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# **Full Length Research Paper** Selection of Efficient AM Fungi for Two Experimental Plants to Understand the Growth Response

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A B S T R A C T
Arbuscular mycorrhizal (AM) fungi found in rhizosphere of several vascular plants and have
important roles on sustainable agriculture as well as agricultural ecosystems management. The
beneficial effect of indigenous AM fungi on the nutrition of agricultural plants depends on both
the abundance and type of fungi present in the soil. However, the potential for employing AM
fungi on a wide scale in agriculture is dependent on the development of crop-growth-promoting
strains of AM, which are superior to native soil population of AM fungi. Therefore, experiment is
necessary to understand the AM fungi suitable for fiber yielding plants present in the rhizosphere
of the crop with the advancement of civilization, the use of plant fibers has gradually increased and their importance today is very great.

# 1.Introduction

Application of mycorrhizal biotechnology to crop production has the potential to reduce inputs such as pesticides or fertilizers and insure the sustainability of agro ecosystems (Hamel, 1996; Chandrasekaran M *et al.*, 2019). As many reports have proved that AMF inoculation is effective to increase crop yield under experimental conditions (Sharma S 2017). Therefore, it is necessary to select efficient AM fungi for the inoculation to the crop plants. Arbuscular mycorrhizal association can be characterized as inducible mutualistic symbiosis involving bi-directional transfer of resources (Smith and Gianinazzi-Pearson, 1988). The plant receives minerals from fungi in return for carbon products from photosynthesis, lipids and protection (Strck *et al.*, 2003). The AM fungi are obligate partners; while most plants are facultative (Smith and Giarl, 1988). Benefits of AM fungi to the host are numerous, growth and photosynthetic rates increases with mycorrhizal colonization in some species (Arain, et al., 2009; Sadhana B. 2014). Arbuscular mycorrhizal plants often have resistance to biotic and other abiotic challenges (Bayat *et al.*, 2009; Kuila D, Ghosh S. 2022 and Tonssaint *et al.*, 2007).

The combined benefit to the plant leads to more vigorous productive, adaptable and competitive individuals. With the advancement of civilization, the use of plant fibers has gradually increased and their importance today is very great. The objective of this experiment is to compare the effectiveness of common AM fungal species associated with fiber yielding plants and to check the efficacy of AM fungal species.

# 1.1 Objective

The objective of this experiment is to compare the effectiveness of common AM fungal species associated with fiber yielding plants and to check the efficacy of AM fungal species.

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# 2. Materials and method

### 3.1 Soil and plant materials

The physical and chemical soil characteristics used for pot experiment were tested in soil testing laboratories at Jalavahini Management Private Limited, Dharwad district, India . The soil was steam sterilized for one hour on two consecutive days. Fibre yielding plants seeds were collected from Seed Development Unit, University of Agricultural Sciences, Dharwad, India. Seeds were germinated in small plastic cups containing sterilized soil. Before sowing, the seeds were surface sterilized in 2% sodium hypochlorite and washed in distilled water for 2-3 times.

<b>able 1.</b> The physico-chemical characteristics of soil used for experiments.
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Parameters	Results				
Soil	Sandy loam				
рН (1:2.5)	8.1				
Conductivity (Fc) us/cm	320				
Moisture (%)	4.86				
Total organic carbon (%)	1.71				
Nitrogen (%)	0.08				
Potassium (%)	7.94				
Phosphorus (%)	4.52				
Magnesium (%)	0.121				
Calcium (%)	0.472				
Zinc ( ppm )	3.86				
Copper ( ppm )	0.03				
Manganese ( ppm )	0.97				
Iron ( ppm )	8.24				

### 3.2 Experimental design

The experimental pots were filled with growth media (soil and sand in 3:1 ratio). The soil based AM fungal inoculum (10 g) containing AMF infected root bits, mycelia and spore/sporacarps (250-300/10g inoculum) was placed as a thin layer just 2 cm below the soil surface. The seeds of all the experimental plants were surface sterilized by keeping them in 1% mercuric chloride solution for 2 to 3 min and then wash thrice with distilled water. Then these surface sterilized seeds were sown in the pre-prepared pots. The control treatment is maintained without any AM fungal inoculum the details of the treatments are as mentioned below.

