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Effect of Arbuscular Mycorrhizal Fungi (AMF) on Fatty Acid Contents of Selected Sunflower (*Helianthus annuus* L.) Hybrids under Various Levels of Rock Phosphate

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Abstract: The experiment was carried out in a net house of Department of Botany, University of Peshawar Pakistan, to find out the effects of arbuscular mycorrhizal fungi (AMF) along with the application of various levels of rock phosphate (RP) fertilizer on fatty acid contents of selected sunflower hybrids (*Helianthus annuus* L.) in P-deficient soil. Results showed that the plants with mycorrhiza and without rock phosphate fertilizer (RP-0) and plants at low RP levels both had significantly better performance as compared to non-inoculated plants in terms of level of unsaturated fatty acid like linoleic acid and oleic acid. But at high RP level (RP3) the non mycorrhizal plants had low fatty acid contents. It is clear that the combination of AMF and different levels of RP enhanced the fatty acid contents of sunflower. Similarly, the mycorrhizal response varied with increasing rock phosphate in different hybrids.

Key words: AMF · Rock phosphate · Fatty acid contents · Sunflower hybrids

INTRODUCTION

Use of Chemical fertilizers are detrimental to the soil, as over usage of chemical will destroy soil microorganisms, killing the soil overtime which become sterile. The erosion of the soil in agricultural environment is mostly due to the overuse of chemicals. Chemical fertilizers also have huge deficiencies in many micro-nutrients, particularly trace elements.

Mycorrhiza is universal mutualistic associations between soil fungi and vascular plants and is essential in improving plant growth and soil quality. It improves the resilience of plant communities against environmental, nutritional and drought stresses [1].

The Arbuscular Mycorrhiza Fungus (AMF) is a biological mutualistic association between fungus of the phylum Glomeromycota and roots of higher plants [2]. AMF association plays an integral role in growth and development of most of the agricultural plants [3-8] especially in phosphorus deficient soils. AMF is the common biotic factor of ecosystem which establishes symbiotic relationship with terrestrial plants and increase plant growth, plant protection, quality of the soil and mineral and water uptake [9, 10].

Sunflower (*Helianthus annuus* L.) is one of the important oilseed crop in the world and ranks fourth in production of vegetable oil [11]. Pakistan is the third largest importer of edible oil in the world [12]. Sunflower is cultivated on more than 22 million hectare (ha) of land worldwide with production of 26 million tones of seeds [13]. In Pakistan sunflower is cultivated on 0.7 million hectares area with production of 144 thousand tons of edible oil [14].

In the Khyber Pakhtunkhwa province sun flower was cultivated on of 559 ha with production of 1608 kg/ha during years 2009-10 [15].

All plants need phosphorus during growth period, since it is essential component of cell membrane, nucleic acids (DNA, RNA), ATP, co-enzymes, phospholipids and phosphoproteins and it plays vital role in many metabolic activities like energy transfer, photosynthesis and respiration etc [16].

Soils of Pakistan like most of the arid and semiarid soils of world are mostly Phosphorus (P) deficient due to their alkaline and calcareous nature, affecting plants adversely [17, 18[. Phosphorus contents of an average soil is about 0.05%, out of which only 0.1% of the total P is brought in use by the plants because of its low

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solubility. To overcome this problem plants have adopted different strategies to acquire sufficient phosphorus. Arbuscular mycorrhizal fungal association is one among these adaptations [19, 20]. Due to scarcity of phosphorus content in the soil and its rapid utilization, efforts are being made to supplement plants with low grade rock phosphate. Pakistan is rich in rock phosphate is mineral. It is in the form of tri-calcium phosphate which is insoluble form and is not directly available to the plants as such, unless it is converted into soluble form [21]. Mycorrhizal inoculation can help plants by solubilizing soil rock phosphate into available forms, which helps in plant growth [22]. Throughout the world scientists are now focused on developing alternative technologies to minimize dependence on chemical fertilizers. Although remarkable research work has been done on various aspects of AM, but in Asian countries including Pakistan, oil yield and fatty acid profile of oil seed crops are least addressed. It is therefore needed not only to introduce high yielding hybrids of sunflower in the country, but also to apply various manipulations, including use of AMF to increase edible oil production Therefore, in the present research work it is intended to evaluate the role of AM fungal technology on oil yield of selected sunflower (*Helianthus annuus* L.) hybrids at various levels of rock phosphate.

