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ccastro@uady.mx

Universidad Autónoma de Yucatán

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Harikumar, V.S.

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# BIOMETRIC PARAMETERS OF FIELD GROWN SESAME INFLUENCED BY ARBUSCULAR MYCORRHIZAL INOCULATION, ROCK PHOSPHATE FERTILIZATION AND IRRIGATION<sup>1</sup>

[PARÁMETROS BIOMÉTRICOS DEL CRECIMIENTO DE CAMPO DE AJONJOLÍ INFLUIDOS POR LA INOCULACIÓN DE MICORRIZAS, FERTILIZACIÓN CON ROCA FOSFÓRICA E IRRIGACIÓN]

V.S. Harikumar

Department of Post Graduate Studies & Research in Botany, Sanatana Dharma College (University of Kerala), Alappuzha-688 003, Kerala, India.

Email: vsharikumar@gmail.com

\*Corresponding author

## SUMMARY

The aim of the study was to assess the effect of inoculation with arbuscular mycorrhizal fungi (AMF) and rock phosphate (RP) fertilization on biometric parameters and mycorrhizal colonization of field grown sesame under rainfed and irrigated conditions. Inoculation of AMF *Funneliformis dimorphicus* improved the biometric parameters of the crop such as leaf area (LA), leaf area index (LAI), specific leaf weight (SLW), net assimilation rate (NAR), oil index (OI) as well as mycorrhizal colonization (%F) in roots. Mycorrhizal inoculation however, did not give any positive response on harvest index (HI). LA, LAI and OI and %F showed a general increment in treatments of no added P (P<sub>0</sub>), while the other parameters such as SLW and NAR were improved by the application of RP at half the recommended dose (P<sub>50</sub>). HI did not respond to RP fertilization. Most of the parameters (LA, LAI, NAR, %F) showed higher values under rainfed condition than irrigated condition whereas, SLW, HI and OI improved significantly under irrigated condition. Results indicated that the inoculation of AMF to field grown sesame can compensate for 50% of the recommended P fertilizer under a need based irrigation schedule, without affecting the biometric parameters.

**Key words:** AMF; rock phosphate; *Sesamum indicum*; biometric parameters.

## RESUMEN

El objetivo de este estudio fue medir el efecto de la inoculación con hongos micorrízicos arbusculares (HMA) y fertilización con roca fosfórica (RF) en los parámetros biométricos y la colonización micorrízica en ajonjolí que creció en campo bajo condiciones de temporal y riego. La inoculación del HMA *Funneliformis dimorphicus* mejoró los parámetros biométricos del cultivo, tales como el área foliar (AF), el índice de área foliar (IAF), peso específico de la hoja (PEH), tasa de asimilación neta (TAN), índice aceites en semilla (IAS) así como la colonización micorrízica (%F) en las raíces. Sin embargo, la colonización micorrízica no aportó una respuesta positiva al índice de cosecha (IC). Los parámetros AF, IAF, IAS y %F mostraron un incremento general en los tratamientos donde no se agregó P (P<sub>0</sub>), mientras que otros parámetros como PEH y TAN mejoraron con la aplicación de RF a la mitad de la dosis recomendada (F<sub>50</sub>). El IC no respondió a la fertilización con RF. La mayoría de parámetros (AF, IAF, TAN, %F) mostraron valores mayores bajo condiciones de temporal que bajo condiciones de riego, mientras que el PEH, IC e IAS mejoraron significativamente bajo condiciones de riego. Los resultados indicaron que la inoculación de las HMA al ajonjolí que crece en campo puede compensar el 50% del fertilizante P recomendado bajo un esquema de riego según se requiera, sin afectar los parámetros biométricos.

**Palabras clave:** HMA; roca fosfórica; *Sesamum indicum*; parámetros biométricos.

## INTRODUCTION

Sesame (*Sesamum indicum* L.) is a major field crop cultivated in tropical, subtropical and southern temperate regions of the world for its seeds, which is a

major source of edible oil. The stability and keeping quality, as well as resistance to rancidity makes sesame oil a much preferred cooking medium, apart from its use in soaps, cosmetics perfumes and insecticides (Anilakumar *et al.*, 2010). The grain of sesame is eaten

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fried and mixed with sugar or jaggery as sweet meats. Oil cake of sesame is a rich source of protein, carbohydrate and mineral nutrients, such as calcium and phosphorus, and is eaten avidly by humans. India ranks first, both in total area and production of sesame in the world, with an annual area of 1.68 million ha and total production of 0.83 million t (DES, 2016). Mostly, the crop is raised during summer season as a rainfed crop in low land rice fallows deficient in nutrients.

