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Comparative Potential of Different Native Mycorrhizal and Cellulolytic Fungi in Recovering Soil Biological Quality under Water Deficit

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ABSTRACT
Soil biological quality has a primary role in many soil functions related to productivity and environmental resilience. Arbuscular mycorrhizal and cellulolytic fungi are two beneficial microorganisms colonizing plant roots and have a potential role in plant productivity. This study aimed to determine the effectiveness of native species of the mycorrhizal (*Acaulospora tuberculata* and *Gigaspora* cf. *gigantea*) and cellulolytic (*Talaromyces pinophilus* strain MR107 and *T. pinophilus* isolate OK3SP103P) fungi from the dry land of Aceh on soil biological quality under water deficit. Dual inoculation of both native species significantly improved soil biological quality by increasing mycorrhizal colonization, the fungal population, and soil respiration activity at 45 and 90 days after sowing (DAS), and soil phosphatase activity at 45 DAS. *Acaulospora tuberculata* was more suitable to *T. pinophilus* strain MR107 while *Gi.* cf. *gigantea* well combined to either *T. pinophilus* strain MR107 or *T. pinophilus* isolate OK3SP103P. *Gigaspora* cf. *gigantea* and *T. pinophilus* strain MR107 was the superior native mycorrhizal and cellulolytic fungi respectively in promoting soil biological quality under drought. This study could provide more insight into the sustainability of soil biological quality under drought by native mycorrhizal and cellulolytic fungi.

Key words: Acaulospora; Aceh; cellulolytic; Gigaspora; mycorrhizae; Talaromyces

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INTRODUCTION

The issue of sustainability in dryland agricultural systems where water is the major constraint that affects soil quality and predicted to increase in the future. This detrimental environmental condition directly changes physiological stress for soil microbes and decreases the abundance of microbial communities and their activities (Kaurin *et al*. 2018). Thus, the negative effects of the water stress on the microbial community will in turn influence soil functioning, which is largely supported by their activities (Cao *et al*. 2018; Zhang *et al*. 2019).

As one of the critical soil fertility components, soil microbial communities contribute to nutrient cycling in functions such as organic matter decomposition and mineralization, turnover and release of nutrients for subsequent plant capture and carbon sequestration (Van der Heijden *et al*., 2008). The quantity and nature of microorganism's species, as well as the number of individuals in the soil affected by various environmental stresses (Liu *et al*., 2019). Preserving soil quality is an effort needed to keep long term soil fertility and sustain crop production. the application of beneficial microorganisms was a promising alternative strategy to increase soil biological fertility (Li *et al*., 2017) and addressing many of the challenges drought poses to agricultural productivity (Grover *et al*., 2011; Xu *et al*., 2018).

These fungi enhance plant tolerance to various soil and environmental stresses, including drought (Mbodj *et al*., 2018; Qiao *et al*., 2011) through nutritional and physiological plant improvements (Ruíz-Lozano *et al*., 2003), direct water uptake (Amiri *et al*., 2107; Sandek *et al*., 2019), and changes in soil structure in terms of the quantity and quality of aggregate stability (Mbodj *et al*. 2018). Therefore, these fungi considered to be an effective and sustainable strategy to mitigate the water deficit problem (Amiri *et al*., 2017; Li *et al*., 2018).

However, it is important to realize that AM fungi do not occur in the soil in isolation, but rather interact with numerous other organism groups, in addition to roots (Mechri *et al*., 2014). Some non-arbuscular mycorrhizal fungi, such as cellulolytic (cellulose degraders) fungi, are known to enhance the AM fungi symbiosis with vascular plants, although information about their role in the symbiosis is still limited (Adnan and Al-Asbahi, 2012). Nevertheless, the results of research on the interactions between soil cellulolytic and AM fungi differ widely, even when the same species of cellulolytic fungi are involved. For example, a synergistic effect on AM root colonization due to the interaction between *T. harzianum and G. constrictum* or *G.intraradices*, while no significant effect was observed for *G. claroideum* and *G. mosseae* (Al-Morad *et al*., 2018; Martínez-Medina *et al*., 2011).