MATERIALS AND METHODS

The study was conducted at the Department of Botany, University of Peshawar. Authentic seeds of four hybrids of sunflower i.e NKS-278, Hysun-33, SMH-0917 and SMH-0907 were obtained from Oilseeds Research Program, NARC Islamabad, Pakistan. The soil used was sandy loam with pH 7.8, electric conductivity 0.675 ds/m^2, Nitrogen 0.032% and Phosphorus 0.8mg/kg with low organic matter (0.6%). All 96 pots having 89 cm diameter and 48 cm depth were filled with 6 Kg of this nutrient deficient soil. The field experimental work was carried out in a randomized complete design (RCD) along with eight treatments; each treatment was replicated three times with five plants in each pot. The test pots were inoculated with soil containing spores of different AMF species i.e. *Glomus fasciculatum, G.mosseae, G.aggregatum, Sclerocystis pakistanica*, *Gigaspora gigantea* along with roots of wheat and maize, infected with arbuscular mycorrhiza. Mycorrhizal inoculum preparation, placement and application were done by the method given by Brundrett *et al.* [23]. Fertilizers were applied by following method of Krishna & Bagyaraj [24]. Urea (100 mg/ pot) as nitrogen source was added to all the pots irrespective of treatments. Moreover 20ml of Hoagland nutrient solution without phosphorus (P) was provided to all pots 15 days after seed sowing. Rock phosphate fertilizer (P_2O_5) was obtained from Hazara deposits. Four levels of rock phosphate fertilizer 0%, 25% (100mg P_2O_5/kg soil), 50% (200mg P_2O_5/ kg soil) and 100% (500mg P_2O_5/kg soil) RP recommended (Recommended dose=80kg P_2O_5/ha.) were applied in combination with or without AMF. After harvesting fatty acid composition and oil content analysis of whole seed samples were carried out by Near Infrared Reflectance Spectroscopy model 6500 visible (Foss NIR Systems Inc. Silver Spring MD) at National Institute of Food And Agriculture, Tarnab, Peshawar by using standard method of Association of Official Analytical Chemists [25]. Experimental data was statistically analyzed by applying ANOVA test, procedures outlined by Steel and Torrie [26] and Least Significant Difference (LSD) was used for any significant difference among the treatments.

RESULTS AND DISCUSSION

In the present study four hybrids of sunflower (*Helianthus annuus* L.) were grown with and without mycorrhiza at various levels of rock phosphate (P fertilizer) to study their response to AMF inoculation. The response varied in different hybrids which are in accordance with [27, 28].

The results presented in (Tables 1-5) showed that mycorrhizal colonization enhanced the unsaturated fatty acid contents as compared to non-mycorrhizal control plants. However, results at various rock phosphate levels varied. The mycorrhizal plants showed significant differences at $P<0.05$ as compared to non-mycorrhizal plants at RP0 (control) and at low levels (RP1) of rock phosphate (RP) application, while non-significant differences were observed at higher RP (RP2 & RP3) levels.

Fatty Acid Profile

Unsaturated Fatty Acids: The results showed that the use of AMF along with low doses of rock phosphate promote monounsaturated (oleic acid) and polyunsaturated fatty acids (linoleic acid) which are more beneficial for health as they are responsible for lowering “bad” LDL cholesterol (low density lipoproteins) and simultaneously increasing the "good" HDL cholesterol (high density lipoprotein), while high levels of rock phosphate is antagonistic to AMF.

Appendix 1: ANOVA table for linoleic acid of *Helianthus annuus* L.

SOV	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
Replication	2	0.250	0.125	0.1216	
Hybrid (H)	3	48.018	16.006	15.5666	0.0000
Treat. (T)	7	309.794	44.256	43.0414	0.0000
H x T	21	187.085	8.909	8.6642	0.0000
Error	62	63.750	1.028		
Total	95	608.897			

Coefficient of Variation: 1.40%

Table 1: Linoleic acid (%) in different hybrids of *Helianthus annuus* L. at various levels of rock phosphate (RP). Each value is a mean of three replicates. Values followed by different letters are significantly different ($p < 0.05$)

Treatment	Percent (%) Rock phosphate levels	Hybrids NKS-278	Hysun-33	SMH-0917	SMH-0907	Means T
		HxT				
M	RP0	72.10^{fghi}	72.10^{fghi}	71.70^{hij}	68.70^{lm}	71.15^{cd}
	RP1	71.50^{hijk}	75.7^{abc}	74.30^{bcde}	75.30^{abcd}	74.2^{b}
	RP2	72.00^{ghi}	74.10^{bcde}	73.90^{cde}	74.30^{bcde}	73.57^{b}
	RP3	68.00^{mn}	72.90^{efgh}	73.80^{cde}	71.50^{hijk}	71.55^{c}
NM	RP0	72.00^{ghi}	70.30^{jkl}	71.60^{hijk}	67.00^{n}	70.22^{e}
	RP1	70.00^{kl}	73.40^{efg}	70.70^{ijk}	67.80^{mn}	70.47^{de}
	RP2	74.3^{a}	74.30^{bcde}	74.20^{bcde}	75.30^{abcd}	74.2^{a}
	RP3	74.20^{bcde}	73.80^{cde}	74.00^{ab}	73.70^{def}	73.92^{ab}
	Means(H)	71.6^{b}	73.32^{a}	73.02^{a}	71.70^{b}	