Phosphorus (P) is an important macronutrient for plant growth, development and reproduction, and it is often a factor limiting crop production in most soils of the world (Ågren *et al.*, 2012). The low availability of P in soil for plant uptake is mainly due to poor solubility and its fixation in soils (Illmer and Schinner, 1995). Further, the rate of phosphate absorption by growing roots is much higher than the rate of soil phosphate diffusion, which results in the formation of a depletion zone at the root system level and consequently limits the supply of P to plants. To satisfy crop nutritional requirements, fertilization with commercial soluble P sources are commonly used. However, the concern of a reduced use of agrochemicals and efficient utilization of natural minerals in agroecosystems has prompted the application of rock phosphate (RP). RP is a sparingly soluble P source, considered suitable for acidic soils and often used for improving crop growth (Patil *et al.*, 2011).

Symbiotic association of arbuscular mycorrhizal fungi (AMF) within plant roots are known to benefit the host increasing nutrient acquisition (Karasawa *et al.*, 2012; Dai *et al.*, 2014) and reducing water stress (Heidari and Karami, 2014). It has been well established that inoculation with AMF would improve the solubility and availability of phosphorus from RP (Barea *et al.*, 2002); also, an interaction involving AMF and RP often results in yield augmentation in a number of field crops (Doley and Jite, 2012; Abdel-Razzak *et al.*, 2013).

Summer sesame crops grown in rice fallows of south India, ends under conditions of low fertility and water availability. It is hypothesized that mycorrhizal inoculation coupled with RP fertilization and irrigation could bring better availability and mobility of P, which

improves biometric characteristics of the crop. To test this, a field study has been conducted to assess the effect of AM inoculation, graded level of RP fertilization and irrigation on biometric parameters of field grown sesame.

## MATERIALS AND METHODS

### Experimental

The experiment was conducted during 2009-2010 growing season, in a farmer's field located at Avoor, Kerala, India (9° 14' LN, 76° 28' LE; altitude 9 m). The design of the experiment was a split-split plot based on randomized complete block (RCB), with two levels of AMF (uninoculated-M<sub>0</sub> and inoculated-M<sub>1</sub>), two levels of water management (rainfed I<sub>0</sub> and irrigated I<sub>1</sub>), and five levels of phosphorus (no added P-P<sub>0</sub>, 7.5 kg ha<sup>-1</sup> P-P<sub>50</sub>, and 15 kg ha<sup>-1</sup> P-P<sub>100</sub>). All 12 treatments in the experiment were carried out by triplicate.

### Land preparation and evaluation of soil properties

Field soil was prepared into a fine tilth by ploughing two times with a power tiller. Clods were broken and the residue of the previous crop (rice) was removed. Physical and chemical properties of the soil are given in Table 1. Total indigenous AMF spore density in the soil was 170 spores 100 mL<sup>-1</sup> of soil, consisting of *Acaulospora delicata* (40 spores 100 mL<sup>-1</sup>), *Funneliformis mosseae* (78 spores 100 mL<sup>-1</sup>) and *Diversispora versiformis* (52 spores 100 mL<sup>-1</sup>).

### Mycorrhizal inoculation

In mycorrhizal treatments, the inoculum of AMF *Funneliformis dimorphicus* was used. The inoculum was prepared by multiplying the endophyte using sterilized sand-soil mix (1:1 v/v) as substrate, and *Sorghum* plants as the host. After six weeks of growth, shoots of the host plants were cut off and the substrate containing hyphae, spores and root fragments was air-dried and used as inoculum. The inoculum containing approximately 300 spores 100 mL<sup>-1</sup> was spread as a uniform layer of 2 cm thickness in the furrow created manually for the sowing of seeds.

Table 1. Some physical and chemical properties of the soil of experimental area.

