorous growth, quick re-growth after grazing or cutting, high biomass production potential and free from hydrocynide acid (Khairwal et al., 2007; Bramhaiah et al., 2018). Pearl millet's green foliage is leafy, palatable and nutritious feedstock for dairy cattle. Forage cutting date was found to be determinant factors that affect to a great extend the regrowth habit as well as yield of forage crops. Bukhari et al. (2011) stated that maximum leaf area was noticed when pearl millet was cut at 75 DAS compared to cutting at 45 and 60 DAS. Eissa et al. (2018) concluded that the highest of the plant and the leaf area of the plant gave the highest significant values in the first cut, and then they gradually decreased until they reached the lowest values in the third cut.

Moreover, farmers in remote arid areas are in need of organic sources that could be easily transported. Humic acid is the active constituents of organic fertilizers (Karakurt et al., 2009) and its application may represent an alternative to conventional soil fertilization. The value of humic acid substances cannot be overstated; they are absolutely critical elements, without which health in plant cannot be achieved. Humic substances can both directly and indirectly affect the physiological processes of plant growth (Yang et al., 2005). Humic substances can be useful for living creatures in developing, as carrier of nutrition, as catalysts of biochemmical reactions, and in antioxidant activity (Kulikova et al., 2005). Humic acid is recognized as an exogenous growth regulator/soil activator, and its effects have been evaluated in its limited quantities only on fruits and vegetables, IhsanullahDaur. (2014).Humic substances help in the promotion and sustainable intensification of crop yields, improve soil quality indicators, modify plant physiological responses, and maximize quantitative and qualitative yield parameters. This is due to their high nutrient content, huge surface area, active bio-stimulants, stability against microbial decomposition and a high number of active ingredients. Functional groups, Mosa et al. (2020). Foliar application of magnetic fulvate and humate solutions may enhance the efficiency of phosphorus fertilization under sandy soil conditions. Mohamed (2020)

Phosphorus responses decrease with improvement in soil P-status, as expected. Even at the current high price of P_2O_5, this underscores the need to revise soil fertility limits (Tiwari, 2002). If the initial soil phosphorus level is high, then maintenance application will be enough (Anonymous, 2012). Phosphorus Use Efficiency (PUE) defined as yield per unit of nutrient supplied (from the soil and or fertilizer), which was determined by using the formula of Moll et al., (1982).

Arbuscular Mycorrhizal (AM) fungi are found in the most soils of the world making an association with 80% of all economic plants (Harley and Harley 1987). The positive effects of AM fungi as a symbiotic association with the plants' growth were renowned (Lakshman 2009 and Abdelhameid, 2020). The AM fungi extraradical hyphae can cross the nutrient depletion zone adjacent, to the plant root and thus improve the immobile elements availability as P, Zn and Cu (Elgharably and Allam, 2013) by translocating them from remote locations through the mycorrhizal hyphae to the plant roots, besides mobile elements as N (Grant et al., 2005). Kanwal et al., (2015) suggested that colonization of wheat plants with AM fungi improved biomass, growth and essential nutrients availability. All mycorrhizal strains were effective in improving plant growth. Also, inoculation with this strain could improve P availability in calcareous soil conditions (Abou El Seoud et al. 2020).

Salinity stress causes ion toxicity and nutritional imbalance in plants, disrupting plant physiological processes, thus causing a serious decline in final yield Hajihashemi et al. (2009) and Taha et al. (2021a). Initially, salinity stress causes a significant reduction in seed germination, after which it alters growth and reproductive behavior causing severe yield losses (Hussain et al., 2021) and (Seleiman and Khair, 2018). Furthermore, salt stress disturbs enzymatic activities, photosynthesis, membrane structure, hormonal balance, water, and nutrient uptake, and induces oxidative stress by Taha et al. (2021b) and Seleiman et al. (2021a). Protective substances can be used as fertilizers, for seed preparation, nutrient management, and application of hormones Promising results for salinity stress management (Erdal, et al., 2010 and Hasanuzzaman, et al., 2017).

Therefore, the aim of this study is to establish applicable practice based upon compatibility of humic acid application with Mycorrhizae fungi and phosphorus acid foliar application in order to overcome the low fertility levels of calcareous soils.

2. Materials and Methods

The present investigation was carried out during the summer seasons of 2019/2020 in the Agricultural Experimental Station of the Desert Research Center at Mariout Research Station-Desert Research Center, between longitude 29°47′ and 11°18′ E and latitudes 31°00′ and 15°18′ N. Mariout station, 40 km south – west of Alexandria Egypt.

The experiment was planned in a split- split plot design with three replicates. Combination of factor-A (inoculated seed with (MF) was used as main plot factor (two treatments), factor B (three HumicAcid levels) used as subplots factor and factor C (three P levels) used as sub- sub plots factor. The plot area was (10.5 m^2), 3.5 x 3 m long and wide (1/400 fed).

Mycorrhizal fungi were inoculated at two levels; with and without seed inoculation. While 3 rates of Humic Acid were to the soil (HA) as [(BRAVO HUMAT 2, Composition 8% K from potassium Humate, Solubility 98%, Country of Origin: China): (H_0, H_1 and H_2 means 0, 8 and 16 kg/fed)] was applied during seedbed preparation, just before sowing the crop. The phosphorous foliar application as phosphoric acide (PA) (H_3PO_4 72.5%P_2O_5, 1.6845g cm^{-1} Density, 25 0C, 85% Normality) at rates of (P_0, P_1 and P_2 means 0, 0.75 and 1.5 ml/l), respectively.