Studies revealed that the autochthonous microorganisms had better ability to survive under environmental stress compared to the allochthonous in terms of physiological and nutritional statuses and in increasing plant drought tolerance, attenuating, and compensating for the detrimental effect of water limitation (Ortiz *et al*., 2015; Wang *et al*., 2016). The effect of combined application of native IS and cellulolytic fungi specific to the dry land of Aceh on soil quality has not been ever yet reported. Therefore, this study aimed to analyze the effectivity of the native IS and cellulolytic fungi on soil biological properties under water deficit. The hypotheses of this study were there are significant differences in the effects of AM and

cellulolytic fungi as singly or combination application on soil biological quality in the maize rhizosphere.

MATERIALS AND METHODS

Experimental Design

The experiment was conducted using a 3 x 3 factorial arranged in a completely randomized block design with three native mycorrhizal [non-inoculated (NM), *Acaulospora tuberculata* (M1), and *Gigaspora* cf. *Gigantea* (M2)] and three cellulolytic [non-inoculated (NC), *Talaromyces pinophilus* strain MR107 (C1) and *T. pinophilus* isolate OK3SP103P (C2)] as treatments. Three replicates consists of three experimental units (pots) were used for each treatment, one plant per pot.

Preparation of mycorrhizal and cellulolytic inoculum

The native mycorrhizal spores and cellulolytic isolates were the potential inoculants based on the previous experiment. The inoculants were collected from dry land in Aceh Provinces, Indonesia. The mycorrhizal spores were propagated in maize-seedling pot culture to produce the mycorrhizal inocula consisted of mycorrhizal roots, fungal spores and mycelium, and zeolite medium.

The native cellulolytic fungal inoculants used were molecularly identified as *Talaromyces pinophilus* strain MR107 and *T. pinophilus* isolate OK3SP103P. The inoculants were sub-cultured in slant potato dextrose agar (PDA) before being multiplicated on Mandel medium added 1% w/v glucose and 0.2% v/v Tween 80 for 48 h in shake culture.

Growing condition

The soil was collected from the same location with inoculant sources. A composite soil sample was collected 0–20 cm depth from the topsoil layer, air-dried and sieved by 5.0 mm sieve before analysis and pot filling. Ten kg of dry soil was added into each of 81 black plastic pots (35 cm in diameter and 26 cm in height). The soil pH was 5.77 (1:1 soil: water), organic carbon was 1.81%, N-tot was 0.15%, P-Bray was 4.68 ppm, Kexch was 1.92 $cmol_+/kg$, Ca-exch was 11.94 $cmol_+/kg$, and Mg-exch was 4.75 $cmol_+/kg$.

At each pot, 10 kg/ha siam weed compost (C-N ratio 22.74) was mixed thoroughly with soil and then the growing medium was incubated for 10 days at 100% field capacity (FC). The pots were covered by black plastic during the incubation and placed in a screen house under natural light and average temperature 28-31ºC.

Both inoculants (AM and cellulolytic fungi) were applied at the sowing time. The mycorrhizal inoculants (having an average of 100 spores and 70% of mycorrhizal root colonization) were applied in the appropriate pots just below the three sterilized maize seeds (variety Bisi 2) while the cellulolytic inoculums were applied at the soil surface of each pot around the seeds as much as 10 mL (10^8 CFU/mL) based on the treatment. The plants were thinned to one per pot after seven days. Each pot was covered with black plastic with a small hole in the middle of the pot to the maize seedling appear.

Plants were watered regularly close to water holding capacity (100% FC) during the first four weeks of plant growth before water stress treatment were imposed. At this time, plants were allowed to dry until soil water content was 50% FC and maintained under these conditions for additional 10 weeks. Water loss was compensated by watering every day to reach the water status. Determination of soil FC was based on methods of Zarik *et al.* (2016). Fertilizers applied were 57.5 kg N/ha, 15 kg P/ha, 52 kg K/ha at the time of sowing while 57.5 kgN/ha at 14 days after sowing (DAS).

Microbiological analysis of soil samples

Soil microbiological parameters analyzed consisted of mycorrhizal colonization, microbial count and activities. These parameters were observed twice vis at 45 and 90 DAS. Mycorrhizal colonization assessment followed the method of Vierheilig *et al*. (1998). The staining procedure was made according to Vierheilig *et al*. (2005) modified to parameters of the present study. The percentage of colonization was calculated using the following formula proposed by Pankaj *et al*. (2017):

$$\% \text{ Colonization} = \frac{\text{mycorrhizal root pieces}}{\text{total numbers of root pieces observed}} x\ 100$$

Fungal enumeration was performed by standard plate count technique (Wollum, 1982) with standard 10-fold dilution method. The medium Potato Dextose Agar was used as medium.