LSD value at 5% level of significance for factor H= 0.5851, for factor T =0.8274 and for H x T interaction=1.655

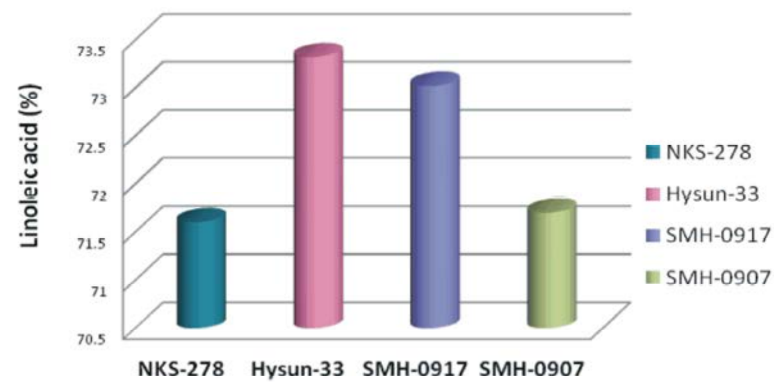

Fig. 1a: Linoleic acid content (%) in different hybrids of *Helianthus annuus* L.

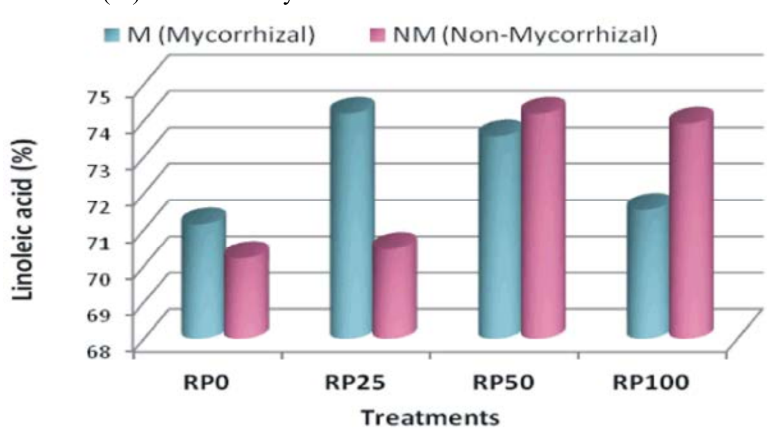

Fig. 1b: Effect of RP treatments on linoleic acid (%) in four hybrids of *Helianthus annuus* L.

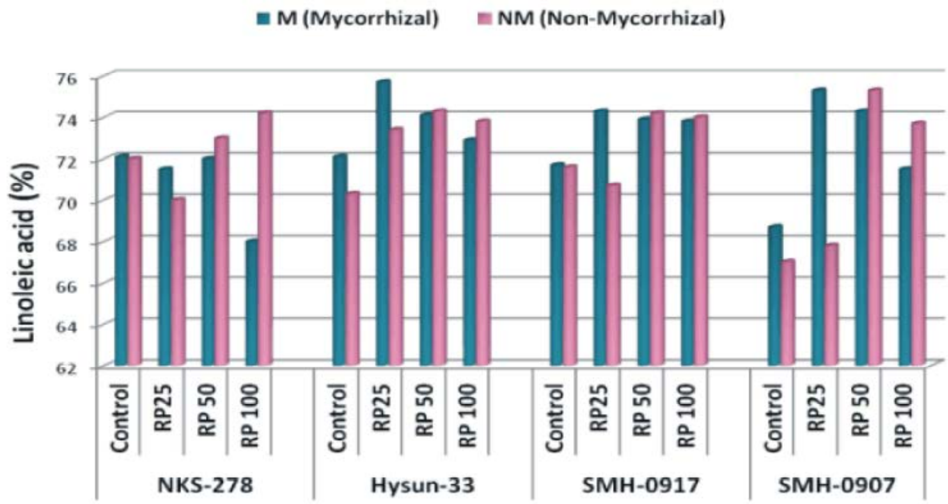

Fig. 1c: Effect of mycorrhiza on linoleic acid (%) under different rock phosphate levels in four hybrids of *Helianthus annuus* L.