The land was prepared using disk plough tillage. The plugging depth was (0 – 20) cm, followed by leveling and ridging. The soil managements were as follows; Compost treatments 20 t ha^{-1}, was added to soil 15 days before cultivation, nitrogen as urea 190 kg/ha, Potassium as as potassium sulphat 115 kg K_2O/ ha were added to soil after every cutting. Inoculation of Mycorrhizae combinations with Humic Acid were done week before the sowing date. Application of phosphorus foliarly was done on the plants month after sowing. Foliar spray of treatments were added three doses in tailoring, elongation and budding stages, of pearl millet plants (Shandaweel 1cultivar),The experiment was irrigated (flood) one time every week depending on growth stages of the crop to avoid water stress. At harvest in three cuttings, the plants were cut by hand just above the ground surface and the following parameters were taken, fodder fresh & dry yield and plant length. The seeds were sown directly at rate of 29 kg/ha in the soil at 20 August (2019).

Chemical contents of shoot

Nitrogen, phosphorus, potassium were determined in the digested dry matter of shoot of pearl millet plants which were oven dried at 700 for 72 hours then fine ground was wet digested to determined N, P, K% according to Chapman and Pratte (1982).

Total nitrogen was determined using Microkjeldahl method, Phosphorus content was determined by spectrophotometer, Potassium percentage was determined by using Flame photometer, Crude protein (Cp) content (%) was calculated based on nitrogen content of the dry matter, using the equation as following:

CP (%)=Nitrogen(%)× 6.25.

Dry matter (DM) content (%) was determined by the following formula, A.O.A.C (1970):

DM (%) = DMY/FFY× 100

Where,

DMY= Dry Matter Yield (ton fed^{-1}).

FFY= Fresh Forage Yield (ton fed^{-1}).

Starch percent is determined by the following formula, A.O.A.C (1970):

Starch (%)=(17.55+ 0.891(D M) - 24.182).

Determination oftotal antioxidant activity

The extract (0.1 ml) was mixed with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and4 mM ammonium molybdate). The tubes were incubated at 95°C for 90 min. The mixture was cooled to room temperature, and then the absorbance of the solution was measured at 695 nm against blank. The total antioxidant activity was expressed as ascorbic acid equivalents in milligrams per gram of the extract Prieto et al. (1999).

Determination of Available Macro and micronutrients in Soil

Available nitrogen in soil samples was extracted by 2M potassium chloride as described by Rowell (1994), Also Available potassium, phosphorous and micronutrients were extracted by DTPA + ammonium bicarbonate solution and measurement according to the method described by (Soltanpour, 1985). The apparent nutrient recovery (ANR) percentage was calculated by the following formula, (Craswell, 1987).

Pearl millet Yield

At harvest time, three times (35, 70 and 105 Days After Sowing (DAS)) 1st, 2nd and 3rd cuts to determine the fresh and dry forage yield kg/fed. All pearl millet plants for each plot plants were harvested to determine: fresh forage yield, dry forage yield and the accumulative of both through the whole season (ton/fed.).

Statistical Analysis

All data were statistically analyzed according to Snedecor& Cochran (1982), where treatment means was compared using L.S.D.test at 0.05 probability level.

The soil and irrigation water were analyzed at the laboratories of Desert Research Center, as shown in Tables (1 and 2).

Table 1. Initial status of some properties of the experimental soil

Soil depth (cm)	Soil Texture	pH	EC(dSm^{-1})	CaCO$_3$ (g kg^{-1})	OM (g kg^{-1})	Available nutrients (mgkg^{-1})			SAR
						N	P	K	
0 - 30	SCL	8.08	1.2	367	10.9	17.3	6.45	26.2	1.23

pH= Acidity; E.C = Electrical conductivity in extract soil (1:5); OM= Total Organic Matter (g kg^{-1});SCL= Sandy Clay Loam and SAR= Sodium Adsorption Ratio.

Table 2. Chemical analysis data of the applied irrigation water

pH	EC(dSm^{-1})	SAR	TDS(ppm)	Soluble cations(mmol l^{-1})				Soluble anions (mmol l^{-1})			
				Na^+	Ca^{++}	Mg^{++}	K^+	$CO_3^=$	HCO_3^-	Cl^-	$SO_4^=$
7.5	2.6	8.67	1380	13.7	2.29	0.22	0.42	nil	6.83	12.6	nil

pH = Acidity; E.C = Electrical conductivity; mmoll^{-1}= milli mole per kilogram, SAR= Sodium Adsorption Ratio and TDS= Total dissolved salt.

3. Results and Discussion

Fresh and dry yields (ton fed^{-1})

With regard to the effect of PA, H A and MF levels on fresh and dry yield of millet for the three cuts and, in turn, on the cumulative yield. The yield with Mycorrhizae, combined with application humic acid (16 kg/fed) and phosphoric acid (1.5 ml/ l) was the most effective treatment in improving fresh and dry forage yield which recorded (80.9 and 21.3ton/ fed), respectively through the whole season. These results are in good accordance with those reported by Abd El-Lattief (2011) and Abou-Amer (2014).

Table 3 showed that fresh and dry forage yield increased at 2nd cut compared with 1st and 3rd cut.

On the other hand, inoculated seed with (MF) increased fresh and dry forage yield about 95 and 110%, respectively compared with uninoculated treatment. This result agree with Abdel-Aziz et al., (1997) and Meftahet al., (2016) who studied effect of cobalt application and mycorrhizal fungi inoculation on growth and some nutrients content of barley and Egyptian clover plants grown in calcareous soil. Because of ArbuscularMycorrhizal Fungi (AMF) play an important role in vegetation restoration because of symbiosis with plant root; they can facilitate mineral absorption by host plant, stability and improve soil structure, affect the population structure and preserve species diversity (Bothe et al., 2010).