### 1. Crotalaria juncea L.

- A. Uninoculated control (UIC)
- B. Mycorrhizal (*Slerocystis dussi*) inoculated
- C. Mycorrhizal (*Acaulospora laevis*) inoculated
- D. Mycorrhizal (Gigaspora margarita) inoculated
- E. Mycorrhizal (*Glomus fasciculatum*) inoculated
- 2. Hibiscus cannabinus L.
  - a. Uninoculated control (UIC)
  - b. Mycorrhizal (*Slerocystis dussi*) inoculated
  - c. Mycorrhizal (*Acaulospora laevis*) inoculated
  - d. Mycorrhizal (*Gigaspora margarita*) inoculated
  - e. Mycorrhizal (*Glomus fasciculatum*) inoculated

All the experimental pots were arranged incompletely randomized block design with triplicate per treatment. The experimental pots were kept free of weeds, insects, pets, rodents etc. the pots were watered every alternate day and 10 ml of Hoagland solution without P was given to each seedling at the interval of 15 days.

### 3.3 Analysis of growth parameters:

Plants were harvested after 60, 90 and 120 days after sowing. The plants parameters like shoot and root length, fresh weight of shoot and root, shoot and root dry weight, stem diameter and number of leaves, the per cent root colonization, spore number per 50 g soil, and phosphorus uptake in shoot were recorded. After the harvest, experimental plants shoot and root was oven dried at 70°C until a constant weight was obtained to determine the dry weight.

#### 3.4 Determination of Mycorrhizal Root colonization

The per cent root colonization was evaluated microscopically followed by clearing of roots in 10% KOH and staining with 0.05% trypan blue in lactophenol according to method described by Phillips and Hayman (1970). The following formula was used to calculate the root colonization (Giovannetti and Mosse, 1980).

Per cent mycorrhizal colonization =	Number of root segments colonized	X 100
	Total number of root segments examine	ed

#### 3.5 Determination of AM fungal spores

Spores were separated from the soil by wet sieving and decanting technique (Gerdemann and Nicolson, 1963). 50 g of soil was mixed with water. The mixture was pour through different sieve size (250, 106,  $45\mu$ m). After, several times of sieve washing the supernatant was collected in Petri dish and spores counted under binocular-microscope.

#### 3.6 Determination of Fiber content

Smallholder plots are usually harvested by hand. The plants are cut at 2 to 3 cm above the soil and left on the ground to dry. The cut, *Crotalaria juncea* L., and *Hibiscus cannabinus* L., is laid in swathes to dry for up to four days.

#### 3.7 Phosphorus content

The phosphorus content in the shoots was determined by the vanado-molybdate phosphoric acid yellow color method outlined by Jackson (1973).

#### 3.8 Statistical Analysis

Analysis of variance (ANOVA) was performed on all data and the means were separated using Duncans multiple Range Test (DMRT), with the help of SPSS student version-9 software.

#### 3. Results

The different AM fungi such as *Glomus fasciculatum, Gigaspora margarita, Acaulospora laevis* and *Sclerocystis dussii* were inoculated to test their efficacy on four fiber yielding plants. All the plant species inoculated with different AM fungi showed increased growth parameters over the control plants. The experimental results revealed that, not only the growth parameters of four experimental plants were increased but also the nutrient uptake and mycorrhizal status was significant compared to non-mycorrhizal plants.

The growth parameters of all the experimental plants were determined at 60, 90 and 120 days after sowing. Initially slowly increased growths were observed but after 95 days significantly increased growth rate had been recorded. The greater values for growth parameters shoot length, fresh weight of shoot, dry weight of shoot, root length, fresh weight of root, dry weight of root, stem diameter, number of flowers, numbers of fruits and increased "P" uptake were recorded in experimental plants with inoculation AM fungus *Glomus fasciculatum* over the reaming treatments. Where as, plants inoculated with *Gigaspora margarita* and *Sclerocystis dussii* have shown to the less but they have significant values when compared to control plants. The intermediate growth rate had been recorded in plants inoculated with AM fungus *Acaulospora laevis*. It indicates that, the AM fungus *Acaulospora laevis* was the second best efficient indigenous AM fungus for the fiber yielding plants. (Table. 2-3).