Linoleic Acid (C18:2): The results showed that linoleic acid content was significantly affected for four hybrids of sunflower (*Helianthus annuus* L.) in hybrids, treatments and interaction between hybrids and treatments at various levels of rock phosphate in mycorrhizal and non-mycorrhizal plants, while the effect of replications was found to be non-significant (Appendix 1). The results showed that among control plants (Table-1 and Fig. 1b) mycorrhiza plants performed better (71.15%) as compared to non-mycorrhizal plants (70.22%) regarding linoleic acid content. Increased linoleic acid content in mycorrhizal plants has also been reported by Abdullah *et al*. [29]. Statistical analysis of data also showed that rock phosphate levels had significant effect on linoleic acid content in sunflower. It is clear from mean data that among the mycorrhizal and non-mycorrhizal treatments at rock phosphate levels showed significant increase in linoleic acid content over the control. However, at low level of rock phosphate (RP1) the mycorrhizal plants showed maximum (74.12 %) linoleic acid as compared to non- mycorrhizal plants (70.47%), while at RP2 and RP3 levels the non-mycorrhizal plants out performed (Table 1 and Fig. 1b). Hybrids also exhibited significant response in terms of linoleic acid i.e Hysun-33 has higher (73.32%) linoleic acid content followed by SMH-0917 (73.02%), SMH-0907 (71.70 %) and NKS-278 (71.6%) and (Table 1 and Fig. 1a). However, interaction data revealed that mycorrhizal response varied with increasing rock phosphate in different hybrids. Maximum linoleic acid (%) was recorded for Hysun-33 (75.7%) followed by SMH-0907 (75.30%) and SMH-0917 (74.30%) at RP1 l evel, while least was recorded for NKS-278 (72.10%) at RP0 as compared to non-mycorrhizal plants.

Oleic Acid (C18:1 cis-9): The results for oleic acid of four hybrids of sunflower (*Helianthus annuus* L.) following different treatments showed that the effect among treatments and interaction between hybrids and treatments were highly significant $P < 0.0008$ and $P < 0.0005$ respectively (Table-2 and Fig. 2c), regarding oleic acid at various levels of rock phosphate in mycorrhizal and non-mycorrhizal plants, while the effect among replications and hybrids were found to be non-significant (Appendix 2). Among the control plants (Table 2, Fig. 2b) mycorrhiza plants performed better (17.02%) as compared to non-mycorrhizal plants (14.47%) regarding oleic acid content. The maximum amount of oleic acid was recorded in RP1 (17.05%) which is statistically at par with RP0 (17.02%). However at high levels of rock phosphate (RP2 & RP3) reverse result noticed i.e. the non-mycorrhizal plants performed better than mycorrhizal plants (Table 2 and Fig. 2b), as high levels of rock phosphate is antagonistic to AMF. Similarly, among the non-mycorrhizal treatments at various rocks phosphate levels results showed significant increase in oleic acid content over the control. However the response varied in different hybrids (Table-2 and Fig. 2b & c). Findings of Khan *et al*. [30] and Zakaria *et al*. [31] also support the present study.

Appendix 2: ANOVA table for oleic acid of *Helianthus annuus* L.

SOV	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
Replication	2	0.438	0.219	0.1950	
Hybrid (H)	3	8.875	2.958	2.6368	0.0575
Treat. (T)	7	32.789	4.684	4.1749	0.0008
H x T	21	69.252	3.298	2.9392	0.0005
Error	62	69.563	1.122		
Total	95	180.917			

Coefficient of Variation: 6.72%

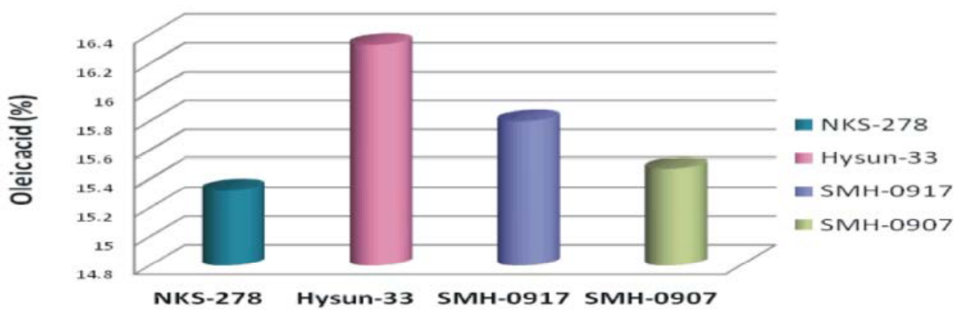

Fig. 2a: Oleic acid (%) in different hybrids of *Helianthus annuus* L.