Also, increasing humic acid application from 8to16 kg /fed significantly increased in fresh yield by 28 to70.5% and with dry yield by 31 to 71%, too. As for the effect of phosphorous foliar application levels as phosphoric acid from 0.75 to 1.5 ml/l were significant increasing the fresh forage yield about 14 to 24.5% and increasing the dry forage yield about 12 to 25.6%, respectively compared with control treatment. Amandeep (2012), Hiroshi et al. (2013) andAbou-Amer (2014) reported that totalyield of green forage increased with the increasing level of P fertilizer on sorghum.

As for the effect of the double interactions, results showed a significant effect on the forage yield of fresh and dry interaction. Noticed that increasing humic acid application from 8 to 16 kg /fed with inoculation significantly increased in fresh yield by 25 to 69% and with dry yield by 16 to 55%, too. In addition to, the combined treatment phosphorus fertilization 0.75 and 1.5 ml/l with inoculation indicating an increase by about 15 and 24.5% with fresh yield and dry yield increasing with raise application foliarly by Phosphoric acid by 10 and 21%, too, compared with uninoculated treatment. In addition, the interaction between phosphorus fertilization (PA) and humic acid application (HA), results indicated significant effect on fresh and dry weight of forage yield.

Table 3. Effect of PA, HA and AMF levels on fresh and dry yields of pearl millet

AMF* (A)	H.A** Levels(B)	P.A*** Levels(C)	FFY				DMY			
			1st	2nd	3rd	CFW	1st	2nd	3rd	CDW
			(ton fed^{-1})							
Without	H_0	P_0	1.68^{L}	10.81^{P}	4.44^{P}	16.93^{P}	0.29^{I}	1.16^{R}	1.02^{N}	2.47^{N}
		P_1	3.34^{K}	11.47^{O}	5.41^{O}	20.22^{O}	0.49^{I}	2.39^{Q}	1.69^{M}	4.57^{M}
		P_2	5.05^{J}	11.93^{N}	7.21^{N}	24.19^{N}	0.66^{HI}	3.69^{P}	1.79^{LM}	6.13^{L}
	H_1	P_0	5.08^{J}	12.02^{N}	7.21^{N}	24.31^{N}	0.68^{HI}	4.45^{O}	1.84^{L}	6.97^{K}
		P_1	6.71^{I}	13.88^{M}	7.30^{M}	27.89^{M}	1.01^{GH}	4.69^{N}	1.86^{L}	7.57^{J}
		P_2	7.07^{I}	15.09^{L}	8.07^{L}	30.23^{L}	1.20^{FG}	4.86^{M}	2.19^{K}	8.25^{I}
	H_2	P_0	8.41^{H}	16.37^{K}	8.12^{K}	32.9^{K}	1.26^{FG}	5.12^{L}	2.25^{K}	8.63^{I}
		P_1	10.06^{G}	16.78^{J}	8.25^{J}	35.09^{J}	1.53^{EF}	5.43^{K}	2.39^{J}	9.35^{H}
		P_2	11.88^{F}	17.75^{I}	8.32^{I}	37.95^{I}	1.62^{EF}	6.08^{J}	2.69^{I}	10.40^{G}
With	H_0	P_0	11.85^{F}	17.62^{I}	8.48^{H}	37.95^{I}	1.60^{EF}	6.33^{I}	2.83^{H}	10.77^{G}
		P_1	11.76^{F}	20.34^{H}	9.08^{G}	41.18^{H}	1.95^{DE}	6.83^{H}	3.61^{G}	12.39^{F}
		P_2	13.60^{E}	21.57^{G}	9.08^{G}	44.25^{G}	2.03^{DE}	6.93^{G}	3.82^{F}	12.78^{F}
	H_1	P_0	15.13^{D}	24.48^{F}	9.76^{F}	49.37^{F}	2.32^{D}	7.27^{F}	4.21^{E}	13.81^{E}
		P_1	15.15^{D}	26.61^{E}	10.01^{E}	51.77^{E}	2.40^{D}	7.34^{E}	4.19^{E}	13.93^{E}
		P_2	15.24^{D}	27.27^{D}	10.75^{D}	53.26^{D}	2.43^{D}	7.49^{D}	4.92^{D}	14.84^{D}
	H_2	P_0	16.77^{C}	27.87^{C}	11.38^{C}	56.02^{C}	3.09^{C}	7.69^{C}	5.37^{C}	16.15^{C}
		P_1	27.56^{B}	31.37^{B}	13.09^{B}	72.02^{B}	4.07^{B}	9.24^{B}	5.60^{B}	18.90^{B}
		P_2	29.51^{A}	37.80^{A}	13.68^{A}	80.99^{A}	5.33^{A}	9.67^{A}	6.31^{A}	21.31^{A}

*AMF= AbuscularMycorhiza Fungi; **HA= Humic acid; H_0, H_1 and H_2 means 0, 8 and 16 kg HA fed^{-1}respectively, ***PA= Phosphoric acid; P_0, P_1 and P_2 means 0, 0.75 and 1.5 ml/l respectively, FFY= Fresh Forage Yield (ton fed^{-1}) and DMY= Dry Matter Yield (ton fed^{-1}), CFM= Cumulative Fresh Weight (ton fed^{-1}) and CDM= Cumulative Dry Weight (ton fed^{-1}); Across each column different superscript indicate significant ($p<0.05$) variation between the mean values.