Mycorrhizal parameters like per cent colonization, and spore number were determined at 60, 90 and 120 days. The mycorrhizal root colonization was found to be varied in each experimental plant it was less in beginning (at 45-60 days) but steadily increased after 90 days. It was observed that at 120 there was maximum colonization. The maximum per cent mycorrhizal colonization (PMC) was recorded in *Corchorus capsularis* L., and very least PMC was observed in the roots of *Gossypium hirsutum* L., whereas, intermediate PMC was recorded in *Hibiscus cannabinus* L., and *Crotalaria juncea* L.,

AM fungal spore number was recorded in all experimental plants. It was found to be highest at 120 days and least was noticed at 60 days, with increase in duration the spore number was increased. Maximum AM fungal spore number was observed in the rhizosphere soils of the experimental plants inoculated with *Glomus fasciculatum* and it was least in plants inoculated with *Slerocystis dussii*, whereas, moderate spore number was noticed in plants inoculated with *Acaulospora laevis* and *Gigaspora margarita*. Among the plant species, the maximum number of AM fungal spores was found in the rhizospheric soils.

The plants were also analyzed for its nutrient continent in shoot, particularly phosphorus. All the AM fungal inoculated plants have shown increased nutrient content when compared to control plants. Maximum increased P uptake was observed in plants inoculated with *Glomus fasciculatum*. The moderately increased P Uptake in shoots was estimated in plants inoculated with *Gigaspora margarita* and it was least in plants inoculated with *Acaulospora laevis* and *Sclerocystis dussii*. Among all

mycorrhizal inoculated plants, *Hibiscus cannabinus* L. had shown significantly increased P uptake over the reaming three fiber yielding plants. The least increased P uptake was reported in mycorrhizal *Gossypium hirsutum* L., whereas, the moderate P uptake was estimated in the reaming two plant species.

The fiber content in all the inoculated and control plants was measured (Table. 2-5). The fiber content in the mycorrhizal plant was greater when compared to control plants (Fig. 2.11). Among all the mycorrhizal plants, the plants inoculated with *Glomus fasciculatum* have shown maximum fiber yield. The least increased fiber content was recorded in plants inoculated with *Sclerocystis dussii*. It can be evident from the above results that, AM fungus *Glomus fasciculatum* was found more efficient and the next best species for the inoculation to the fiber yielding plants was *Acaulospora laevis*. Fiber yield was considerably more in inoculated plants. All the experimental plants have shown increased fiber yield, but with *Glomus fasciculatum* fiber yield was maximum.

**Table: 3.** The effect of AM fungi on the Fiber yield (g/plant or fruit) of *Crotalaria juncea* L. and *Hibiscus cannabinus* L. at different interval plants at 60, 90 and 120 days after sowing.

Treatments	Crotalaria juncea L.	Hibiscus cannabinus L.
Uninoculated (UN)	3.500±0.057e	2.100±0.115e
Slerocystis dussii (SD)	4.433±0.088d	3.267±0.066d
Acaulospora laevis (AL)	5.400±0.115c	4.400±0.115c
Gigaspora margarita (GM)	6.066±0.088b	5.600±0.115b
Glomus fasciculatum (GF)	7.966±0.120a	6.733±0.371a

Values represent the mean ± SD. Means followed by the same letter within a column are not significantly P= 0.05 according to DMRT.

### 4. Discussion

In present study mycorrhizal parameters like per cent root colonization and extra matricular spore count were higher in plants inoculated with AM. The influence of *Glomus fasciculatum* was highest on per cent colonization and spore number in fiber yielding plants; similar observations were also made by Reena and Bagyaraj (1990) in their studies with *Calliandra calothrysus* inoculated with four different VA mycorrhizal fungi. So naturally the fungus having higher root colonization will be better adapted and absorb more nutrients and thus better growth. Rani and Bhaduria (2001) Michail Orfanoudakis, *et al.*, (2010), Mulla (2002), Mulani (2002), Lakshmipathy *et al* (2003) observed higher colonization and up take of more nutrients in medicinal plants. The results from this study indicated that, potential benefits could be obtained from the AM fungi in production of fiber yielding plants for their better use in future. It is concluded from the present experiments that the inoculation of fiber yielding plants to minimize manure dosage in improving the growth, biomass production and the fiber content in fiber yielding plants by inoculating biofertilizer and bioinoculant like AM fungi.