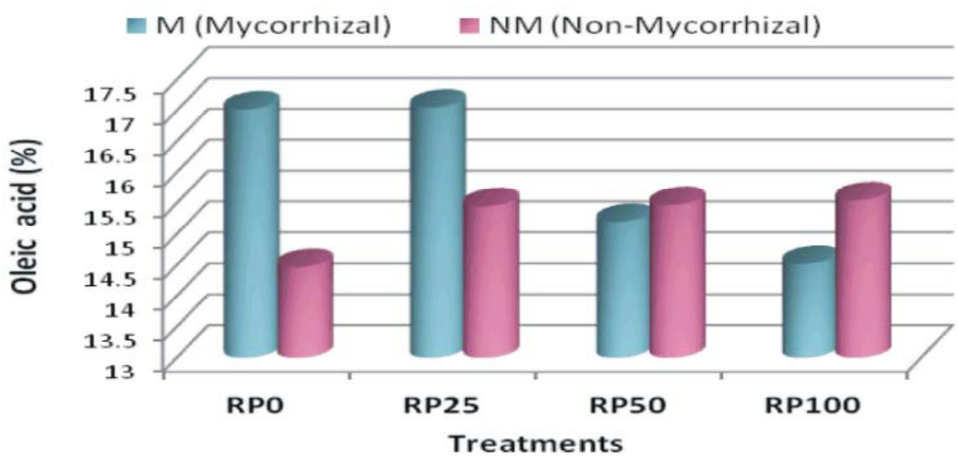

Fig. 2b: Effect of RP treatments on Oleic acid (%) in four hybrids of *Helianthus annuus* L.

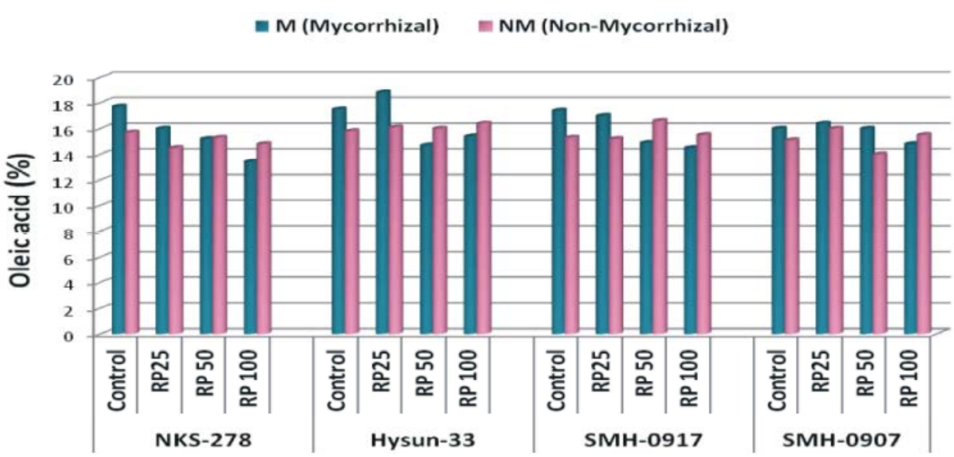

Fig. 2c: Effect of mycorrhiza on Oleic acid (%) under different rock phosphate levels in four hybrids of *Helianthus annuus* L.

Table 2: Oleic acid (%) in different hybrids of *Helianthus annuus* L. at various levels of rock phosphate (RP). Each value is a mean of three replicates. Values followed by different letters are significantly different ($p < 0.05$)

Treatment	Percent (%) Rock phophate levels	Hybrids NKS-278	Hysun-33	SMH-0917	SMH-0907	Means T
		HxT				
M	RP0	17.7 a	17.5 ab	17.4 abc	16 bcd	17.02 ab
	RP1	16 bcdefgh	18.8 a	17 bcd	16.4 bcdef	17.05 a
	RP2	15.2 efghi	14.7 hij	14.9 fghij	16 bcdefgh	15.2 cd
	RP3	13.4 j	15.4 defghi	14.5 hij	14.8 ghij	14.52 d
NM	RP0	15.7 cdefghi	15.8 bcdefgh	15.3 defghi	15.1 efghij	14.47 de
	RP1	14.5 hij	16.1 bcdefgh	15.2 efghi	16 bcdefgh	15.45bcde
	RP2	15.3 defghi	16 bcdefgh	16.6 bcde	14 ij	15.47bcd
	RP3	14.8 ghij	16.4 bcdefg	15.5 defghi	15.5 defghi	15.55 bc
	Means(H)	15.32	16.33	15.8	15.47	

LSD value at 5% level of significance for factor T = 0.8644 and for H x T interaction= 1.729

Appendix 3: ANOVA table for palmitic acid of *Helianthus annuus* L.