Texture	Taxonomy	pH	Organic carbon (%)	Electrical conductivity (dSm <sup>-1</sup> ) (1:2.5)	N	P	K
		(1: 2.5)			(kg ha <sup>-1</sup> )		
Sandy	Entisol	5.2	1.42	0.17	144	10	18

## Sowing and cultural practices

Seeds of sesame (cv. Tilatara) were sown by hand in rows directly over the inoculum in furrows, and lightly covered with soil from the furrow on the day of planting. When the plants reached 15 cm in height, the plant density was thinned to give a spacing of 15 to 25 cm between plants. About 25 plants were maintained per row (5 m long) in a block. RP of sedimentary origin ( $P_2O_5$ -18%; solubility 0.6 in 2% citric acid) with a reactivity (Molar  $CO_3^{2-}$ :  $PO_4^{3-}$  ratio 0.053) was applied in graded levels in fertilizer treatments as mentioned earlier. In irrigated treatments, irrigation was given at 15 days interval using a sprinkler till the field capacity (Veihmeyer and Hendrickson, 1931) reaches 20 to 30%. Irrigation continued up to the time the pods begin to mature. Rainfed plants depended on dew as a source of moisture and did not receive irrigation throughout the growth period. Weeds were controlled by hand as required.

## Sampling

Three plants from each treatment were harvested out with the roots intact at 75 days of growth for monitoring the parameters.

## Evaluation of biometric parameters

Biometric parameters were evaluated at the maturity of the crop. For leaf area (LA) measurements, the entire leaves from each sampled plant were subjected to analysis using a leaf area meter (LICOR LI3100). Leaf area index (LAI) was worked out by dividing the total leaf area of the crop by total ground area under the crop, specific leaf weight (SLW) was calculated by dividing dry weight of the leaf with area of the leaf, net assimilation rate (NAR) was estimated as the increase of plant material per unit of assimilatory material per unit time and harvest index (HI) was taken as the ratio of seed weight to its total biological yield (Srivastava and Prasad, 2010). Sesame oil from the dried seeds was extracted with petroleum ether in a Soxhlet system as per the AOCS method (AOCS, 1993). The oil extract was evaporated by distillation at a reduced pressure in a rotary evaporator at 40°C and the solvent was totally removed. Oil index (OI) was calculated according to the formula suggested by Kohel (1978).

## Mycorrhizal colonization

Fresh fine roots of treatment plants were washed thoroughly under running tap water to remove soil particles and adherent debris. Roots were cut into 1 cm fragments, from these about 30 fragments were selected at random. Root fragments were cleared with 10% KOH for 15 min at 90°C, placed in 1% HCl for 10 min, and then stained with 0.05% lactic-glycerol-Trypan Blue for 5 min at 100°C (Phillips and Hayman,

1970). Root fragments were mounted on clean microscopic slides in a mixture of glycerol and lactic acid (v/v), covered by glass cover slips and tested under a compound microscope (Nikon Eclipse E 400). Frequency of AM colonization (%F) was calculated by the following equation (Trouvelot *et al.*, 1986):

$$F\% = (\text{Number of fragments mycorrhizal/total number}) \times 100$$

## Statistical analyses

An ANOVA for a split-split plot design was performed using SYSTAT 9. Mean comparison was done using LSD test at 95% level of probability. All differences reported are significant at  $P \leq 0.05$ .

# RESULTS AND DISCUSSION

## Biometric parameters

Mean value across treatments, LA of sesame was greater in mycorrhiza inoculated plants than in uninoculated (193.25 cm<sup>2</sup> and 117.24 cm<sup>2</sup>, Table 2) possibly due to an improved growth resulting in a total increase of photosynthetic area. Increased LA due to mycorrhizal inoculation has been corroborated elsewhere in field crops, such as sorghum (Afshar *et al.*, 2014) and maize (Hagh *et al.*, 2016). Mean LA indicated a general increment in treatments of no added P than fertilized with RP (192.57 cm<sup>2</sup> and 136.58 cm<sup>2</sup>). It seems that out of the P (35 kg h<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>) applied to the previous crop (rice), the residual quantity left in the soil after its utilization by the crop is adequate enough for growth leading to an increase in LA of sesame in the presence of a functional mycorrhizal system. According to Thompson (1991), improved plant growth response to AM colonization is mostly achieved in soils of limited available P.

Average LA in rainfed sesame increased to 81.73% compared to irrigated ones, suggesting that superimposition of irrigation did not give any significant effect on LA in sesame. It is hypothesized that irrigation causes dilution of nutrients in the rhizosphere and allowed its movement to the deeper strata of soil beyond the absorptive zone of mycorrhizal roots (Smith *et al.*, 2011). Further, soil moisture acts as an abiotic filter on AM fungal community assembly by regulating AM colonization in roots, which affects P uptake (Deepika and Kothamasi, 2015) and leaf growth.