The combined treatment phosphorus fertilization (PA) and humic acid application (HA) (1.5 ml/l with 16 kg HA/fed) showed the highest fresh of forage yield indicated increase of about 33.8%.With respect to the triple interaction, results showed a significant effect on the forage yield. So the combined treatment (16 kg HA+ 1.5 ml PA/l with inoculation by Mycorrhizae) showed the highest yield of both fresh and dry 378% and 761%, respectively compared with control treatment. Such promoting effect on forage yield may be resulted from the ability of humic acid to sustain photosynthetic tissues which in turn increase the total dry weight (Turkmen et al., 2005). The obtained in this section are ascertained by the findings of Motaghi & Nejad (2014), Tuba et al. (2015) and Manal et al. (2016).

Nitrogen and Phosphorus content of millet (kg fed^{-1})

Data in Tables 4 & 5 indicate that increasing rates of humic and phosphoric acid addition with inoculated seeds by mycorrhizae treatments increased the N and P content of pearl millet. The N concentration and uptake were the highest in the H_2 and P_2 with inoculation by mycorrhizae where recorded 3.88% and 245 kg N fed respectively in 3rd cut compared 1st and 2nd cuts. The raised concentration and uptake of N were found in the presence of mycorrhizae as average for the three studied cuts by 91 and 274% respectively compared with uninoculated treatment.

These results are in agreement with those found by Meftah et al., (2016). Because of Mycorrhizae plants roots hyphae can increase the branching of root system in rhizosphere so that mycorrhizae plants roots have more absorption efficiency compared to non-mycorrhizae ones. Also, the increasing in humic acid addition rates from 8 to 16 kg HA fed^{-1} led to an increase in the concentration of N by 28 to 64% and N uptake increased from 60 to 164% respectively compared with control. Similar results obtained by Tahiret al. (2011).

Although the enhanced nutrient uptake may be due to the polyelectrolyte and macro-ionic nature of HA that increases osmotic process, which in turn enhances ion exchange, improves root nutrient uptake, and increases transport through the cell membrane. However, HA is a complex source of plant nutrients and in addition, it serves as a substrate for beneficial microbes in the soil. Previous reports by Khaled and Fawy (2011), Bakhashwain et al., (2013), and Du et al., (2013) using small amounts of HA support our results, in which HA imparted a positive effect on water retention in soil, improved leaf N content and nutrient uptake, and higher rates of photosynthesis. With respect to the P concentration and uptake were the highest in the H_2 and P_2 with inoculation by mycorrhizae where recorded 0.55% and 51 kg P_2O_5 fed^{-1}. The raise concentration and uptake of P were found in the presence of mycorrhizae as average for the three studied cuts by 45 and 248% respectively compared with uninoculated treatment.

Also, the increasing in humic acid addition rates from 8 to 16 kg HA fed^{-1} led to an increase in the concentration of P by 15 to 22% and P uptake increased from 42 to 121% respectively compared with control. The results were summarize by increasing the P concentration and uptake in dry matter of pearl millet by increasing the rates of adding phosphoric acid (PA) foliarly from 0.75 to 1.5 ml/lled to an increase in the concentration of P by 19 to 34% and P uptake increased from 32 to 61% respectively compared with control.

Table 4. Effect of PA, H A and AMF levels on N and P concentration of pearl millet

AMF* (A)	H.A** Levels(B)	P.A*** Levels©	N 1st	N 2nd	N 3rd	N Means	P 1st	P 2nd	P 3rd	P Means
			(%)							
Without	H_0	P_0	0.59^{J}	0.63^{L}	0.74^{O}	0.65^{O}	0.21^{F}	0.14^{Q}	0.07^{H}	0.14^{K}
		P_1	0.61^{J}	0.84^{K}	0.80^{N}	0.75^{N}	0.43^{C}	0.15^{P}	0.15^{G}	0.24^{I}
		P_2	0.65^{IJ}	0.98^{J}	0.94^{M}	0.86^{M}	0.44^{C}	0.17^{N}	0.17^{FG}	0.26^{H}
	H_1	P_0	0.78^{H}	1.01^{J}	1.08^{L}	0.95^{L}	0.31^{E}	0.16^{O}	0.15^{G}	0.21^{J}
		P_1	0.77^{HI}	1.01^{J}	1.14^{K}	0.97^{L}	0.45^{C}	0.18^{M}	0.18^{EFG}	0.27^{GH}
		P_2	0.84^{GH}	1.20^{I}	1.28^{J}	1.10^{K}	0.54^{A}	0.20^{K}	0.20^{DEF}	0.31^{F}
	H_2	P_0	0.91^{G}	1.21^{I}	1.36^{I}	1.16^{J}	0.43^{C}	0.19^{L}	0.18^{EFG}	0.27^{GH}
		P_1	0.92^{G}	1.26^{HI}	1.67^{H}	1.28^{I}	0.43^{C}	0.21^{J}	0.20^{DEF}	0.28^{G}
		P_2	0.92^{G}	1.30^{GH}	1.92^{G}	1.38^{H}	0.49^{B}	0.32^{H}	0.21^{CDE}	0.34^{E}
With	H_0	P_0	1.15^{F}	1.30^{GH}	1.70^{H}	1.38^{H}	0.36^{D}	0.27^{I}	0.21^{CDE}	0.28^{G}
		P_1	1.25^{EF}	1.34^{FG}	1.92^{G}	1.50^{G}	0.45^{C}	0.37^{G}	0.21^{CDE}	0.34^{E}
		P_2	1.33^{DE}	1.40^{F}	2.14^{F}	1.62^{F}	0.50^{B}	0.42^{E}	0.21^{CDE}	0.38^{D}
	H_1	P_0	1.34^{DE}	1.54^{E}	2.59^{E}	1.82^{E}	0.43^{C}	0.39^{F}	0.21^{CD}	0.34^{E}
		P_1	1.36^{DE}	1.53^{E}	2.59^{E}	1.83^{E}	0.44^{C}	0.43^{D}	0.22^{BCD}	0.36^{D}
		P_2	1.44^{CD}	1.61^{D}	3.04^{D}	2.03^{D}	0.49^{B}	0.45^{C}	0.25^{B}	0.40^{C}
	H_2	P_0	1.49^{C}	1.72^{C}	3.45^{C}	2.22^{C}	0.43^{C}	0.43^{D}	0.24^{BC}	0.37^{D}
		P_1	1.81^{B}	1.88^{B}	3.49^{B}	2.39^{B}	0.52^{AB}	0.50^{B}	0.29^{A}	0.44^{B}
		P_2	1.96^{A}	2.11^{A}	3.88^{A}	2.65^{A}	0.55^{A}	0.53^{A}	0.29^{A}	0.46^{A}