SOV	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
Replication	2	2.215	1.108	1.1351	0.3280
Hybrid (H)	3	0.177	0.059	0.06	
Treat. (T)	7	11.015	1.574	1.6126	0.1486
H x T	21	25.148	1.198	1.2273	0.2617
Error	62	60.498 0.976			
Total	95	99.053			

Coefficient of Variation: 25.77%

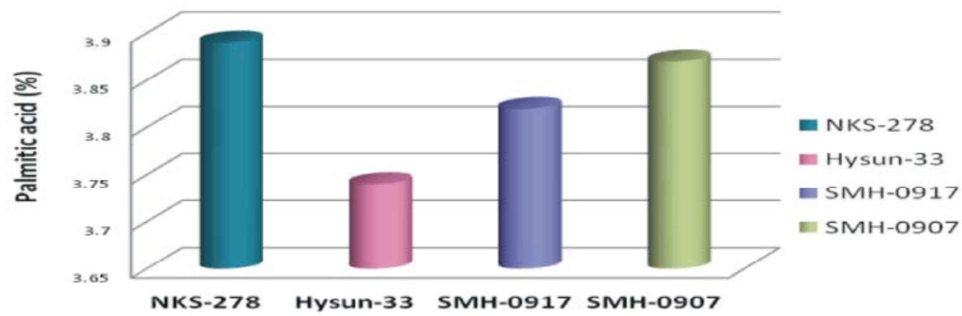

Fig. 3a: Palmitic acid (%) in different hybrids of *Helianthus annuus* L.

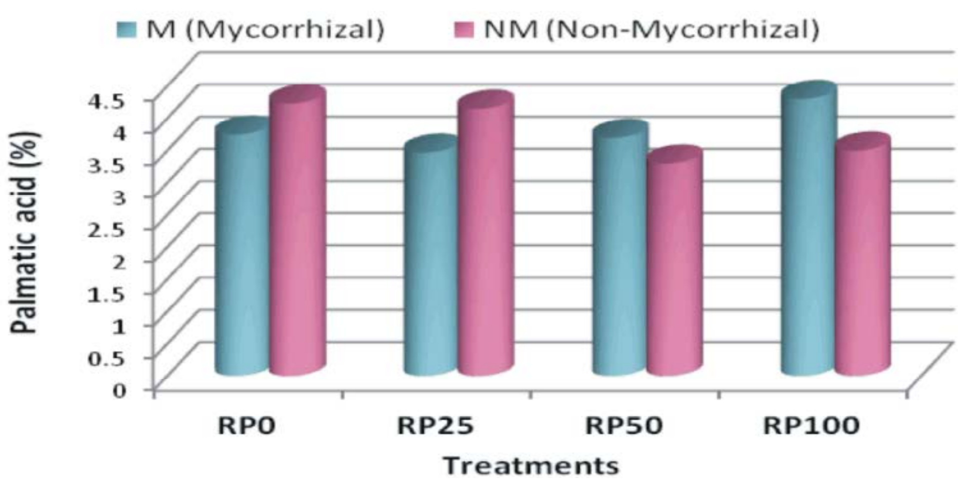

Fig. 3b: Effect of RP treatments on Palmitic acid (%) in four hybrids of *Helianthus annuus* L.