LAI increased in concomitant with the increase in LA in mycorrhiza inoculated treatments than in uninoculated ones (12.84 vs 6.85, Table 2). This could be due to an increase in productivity of plant canopy, which resulted in an increased LAI (Hirose *et al.*, 1999). In the present study, neither P addition, nor

irrigation caused any significant effect on LAI, as the highest value (28.30) being observed in P<sub>0</sub> under rainfed condition. This contrasts the finding of Albaugh *et al.* (1998), who observed an increase in LAI up to 101% with fertilizer and 16% with irrigation in loblolly pine. A possible explanation to the negative response of P and irrigation is that, an increased LAI could maintain the source/sink strength for water and CO<sub>2</sub> exchange, even at low P and poor water availability, which has reflected on the growth and vigour of the crop.

SLW increased to 22.03% in mycorrhiza inoculated treatments and addition of RP at P<sub>50</sub> level made an increase of 6.34%. Further, irrigation of plants increased SLW to 11.29%. The interactive effects of mycorrhiza, RP and irrigation resulted in the maximum increase of SLW (1.18), suggesting that mycorrhizal inoculation and addition of RP at half the recommended dose and irrigation could enlarge the possibility of improving photosynthesis Pn as Pn increases with an increase in SLW (Pearse *et al.*, 1969).

The two fold increase in NAR was observed in mycorrhiza inoculated sesame plants compared to uninoculated plants (Table 2). This is in congruence with the findings of Kumar (2016), where the NAR in okra was greater by 17% with AMF inoculation. Karikari and Arkorful (2015) observed that NAR was not significantly influenced by soil P. Conversely, in the present study, NAR increased to 15.38% when plants were fertilized with RP. The highest value for NAR was obtained in plants receiving half the recommended dose (P<sub>50</sub>) of RP fertilizer. Irrigation did

not cause any positive effect on this parameter, confirming the finding of Pandey *et al.* (1984) that increasing moisture stress resulted in progressive reduction in growth indices in grain legumes.

The mean values recorded for HI in sesame showed that neither mycorrhizal inoculation nor RP fertilization had a significant effect on this parameter (Table 3). This contrasts the finding of Hagh *et al.* (2016), who reported a positive effect of mycorrhizal inoculation on HI under low level of soil P in field grown maize. HI being a measure of crop reproduction efficiency, is influenced by a number of factors. Extreme temperature (Unkovich *et al.*, 2010), drought (Ruttanaprasert *et al.*, 2016), during crop reproductive development or a balance between pre-and post anthesis water use of the field grown crop are some of the factors influencing HI. Further, excess of nitrogen can enhance the allocation of photosynthates to structural carbon, which cannot be mobilized to seeds later, resulting in a decline in HI (Unkovich *et al.*, 2010). Therefore, the individual or cumulative effects of these factors could have contributed to a decline in HI observed in the present study. However, irrigation caused a significant improvement of HI (Ahmad *et al.*, 2015).

Mean OI across treatments recorded a higher value in mycorrhiza inoculated plants than uninoculated plants (1.42 and 1.07, Table 3). Mycorrhizal plants are reported to improve oil content of seeds (Heidari and Karami, 2014), possibly by equipping the plants to overcome stress by a better uptake of water and nutrients, particularly during the seed filling stage which in turn reflects on OI.

Table 2. Leaf area (LA-cm<sup>2</sup>), leaf area index (LAI), specific leaf weight (SLW-mg cm<sup>-2</sup>) and net assimilation rate (NAR- g dm<sup>-2</sup> day<sup>-1</sup>) as influenced by mycorrhizal inoculation, RP fertilization and irrigation in sesame.