*AMF= AbuscularMycorhizae Fungi; **HA= Humic acid; H_0, H_1 and H_2 means 0, 8 and 16 kg HA fed^{-1}respectively, ***PA= Phosphoric acid; P_0, P_1 and P_2 means 0, 0.75 and 1.5 ml/l respectively, across each column different superscript indicate significant ($p<0.05$) variation between the mean values

Table 5. Effect of PA, H A and AMF levels on N and P content of pearl millet

AMF* (A)	H.A** Levels(B)	P.A*** Levels©	N 1st	N 2nd	N 3rd	N Means	P 1st	P 2nd	P 3rd	P Means
			(Kg fed^{-1})							
Without	H_0	P_0	1.69^{L}	7.27^{N}	7.59^{N}	5.51^{N}	0.57^{L}	1.66^{Q}	0.73^{L}	0.99^{N}
		P_1	2.97^{L}	20.09^{M}	13.52^{M}	12.19^{M}	2.10^{K}	3.62^{P}	2.52^{K}	2.75^{M}
		P_2	4.32^{KL}	35.96^{L}	16.79^{LM}	19.02^{L}	2.87^{K}	6.44^{O}	3.10^{JK}	4.14^{L}
	H_1	P_0	5.31^{JKL}	44.82^{K}	19.91^{KL}	23.34^{KL}	2.10^{K}	7.25^{N}	2.70^{K}	4.02^{L}
		P_1	7.85^{IJKL}	47.31^{K}	21.13^{K}	25.43^{K}	4.55^{H}	8.56^{M}	3.27^{IJK}	5.46^{K}
		P_2	10.13^{IJKL}	58.07^{J}	27.94^{J}	32.05^{J}	6.53^{G}	9.61^{L}	4.35^{HI}	6.83^{IJ}
	H_2	P_0	11.43^{IJKL}	62.18^{J}	30.60^{J}	34.74^{J}	5.45^{GH}	9.66^{L}	4.08^{HIJ}	6.40^{J}
		P_1	14.04^{HIJKL}	68.42^{I}	39.92^{I}	40.79^{I}	6.52^{G}	11.63^{K}	4.86^{GH}	7.67^{I}
		P_2	14.77^{HIJ}	79.21^{H}	51.68^{H}	48.55^{H}	7.95^{F}	19.34^{I}	5.74^{G}	11.01^{G}
With	H_0	P_0	18.43^{GHI}	82.19^{H}	48.06^{H}	49.56^{H}	5.79^{GH}	16.91^{J}	5.85^{G}	9.52^{H}
		P_1	24.40^{FGH}	91.79^{G}	69.30^{G}	61.83^{G}	8.85^{F}	24.96^{H}	7.46^{F}	13.76^{F}
		P_2	27.00^{EFG}	97.00^{F}	81.87^{F}	68.62^{F}	10.05^{DE}	29.42^{F}	7.88^{EF}	15.78^{E}
	H_1	P_0	31.17^{DEF}	111.99^{E}	109.17^{E}	84.09^{E}	9.94^{DE}	28.53^{G}	8.69^{DE}	15.72^{E}
		P_1	32.69^{DE}	112.33^{E}	108.62^{E}	84.53^{E}	10.53^{DE}	31.44^{E}	9.37^{D}	17.11^{D}
		P_2	34.87^{D}	120.86^{D}	149.66^{D}	101.80^{D}	12.05^{D}	33.51^{C}	12.15^{C}	19.24^{C}
	H_2	P_0	45.96^{C}	132.06^{C}	185.37^{C}	121.13^{C}	13.20^{C}	32.72^{D}	12.72^{C}	19.54^{C}
		P_1	73.63^{B}	173.30^{B}	195.44^{B}	147.46^{B}	21.18^{B}	46.25^{B}	16.27^{B}	27.90^{B}
		P_2	104.83^{A}	204.51^{A}	244.70^{A}	184.68^{A}	29.12^{A}	51.20^{A}	18.31^{A}	32.88^{B}

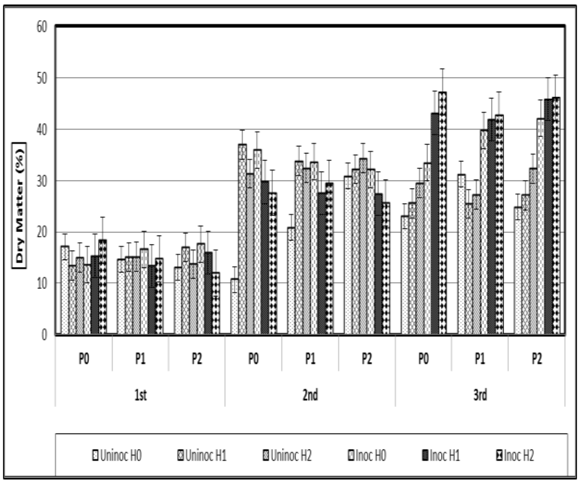
*AMF= Abuscular Mycorhizae Fungi; **HA= Humic acid; H_0, H_1 and H_2 means 0, 8 and 16 kg HA fed^{-1}respectively, ***PA= Phosphoric acid; P_0, P_1 and P_2 means 0, 0.75 and 1.5 ml/l respectively, Across each column different superscript indicate significant ($p<0.05$) variation between the mean values.