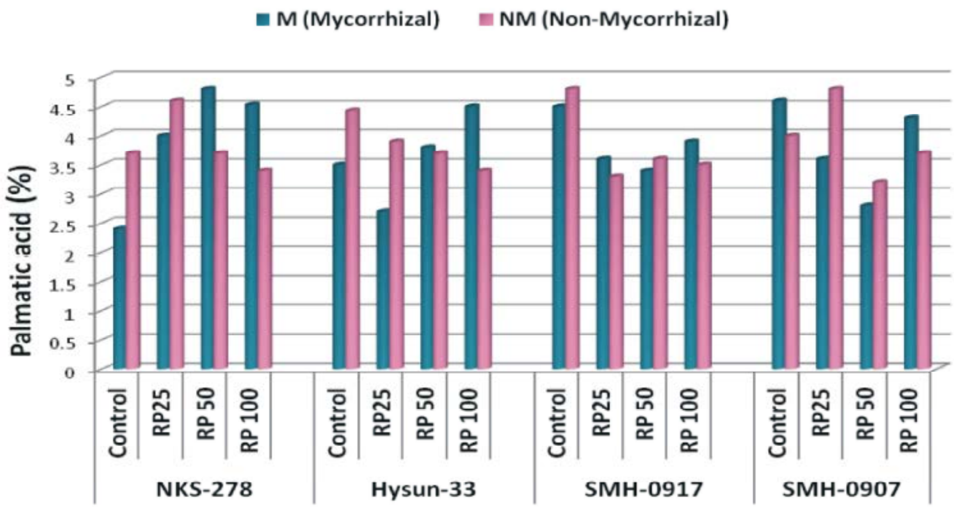

Fig. 3c: Effect of mycorrhiza on Palmitic acid (%) under different rock phosphate levels in four hybrids of *Helianthus annuus* L.

Table 3: Palmitic acid (%) in different hybrids of *Helianthus annuus* L. at various levels of rock phosphate (RP). Each value is a mean of three replicates. Values followed by different letters are significantly different ($p < 0.05$).

Treatment	Percent (%) Rock phosphate levels	Hybrids NKS-278	 Hysun-33	 SMH-0917	 SMH-0907	Means T
		HxT				
M	RP0	2.40	3.50	4.50	4.60	3.75
	RP1	4.00	2.70	3.60	3.60	3.47
	RP2	4.80	3.80	3.40	2.80	3.70
	RP3	4.53	4.50	3.90	4.30	4.30
NM	RP0	3.70	4.43	4.80	4.00	4.23
	RP1	4.60	3.90	3.30	4.80	4.15
	RP2	3.70	3.70	3.60	3.20	3.30
	RP3	3.40	3.40	3.50	3.70	3.50
	Means(H)	3.89	3.74	3.82	3.87	

Saturated Fatty Acids: The use of AMF along with high doses of rock phosphate brought increase in the production of saturated fatty acids like Palmitic acid and Stearic acid in sunflower seeds. This research work has substantial health significance as saturated fatty acids are less beneficial for health as they are responsible for increasing "bad" LDL cholesterol (low density lipoproteins).

Palmitic Acid (C16:0): The show that effect among replications, hybrids, treatments and interaction between hybrids and treatments were non-significant regarding palmitic acid at various levels of rock phosphate in mycorrhizal and non-mycorrhizal plants (Appendix 3), as recorded values are statistically at par with each other in terms of Palmitic acid (Table 3 and Fig. 3 a & c). As evident from the result (Table 3) that non-mycorrhizal plants (4.23%) showed better response as compared to mycorrhizal control plants (3.75%) regarding the palmitic acid content.

The perusal of treatment means data (Table 3 and Fig. 3b) regarding Palmitic acid revealed that the mycorrhizal responses were most pronounced at high level of applied fertilizer (RP2 & RP3). However at RP0 & RP1 levels of rock phosphate the non-mycorrhizal plants outperformed (Table 3, Fig. 3b).

Stearic Acid (C18:0): The results showed that presence of Stearic acid was significantly affected among treatments and interaction between hybrids and treatments $P<0.0008$ and $P<0.0037$ respectively, at various levels of rock phosphate in mycorrhizal and non-mycorrhizal plants, while the effect among replications and hybrids were found to be non-significant (Appendix 4).

Appendix 4: ANOVA for Stearic acid of *Helianthus annuus* L.

SOV	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
Replication	2	1.273	0.637	0.6407	
Hybrid (H)	3	5.344	1.781	1.7928	0.1578
Treat. (T)	7	28.958	4.137	4.1637	0.0008
H x T	21	50.554	2.407	2.4229	0.0037
Error	62	61.600	0.994		
Total	95	147.729			

Coefficient of Variation: 18.11%

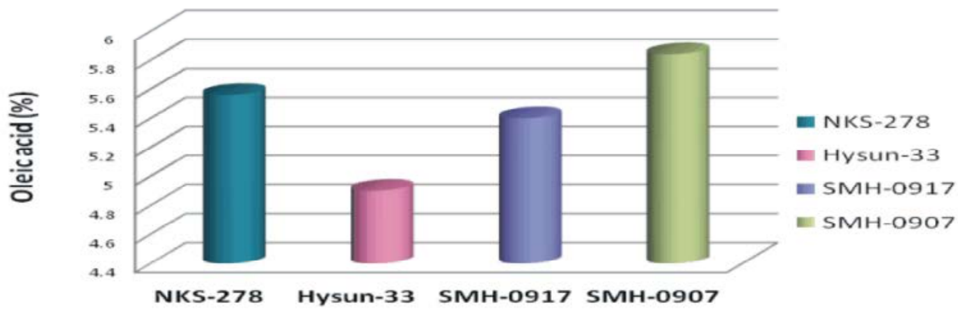

Fig. 4a: Stearic acid (%) in different hybrids of *Helianthus annuus* L.