### 5. Conclusion

The greater values for growth parameters shoot length, fresh weight of shoot, dry weight of shoot, root length, fresh weight of root, dry weight of root, stem diameter, number of flower and increased "P" uptake were recorded in experimental plants with inoculation AM fungus *Glomus fasciculatum* over the reaming treatments. The plants inoculated with *Glomus fasciculatum* have shown maximum fiber yield. The least increased fiber content was recorded in plants inoculated with *Sclerocystis dussii*. It can be concluded that, AM fungus *Glomus fasciculatum* was found to be more efficient for the growth and yield of the experimental plants and the next best species for the inoculation to the fiber yielding plants was *Acaulospora laevis*.

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**Table: 1.** Effect of AM fungi on growth parameters of *Crotalaria juncea* L., phosphorus uptake in shoot and per cent mycorrhizal colonization, spore number at 60, 90 and 120 days after sowing.

Treatments	SL	FWS	DWS	RL	FWR	DWR	STD	РС	NFL	NFR	SP	P-uptake
60 DAYS												
Uninoculated (UN)	27.80	9.000	3.833	10.10	2.666	0.966	1.033	0.000	0.00	0.00	0.000	0.050
	±0.100e	±0.400e	±0.066e	±0.404e	±0.120e	±0.024e	±0.033e	±0.000e	±0.000	±0.000	±0.000e	±0.000e
Slerocystis dussii (SD)	32.76	10.56	4.533	13.16	3.800	1.166	1.033	32.00	0.00	0.00	74.66	0.080
	±0.088d	±0.20d	±0.176d	±.0176d	±0.100d	±0.033d	±0.033d	±0.000d	±0.000	±0.000	±1.763d	±0.000d
Acaulospora laevis	34.33	11.13	5.266	13.13	4.000	1.866	1.100	39.00	0.00	0.00	83.66	0.090
(AL)	±0.133c	±0.066c	±0.120c	±0.033c	±0.057c	±0.033c	±0.057c	±0.577c	±0.000	±0.000	±1.453c	±0.000c
Gigaspora margarita	35.80	12.63	6.600	13.76	4.166	1.600	1.200	44.66	0.00	0.00	86.66	0.100
(GM)	±0.100b	±0.088b	±0.115b	±0.088b	±0.033b	±0.115b	±0.057b	±1.333b	±0.000	±0.000	±1.201b	±0.00b
Glomus fasciculatum	39.63	14.76	9.666	18.76	4.766	2.033	1.5000	53.33	0.00	0.00	115.0	0.120
(GF)	±0.088a	±0.088a	±0.120a	±0.088a	±0.088a	±0.033a	±0.057a	±0.666a	±0.000	±0.000	±2.081a	±0.000a
90 DAYS												
Uninoculated (UN)	35.76	12.76	7.633	17.30	4.200	2.066	1.066	0.000	0.333	0.000	0.000	0.070
	±0.088e	±0.088e	±0.088e	±0.450e	±0.230e	±0.033e	±0.066e	±0.000e	±0.333e	±0.000e	±0.000e	±0.000e
Slerocystis dussii (SD)	43.63	18.33	10.40	20.70	6.600	3.600	1.233	47.33	0.666	0.000	94.00	0.110
	±0.088d	±0.033d	±0.115d	±0.264d	±0.115d	±0.115d	±0.033d	±0.881d	±0.333d	±0.000d	±1.154d	±0.000d
Acaulospora laevis	45.76	20.60	11.23	24.20	8.100	4.066	2.033	50.33	1.000	0.666	113.0	0.100
(AL)	±0.088c	±0.115c	±0.202c	±0.503c	±0.057c	±0.066c	±0.033c	±1.154c	±0.000c	±0.333c	±1.527c	±0.000c
Gigaspora margarita	48.56	23.60	12.50	25.23	9.033	4.400	1.333	54.66	1.666	0.333	121.6	0.130
(GM)	±0.176b	±0.057b	±0.057b	±0.145b	±0.218b	±0.115b	±0.066b	±0.666b	±0.333b	±0.333b	±1.201b	±0.000b
Glomus fasciculatum	53.70	26.93	14.53	30.63	11.96	5.766	1.766	66.00	2.666	1.333	147.6	0.150
(GF)	±0.057a	±0.284a	±0.176a	±0.088a	±0.185a	±0.088a	±0.088a	±1.201a	±0.333a	±0.333a	±0.881a	±0.000a
					120	DAYS						
Uninoculated (UN)	47.56	24.76	13.43	26.66	10.60	5.166	1.700	0.000	3.333	2.000	0.000	0.080
	±0.202e	±0.088e	±0.272e	±0.185e	±0.115e	±0.033e	±0.057e	±0.000e	±0.666e	±0.577e	±0.000e	±0.000e
Slerocystis dussii (SD)	54.66	29.66	18.16	30.76	13.26	6.466	1.866	54.33	4.666	3.333	137.6	0.130
	±0.185d	±0.185d	±0.033d	±0.088d	±0.066d	±0.066d	±0.033d	±0.333d	±0.667d	±0.333d	±1.527d	±0.000d
Acaulospora laevis	60.46	34.76	21.00	34.76	15.20	7.866	2.066	72.66	7.666	4.333	150.3	0.150
(AL)	±0.120c	±0.088c	±0.351c	±0.088c	±0.057c	±0.033c	±0.033c	±0.667c	±0.881c	±0.333c	±1.527c	±0.000c
Gigaspora margarita	57.00	31.50	20.13	32.30	15.06	7.066	2.033	81.66	6.000	5.333	141.6	0.140
(GM)	±0.033e	±0.152a	±0.371a	±0.200a	±0.318a	±0.088a	±0.033a	±0.666a	±1.154a	±0.666a	±8.736a	±0.000a
Glomus fasciculatum	67.83	37.76	24.66	40.13	17.76	10.20	2.133	64.00	9.666	7.000	178.3	0.170
(GF)	±0.300d	±0.088b	±0.185b	±0.033b	±0.088b	±0.057b	±0.033b	±0.577b	±0.333b	±0.577b	±6.027b	±0.000b