These results are confirmed by those obtained by Abou-Aly and Mady (2009) and Ali, et al. (2009).

In general, it is obvious from that application of bio-fertilizer, humic and phosphoric acid gave considerable improvement in nutrients uptake of straw millet when compared with control. In this respect Daur and Bakhashwain (2013) and Daur, (2014) documented that enhanced uptake of macronutrients (NP) was due to the stimulatory effect of humic substances. Recently, Tuba, et al., (2015) added that application of humic acid showed promising effects on nutrient contents of millet shoot**.**

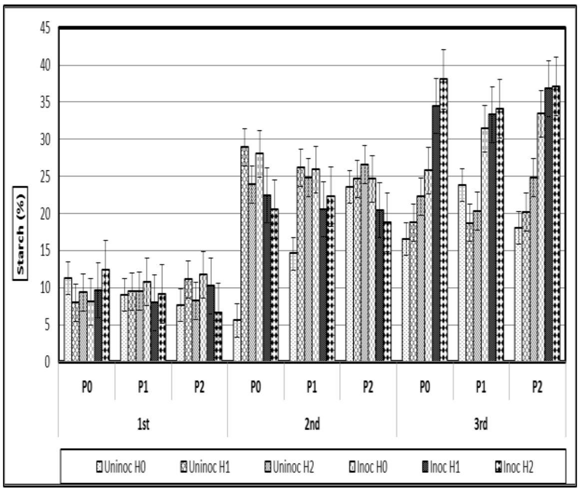
Starch and dry matter content of millet

Regarding total dry matter and starch in forage yield, the same positive trend on straw was observed with application of himic and phosphoric acid fertilizer as a compared with unfertilized plant. Starch and dry matter content in dry forage yield were significantly influenced by mineral phosphorus, humic acid and mycorrhizae (Fig. 1&2).However, such effect was obvious by humic acid application. The maximum dry matter and starch was (47.2 and 38.07%) respectively obtained by applying humic acid with rate of 16 kg HA fed^{-1}. These results were in full agreement with those obtained by Khaled and Fawy (2011), Abou-Aly and Mady (2009) and Tuba Arjumend et al. (2015).



*AMF= Abuscular Mycorrhizae Fungi; means without and with inoculation respectively, **HA= Humic acid; H_0, H_1 and H_2 means 0, 8 and 16 kg HAfed^{-1} respectively, ***PA= Phosphoric acid; P_0, P_1 and P_2 means 0, 0.75 and 1.5 ml/l, respectively, Dry Matter (%), 1st, 2nd and 3rd cuts.

Fig. 1. Effect of PA, H A and AMF levels on dry matter content of millet

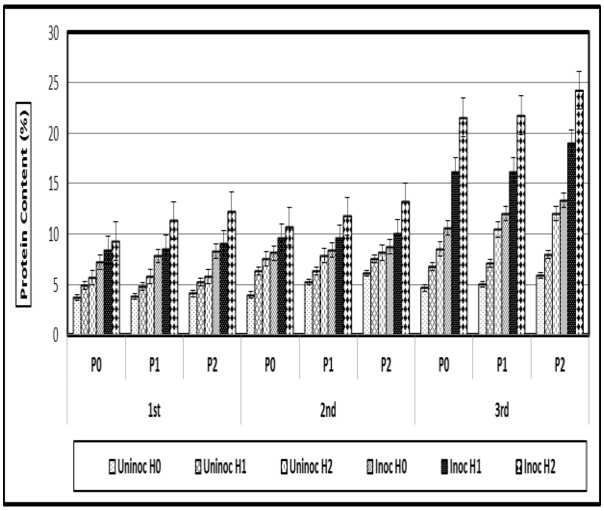


*AMF= Abuscular Mycorhizae Fungi; means without and with inoculation respectively, **HA= Humic acid; H_0, H_1 and H_2 means 0, 8 and 16 kg HA fed^{-1}respectively, ***PA= Phosphoric acid; P_0, P_1 and P_2 means 0, 0.75 and 1.5 ml/l, respectively, Starch (%), 1st, 2nd and 3rd cuts.

Fig. 2. Effect of PA, H A and AMF levels on starch content of millet

Protein content of millet

Crude protein (CP) content (%) was calculated based on nitrogen content of the dry matter, using the equation as follows: CP (%) = nitrogen (%) × 6.25. The protein content of forage yield is an indicator of quality characteristics. The effect of the mixture of mineral phosphorus, humic acid and inoculated with mycorrhizae on the protein content was calculated (Fig. 3).



*AMF= Abuscular Mycorhizae Fungi; means without and with inoculation respectively, **HA= Humic acid; H_0, H_1 and H_2 means 0, 8 and 16 kg HA fed^{-1}respectively, ***PA= Phosphoric acid; P_0, P_1 and P_2 means 0, 0.75 and 1.5 ml/l, respectively, Protein (%), 1st, 2nd and 3rd cuts.