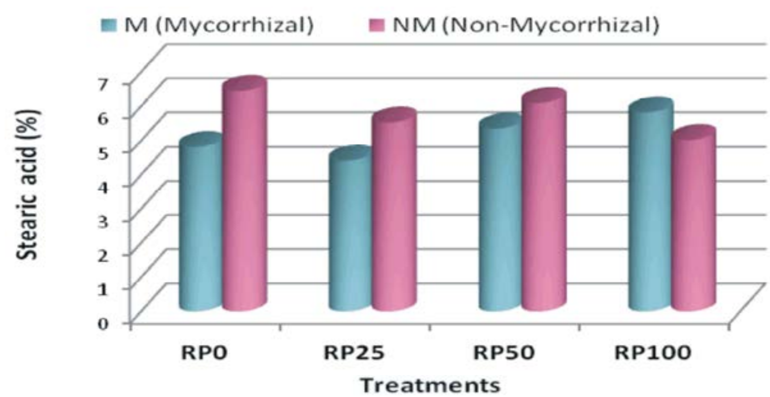

Fig. 4b: Effect of RP treatments on Stearic acid (%) in four hybrids of *Helianthus annuus* L.

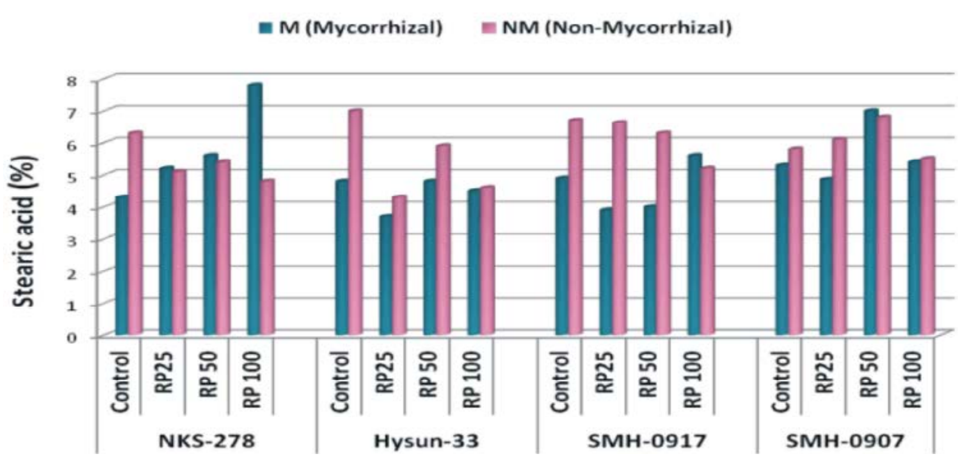

Fig. 4c: Effect of mycorrhiza on Stearic acid (%) under different rock phosphate levels in four hybrids of *Helianthus annuus* L.

The perusal of treatment mean data (Table 4 and Fig. 4b) regarding Stearic acid revealed that the mycorrhizal responses were most pronounced at high level of applied fertilizer (RP3). However at RP0, RP1 and RP2 levels of rock phosphate the non-mycorrhizal plants outperformed (Table-4 and Fig.4b). Stearic acid contents were maximum in SMH-0907 (5.84%) followed by NKS-278 (5.56%), SMH-0917 (5.40%) and Hysun-33 (4.9%) at various levels of rocks phosphate (Table 4 and Fig. 4a).

Similarly, mean values for Hybrids × Treatment interaction data (Table-4 and Fig. 4c) revealed that mycorrhizal response varied with increasing rock phosphate in different hybrids. Results clearly showed that minimum stearic acid content was recorded for Hysun-33 (3.70%) and SMH-0917 (3.90%) followed by SMH-0907 (4.86%) at RP1 level and NKS-278 (4.305) at RP0 as compared to non-mycorrhizal plants.

CONCLUSIONS

The present study strongly suggests that the AMF-rock phosphate combination produces better results in the enhancement of the edible oil contents of sunflower hybrids even in P-deficient soils. The use of biofertilizer is not only eco-friendly but also economical as it reduces our dependence on expensive chemical fertilizers. Moreover, it is suggested that out of four hybrids used in experiment Hysun-33 is more suitable to agronomical conditions of Peshawar climate due to its high oil yield.

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