Note: SL: Shoot length, FWS: Fresh weight of Shoot, DWS: Dry weight of Shoot, RL: Root Length, FWR: Fresh weight of Root, DWR: Dry weight of Root, STD: Stem Diameter, PC: per cent of mycorrhizal colonization, NFL: Number of Flowers, NFR: Number of fruits, SP: Spore Number, P: Phosphorous. Values represent the mean ± SD. Means followed by the same letter within a column are not significantly P= 0.05 according to DMRT

**Table: 2.** Effect of AM fungi on growth parameters of *Hibiscus cannabinus* L., phosphorus uptake in shoot and per cent mycorrhizal colonization, spore number at 60, 90 and 120 days after sowing.

Treatments	SL	FWS	DWS	RL	FWR	DWR	STD	РС	NFL	NFR	SP	P-uptake
60 DAYS												
Uninoculated (UN)	18.50	4.300	1.300	7.366	1.633	0.926	1.233	0.000	0.000	0.000	0.000	0.050
	±0.057e	±0.057e	±0.057e	±0.088e	±0.033e	±0.006e	±0.033e	±0.000e	±0.000	±0.000	±0.000e	±0.000e
Slerocystis dussii (SD)	23.66	12.40	3.833	10.33	2.366	1.333	1.433	34.33	0.000	0.000	71.33	0.080
	±0.088d	±0.057d	±0.033d	±0.088d	±0.120d	±0.088d	±0.033d	±1.453d	±0.000	±0.000	±1.763d	±0.000d
Acaulospora laevis (AL)	26.53	13.46	4.600	12.40	2.600	1.600	1.766	39.66	0.000	0.000	82.33	0.08
	±0.145c	±0.066c	±0.650c	±0.057c	±0.115c	±0.000c	±0.033c	±0.333c	±0.000	±0.000	±2.185c	±0.000c
Gigaspora margarita	29.46	14.53	4.533	13.43	2.766	1.733	1.633	41.00	0.000	0.000	97.33	0.090
(GM)	±0.145b	±0.088b	±0.176b	±0.033b	±0.033b	±0.033b	±0.066b	±0.577b	±0.000	±0.000	±1.763b	±0.000b
Glomus fasciculatum	36.56	17.70	6.966	15.70	3.133	1.933	2.066	51.66	0.000	0.000	127.6	0.110
(GF)	±0.120a	±0.152a	±0.120a	±0.057a	±0.066a	±0.033a	±0.033a	±1.453a	±0.000	±0.000	±1.333a	±0.000a
90 DAYS												
Uninoculated (UN)	28.76	10.40	3.366	10.73	4.233	1.766	1.533	0.000	0.000	0.000	0.000	0.070
	±0.088e	±0.057e	±0.088e	±0.120e	±0.133e	±0.088e	±0.066e	±0.000e	±0.000e	±0.000	±0.000e	±0.000e
Slerocystis dussii (SD)	37.96	23.56	8.266	13.63	6.266	2.700	1.833	47.00	1.333	0.000	102.0	0.100
	±0.233d	±0.202d	±0.409d	±0.088d	±0.120d	±0.152d	±0.066d	±0.577d	±0.333d	±0.000	±1.527d	±0.000d