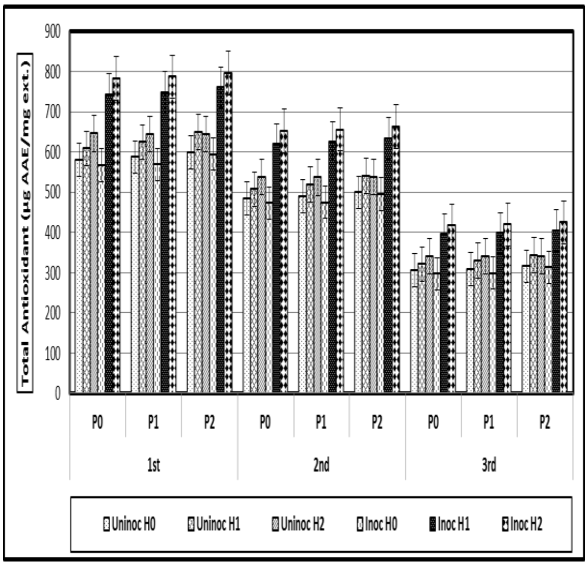
Fig. 3. Effect of PA, H A and AMF levels on the protein content

Increased rates of addition of humic and phosphoric acid with mycorrhizae treatments increased the protein content. Whereas, inoculation with MAF increased protein by 91.5% compared to without. Also, increasing the addition of humic acid from 8 to 16 kg feddan^{-1} increased the biochemical content of protein by 28.8 to 63.8%, respectively. The results also indicated that the protein increased in succession with the increase of phosphoric acid spray by 20.8% compared to the lower rates.This result agrees with Daur (2014). Because of the mycorrhizal hyphae to the plant roots, besides mobile elements as N (Grant et al., 2005), Kanwal et al. (2015) suggested that colonization of wheat plants with AM fungi improved biomass, growth and essential nutrients availability. Humic is due to their high nutrient content, huge surface area, active bio-stimulants, stability against microbial decomposition and a high number of active ingredients and functional groups, (Mosa et al. 2020).

Total antioxidant activity of millet

Figure 4 indicates that increasing rates of humic and phosphoric acid addition with mycorrhizae treatments increased total antioxidantActivity (TAA) of leaf. TAA recorded the highest in the H_2 and P_2 with inoculation about796, 663 and 425(µgAAE/mg ext.) in 1st, 2nd and 3rd cuts, respectively. Mycorrhizae raised TAA as about 13.8% compared unioculaed treatment. These results agree with Hashem et al. (2015a) and (2015b) because AMF increased their growth under salt-stressed conditions.

Increasing the addition of humic from 8 to 16 kg fed^{-1} improves the biochemical content as TAC increased by 18.4 to 23.23% respectively.The effect of humic substances in the alleviation of abiotic stress effects in plants is ascribed to the elevation in enzymatic and non-enzymatic antioxidants, formation of compatible solutes and changes in ionic balance. In various abiotic stresses, reactive oxygen species (ROS) are one of the significant causes responsible for cellular damage (Kıran, et al., 2019 and Rehab et al., 2020). It may also enhance the foliar application of phosphorous (P) enhances leaf area index and plant biomass and reduces salinity-induced damages (Khan, et al., 2013).



*AMF= AbuscularMycorhizae Fungi; means without and with inoculation respectively, **HA= Humic acid; H_0, H_1 and H_2 means 0, 8 and 16 kg HA fed^{-1}respectively, ***PA= Phosphoric acid; P_0, P_1 and P_2 means 0, 0.75 and 1.5 ml/lrespectively, Total Antioxidant (µgAAE/mg ext.), 1st, 2nd and 3rd cuts.

Fig. 4.Effect of PA, H A and AMF levels on the total antioxidantactivity

Effect of Humic acid and inoculated with micorrhiza fungi on soil properties

Data in Table 6 showed that micorrhizal fungi and humic acid positive effect on some soil properties. Increasing of humic acid with inoculate of seeds with micorrhizae reduced pH from 8.32 to 7.96, caused to decrease to EC due to activate role as produce some acids which help roots to soluble and absorption of many nutrient, to the plant through all stages growth. The increased P content in different millet parts with humic acid application maybe due to the fact that humic acid increased phosphorus availability and uptake. The increase in the addition of humus and phosphoric acid in the presence of the microrhiza increased the ability of the plant to produce antioxidants against salt stress, and the action of humic to retain salt and improve soil properties against the accumulation of salts around the plant root, which may cause stress and reduce production. These data agree with the finding of (Guppy, et al., 2005). Bans Bulent et al. (2009) and Tahir, et al., (2011) who reported that humic acid significantly improved millet potassium content under calcareous soil.

Table 6. Some properties of the experimental soil after harvesting

Treat.	Soil Texture	pH	EC (dSm^{-1})	$CaCO_3$(g kg^{-1})	OM(g kg^{-1})	Available nutrients ($mgkg^{-1}$) N	P	Alkaline phosphatase (µgP.nitrophenol/ ml/hr)
T1	SCL	8.32	1.24	365	7.8	12.3	2.76	10.2
T2		8.19	0.81	364	10.5	13.3	5.86	10.2
T3		8.11	0.79	365	12.6	14.0	8.11	13.5
T4		8.13	0.71	363	14.7	12.8	4.8	82.2
T5		8.08	0.71	361	14.7	18.2	7.94	87.2
T6		7.96	0.69	360	17.4	18.2	11.3	89.3

pH= Acidity; E.C = Electrical conductivity in extract soil (1:5); OM= Total Organic Matter (g kg^{-1});SCL= Sandy Clay Loam and SAR= Sodium Adsorption Ratio. T_1= without inoc. + H_0, T_2= without inoc. + H_1, T_3= without inoc. + H_2, T_4= with inoc. + H_0, T_5= with inoc. + H_1 and T_6= with inoc. + H_2.