Mycorrhiza (M)	Fertilizer (F)	Irrigation (I)	LA	LAI	SLW	NAR
M <sub>0</sub> <sup>†</sup>	P <sub>0</sub> <sup>‡</sup>	I <sub>0</sub> <sup>§</sup>	229.33b	9.62c	0.58cdef	0.27ab
		I <sub>1</sub> <sup>§§</sup>	40.30h	2.66f	0.67cde	0.18ab
	P <sub>50</sub> <sup>‡‡</sup>	I <sub>0</sub>	102.00f	6.80de	0.48ef	0.16ab
		I <sub>1</sub>	56.60gh	3.73f	0.64cdef	0.16ab
	P <sub>100</sub> <sup>‡‡‡</sup>	I <sub>0</sub>	138.00e	9.16cd	0.73bcd	0.11b
		I <sub>1</sub>	137.22e	9.13cd	0.46ef	0.19ab
M <sub>1</sub> <sup>††</sup>	P <sub>0</sub>	I <sub>0</sub>	425.00a	28.30a	0.54cdef	0.07b
		I <sub>1</sub>	75.66g	5.00ef	0.76bc	0.50ab
	P <sub>50</sub>	I <sub>0</sub>	188.33c	12.53b	0.52def	1.00a
		I <sub>1</sub>	146.00e	9.70c	1.18a	0.42ab
	P <sub>100</sub>	I <sub>0</sub>	171.67cd	11.43bc	0.90b	0.18ab
		I <sub>1</sub>	152.86de	10.13c	0.43f	0.14b

<sup>†</sup>M<sub>0</sub>, no mycorrhizal inoculation, <sup>††</sup>M<sub>1</sub>, inoculation with mycorrhizal fungi (*Funneliformis dimorphicus*), <sup>‡</sup>P<sub>0</sub>, no phosphorus fertilizer, <sup>‡‡</sup>P<sub>50</sub>, 50% of the required chemical phosphorus fertilizer, <sup>‡‡‡</sup>P<sub>100</sub>, 100% of the required chemical phosphorus fertilizer, <sup>§</sup>I<sub>0</sub>, no irrigation, <sup>§§</sup>I<sub>1</sub>, irrigation at 15 days interval.

Table 3. Harvest index (HA-%) and oil index (OI- g oil 1000<sup>-1</sup> seeds) as influenced by mycorrhizal inoculation, RP fertilization and irrigation in sesame.

Mycorrhiza (M)	Fertilizer (F)	Irrigation (I)	HI	OI
M <sub>0</sub> <sup>†</sup>	P <sub>0</sub> <sup>‡</sup>	I <sub>0</sub> <sup>§</sup>	0.12cd	1.16cde
		I <sub>1</sub> <sup>§§</sup>	0.11d	0.81f
	P <sub>50</sub> <sup>¶¶</sup>	I <sub>0</sub>	0.17a	1.15cde
		I <sub>1</sub>	0.13bc	1.26c
	P <sub>100</sub> <sup>¶¶¶</sup>	I <sub>0</sub>	0.08e	1.2cd
		I <sub>1</sub>	0.14b	0.87def
M <sub>1</sub> <sup>††</sup>	P <sub>0</sub>	I <sub>0</sub>	0.08e	1.70b
		I <sub>1</sub>	0.18a	2.11a
	P <sub>50</sub>	I <sub>0</sub>	0.13bc	0.96cdef
		I <sub>1</sub>	0.11d	0.85ef
	P <sub>100</sub>	I <sub>0</sub>	0.08e	1.18cde
		I <sub>1</sub>	0.13bc	1.71b

<sup>†</sup>M<sub>0</sub>, no mycorrhizal inoculation, <sup>††</sup>M<sub>1</sub>, inoculation with mycorrhizal fungi (*Funneliformis dimorphicus*), <sup>‡</sup>P<sub>0</sub>, no phosphorus fertilizer, <sup>¶¶</sup>P<sub>50</sub>, 50% of the required chemical phosphorus fertilizer, <sup>¶¶¶</sup>P<sub>100</sub>, 100% of the required chemical phosphorus fertilizer, <sup>§</sup>I<sub>0</sub>, no irrigation, <sup>§§</sup>I<sub>1</sub>, irrigation at 15 days interval.

Mycorrhizal benefit on OI was at its best in treatments of no added P (P<sub>0</sub>), substantiating the fact that high P in soils adversely affect mycorrhizal performance (Ergin and Gülser, 2016). An irrigation schedule at 15 days interval significantly improved OI in mycorrhiza inoculated plants, which is in agreement with the finding of Heidari and Karami (2014), that interaction of water stress and mycorrhizal inoculation has a significant effect on oil content in sunflower.