Acaulospora laevis (AL)	40.73	28.66	18.43	14.90	7.566	3.400	1.866	53.66	1.000	0.000	115.3	0.110
	±0.120c	±0.120c	±0.120c	±0.404c	±0.120c	±0.115c	±0.088c	±0.881c	±0.000c	±0.000	±1.763c	±0.000c
Gigaspora margarita	43.76	30.76	12.96	17.06	8.466	3.866	1.900	59.66	1.666	0.000	125.3	0.130
(GM)	±0.088b	±0.088b	±0.233b	±0.166b	±0.176b	±0.176b	±0.057b	±1.201b	±0.333b	±0.000	±2.027b	±0.000b
Glomus fasciculatum	64.86	40.30	23.50	23.43	14.56	7.833	2.066	74.33	2.666	0.666	150.6	0.150
(GF)	±0.033a	±0.602a	±1.732a	±1.097a	±1.126a	±0.876a	±0.033a	±1.201a	±0.666a	±0.000a	±3.844a	±0.000a
						120 DAYS						
Uninoculated (UN)	41.56	18.63	5.600	12.76	5.066	2.366	1.700	0.000	0.666	0.666	0.000	0.090
	± 0.202e	±0.218e	±0.300e	±0.088e	±0.033e	±0.260e	±0.152e	±0.000e	±0.333e	±0.333e	±0.000e	±0.000e
Slerocystis dussii (SD)	53.36	34.66	17.76	21.63	9.700	4.100	2.066	62.00	1.666	0.666	133.3	0.120
	±0.622d	±0.120d	±0.088d	±0.088d	±0.404d	±0.251d	±0.088d	±1.154d	±0.333d	±0.333d	±4.255d	±0.000d
Acaulospora laevis (AL)	61.83	37.80	17.33	23.56	10.60	4.766	2.100	74.66	2.333	1.333	144.3	0.130
	±0.033c	±0.351c	±0.120c	±0.120c	±0.321c	±0.088c	±0.057c	±1.763c	±0.333c	±0.333c	±2.728c	±0.000c
Gigaspora margarita	64.80	40.76	21.10	26.56	12.96	5.400	2.166	74.66	4.000	2.000	158.6	0.150
(GM)	±0.057b	±0.088b	±0.472b	±0.120b	±0.393b	±0.057b	±0.066b	±3.179b	±0.577b	±0.577b	±4.910b	±0.000b
Glomus fasciculatum	78.70	46.50	25.06	30.96	17.26	7.866	2.266	89.66	6.333	3.333	196.3	0.180
(GF)	±0.152a	±0.173a	±0.721a	±0.185a	±0.417a	±0.185a	±0.088a	±1.201a	±0.333a	±0.333a	±0.881a	±0.000a

Note: SL: Shoot length, FWS: Fresh weight of Shoot, DWS: Dry weight of Shoot, RL: Root Length, FWR: Fresh weight of Root, DWR: Dry weight of Root, STD: Stem

Diameter, PC: per cent of mycorrhizal colonization, NFL: Number of Flowers, NFR: Number of fruits, SP: Spore Number, P: Phosphorous. Values represent the mean ± SD. Means followed by the same letter within a column are not significantly P= 0.05 according to DMRT

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