It was observed that increasing N and P application to the soil highly increased N and P content in soil than the control. Also, increase of N and P content in soil was increasing by humic acid application. Maximum soil N and P available content were18.2 and 11.3mg kg^{-1} by applying 16 kg HA/fed. However, soil N and P were declined gradually as a result of plant uptake of N and P throughout the growing season and due to increase of millet of green fodder yield in the first cutting than the second and third cuttings. Also, application of P foliarlyshowed positive effect on soil N and P content (Hassan, 2003, Abou-Amer, 2007 and Mohamed, 2020).

Phosphorus soil content was increased with inoculated MFA application. This may be due to the low P content of calcareous soil. Micorrhiza caused to phosphatase enzyme by 89 Alkaline phosphatase (µg P. nitrophenol/ml/hr). This may be due to the absorption of the plant to available P soil and regrowth of millet (Berry and Miller, 1989). Arbuscular Mycorrhizal Fungi (AMF) plays an important role in vegetation restoration because of symbiosis with plant root; they can facilitate mineral absorption by host plant, stability and improve soil structure, affect the population structure and preserve species diversity (Bothe et al., 2010).

However, the highest rates of P in calcareous soil enhance P availability; because the lime in calcareous soil reacts with soil solution P to form a strong calciumphosphate bond at the surface of the lime. Therefore, high P fertilizer rates are required for crops grown in calcareous soil, with increasing rates needed as limecontent in these soils increases (Bryan and Jason, 2005). Also, the positive effect of HA applications increase by theavailably P in soil. In this respect, Gahoonia et al. (1992), studied soil P release in the rhizosphere of ryegrass and found that, under NH4-N nutrition, soil P depletion in the vicinity of roots was also correlated with the pH decrease in calcareous soil.

4. Conclusions

From the obtained results it can be concluded that application of humic acid and bio-fertilizers has promoting effect on most yield components. Humic acid application can lessen the mineral fertilizers need for and subsequently reduce environmental pollution. Finally, it can be said that application of humic, phosphoric acid and inoculated by mycorrhizal fungi applying not only increases the yield of forage millet yield, but also millet quality reflexed by high content of starch, dry matter and protein content of strawmillet. Phosphorus foliarly sprays as phosphoric acid form can decrease of phosphorus fixation in some soil. It can play a significant role in achieving the goals of sustainable agriculture in new reclaimed sandy soils.

Overall, this study indicates that the application doses are important for deriving benefit from humic substances under sand soils. These results indicate that the highest yield and yield components revealed byfoliar application of 1.5ml PA/l, 16kghumic acid/ fed with inoculated with mycoorhizae. The increase in the addition of humus and phosphoric acid in the presence of the microrhiza increased the ability of the plant to produce antioxidants against salt stress, and the action of humic to retain salt and improve soil properties against the accumulation of salts around the plant root, which may cause stress and reduce production.

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استجابة الدخن الى التسميد بالفوسفور المعدنى وحامض الهيومك والميكورهيزا تحت ظروف الاراضى الجيرية

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أقيمت تجربة حقلية بمحطة بحوث مريوط على بعد 40 كم جنوب غرب الاسكندرية وهى تابعة لمركز بحوث الصحراء – مصر. لدراسة مدى إستجابة نبات الدخن للتسميد بالفوسفور والهيوميك ولقاح الميكروهيذا.

وقد اوضحت النتائج أن زيادة التسميد بحامض الهيوميك يزداد محصول العلف الناتج من وحدة المساحة كما اوضحت أيضا ان هناك علاقة طردية بين زيادة الانتاج بزيادة الاضافات من حامض الفوسفوريك فى وجود لقاح الميكروهيذا . لذلك فان المعدل 16 كجم حمض الهيوميك و 1.5 مل حامض فوسفوريك/لتر فى وجود لقاح الميكروهيذا اعطت أعلى إنتاج من محصول العلف الاخضر والجاف وصل 80.9 و 21.3 طن للفدان على التوالى خلال الثلاث حشات . كما ارتفعت تركيزات البروتين الى 12.3 ، 13.2 و24.3% خلال الحشة الاولى والثانية والثالثة على التوالى . ايضا ارتفع تركيز النشا والماده الجافة بشكل ملحوظ نتيجة الدور الفعال الذى تقوم به الميكروهيذا من تيسر الفوسفور بشكل مباشر ومساعدة النبات على امتصاص العناصر الغذائية الاخرى بشكل غير مباشرتوافقا مع الدور الذى يقدمة حامض الهيوميك من تحسين الخواص الطبيعية والكيميائية مما ينعكس اثره على النبات ومن ثم الانتاجية لذلك فان المعاملات التى تحظى بارتفاع ملحوظ فى كمية وجوده الانتاج تحظى ايضا باضافات اعلى من الهيوميك والفوسفور فى وجود فطر الميكورهيذا .كما لوحظ ايضا ارتفاع فى تركيز مضادات الاكسده فى اوراق الدخن بزيادة الاضافات السمادية من الهيوميك والفوسفور فى وجود الميكورهيذا مقارنتا بالكنترول حيث عملتتلك المعاملات نحو دفع النبات نحو زيادة الامتصاص من العناصر المغذية فى محلول التربة و احتباس الماء في الأوراق والتمثيل الضوئي والتمثيل الغذائي تحت ضغط الماء الملحى. حيث زادت المواد الدبالية من كثافة الجذور وتمتص المغذيات عبر أغشية البلازما للجذور.