### Mycorrhizal colonization

Frequency of root length colonized by AMF (%F) increased significantly in inoculated treatments (Fig. 1). Though the plant roots in the uninoculated treatments had a low level of colonization by native AMF, inoculation with an efficient AMF further improved the mycorrhizal status of the crop. Abbot *et al.* (1983) observed a better colonization of introduced AMF when the colonization by native AMF is low, suggesting that the increased mycorrhizal colonization observed in inoculated plants could be resulted from the introduced AMF. Increased root colonization due to inoculation of efficient AMF has been reported earlier in field crops (Arab *et al.*, 2013; Afshar *et al.*, 2014).

Colonization by AMF in plant roots increased with the application of RP, irrespective of levels. However, the beneficial effect of AMF and RP on %F was more in treatments of no added P. Soil prior to the cultivation of sesame had a low level of P, and the application of

RP up to the recommended dose is not high enough to cause any deleterious effect on the mycorrhizal symbiosis of sesame. In this context, it is worthwhile to examine the observation of Bolan *et al.* (1984), who indicated that under severe P limitation in soil, increasing P supply by fertilization may favour the colonization of AMF.

The mean value of mycorrhizal colonization between rainfed and irrigated plants was not significant in the present study, though the %F increased in rainfed treatment in most cases. The interactive effect of AMF and RP under rainfed condition increased %F to the maximum mean value (50.0). The study shows that irrigation did not give any notable response on AM colonization, probably due to the reason that an irrigation schedule at 15 days interval might not have created any anoxic condition impeding mycorrhizal development in sesame (Wu *et al.*, 2013).

### CONCLUSION

Inoculation of AMF to field grown sesame, can improve biometric parameters of the crop at a 50% saving on the required P fertilizer. Though certain parameters (LA, LAI, NAR and %F) are not responsive to irrigation, several parameters can be improved by an irrigation schedule at 15 days interval. Mycorrhizal inoculation and RP fertilizer at half the recommended dose, coupled with need based irrigation, can improve the biometric parameters of field grown sesame.

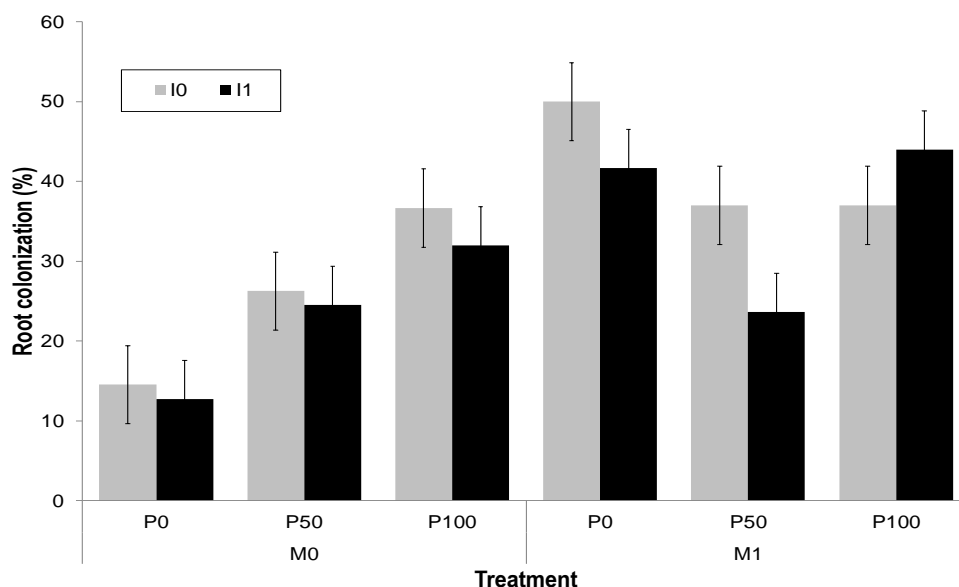


Figure 1. Mycorrhizal colonization in sesame roots as influenced by inoculation with AMF, RP fertilization and irrigation. M<sub>0</sub>, no mycorrhizal inoculation, M<sub>1</sub>, inoculation with mycorrhizal fungi (*Funneliformis dimorphicus*), P<sub>0</sub>, no phosphorus fertilizer, P<sub>50</sub>, 50% of the required chemical phosphorus fertilizer, P<sub>100</sub>, 100% of the required chemical phosphorus fertilizer, I<sub>0</sub>, no irrigation, I<sub>1</sub>, irrigation at 15 days interval.

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