Microbial activity observed in this experiment was respiration following Verstraete method (1981 in Franzlubber *et al*., 1999) and soil phosphatase with the p-NPP method (Tabatabai, 1982).

STATISTICAL ANALYSIS

Data were subjected to statistical analysis using SPSS statistical software version 25.0 to test effects of AMF, cellulolytic fungi, and their interactions on soil biological quality parameters i.e. mycorrhizal colonization, fungal population, soil respiration activity, and soil phosphatase activity. Significant differences between means were compared using Duncan's multiple range test (DMRT) at $P < 0.05$.

RESULTS AND DISCUSSION

AMF colonization

The abundance of AM fungi in roots and soil has been proposed to correlate positively with soil quality and can be a biological indicator of the impact of different agricultural management practices (Mariela *et al.* 2016)

Table 1 showed that the mycorrhizal colonization percentage of maize under drought stress was significantly affected by AM fungi ($P < 0.01$) at 45 and 90 DAS and by interaction of both fungal groups ($P < 0.05$) at 45 and 90 DAS but was not significantly by cellulolytic fungi.

Table 1. The significance of the differences in soil biological parameters resulting from the interactions between inoculation of AMF and cellulolytic fungi

Treatments	Mycorrhizal colonization		Fungi		Respiration		Phosphatase	
	45 DAS	90 DAS	45 DAS	90 DAS	45 DAS	90 DAS	45 DAS	90 DAS
M	**	**	**	**	*	**	**	ns
C	ns	*	**	**	**	**	**	ns
M*C	*	*	**	**	*	**	**	ns

M =AMF; C = Cellulolytic Fungi; DAS= days after sowing; ns=not significant; *= significantly difference; ** = very significantly difference

As expected, the mycorrhizal colonization of maize inoculated with both native AMF and CF has been increased under drought stress as either single or co-inoculation at 45 and 90 DAS (Figure 1). *Gi.*cf *gigantea* was the AMF inoculant which gave better effect to increase 36 to 38% the mycorrhizal colonization after combining with both CF (*T. pinophillus* strain MR107 and *T. pinophilus* isolate OK3SP103P) at 90 DAS (Figure 1a). This result showed

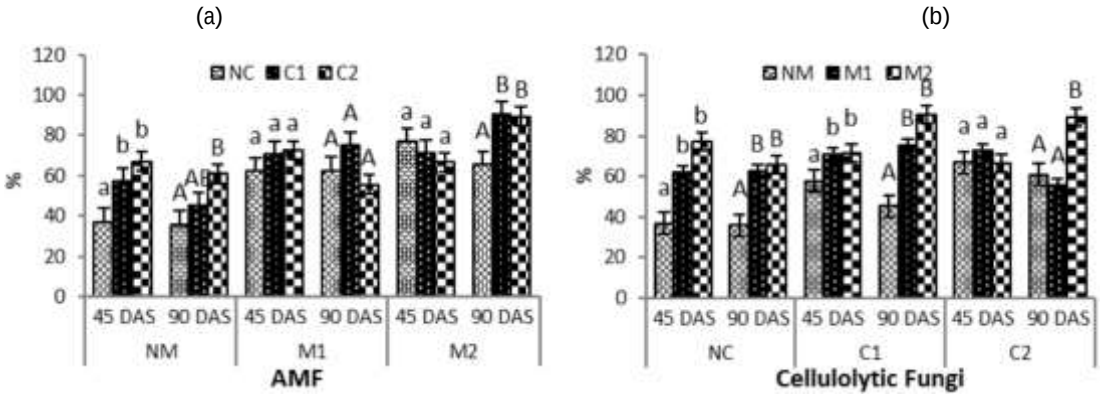

Fig. 1. Mycorrhizal colonization of maize roots as affected by different AMF and cellulolytic fungi under water stress. NM = no AMF; M1 = *Acaulospora tuberculata*; M2 = *Gigaspora* cf *gigantea*; NC = no cellulolytic fungi; C1 = *T. pinophillus* strain MR107; C2 = *T. pinophilus* isolate OK3SP103P. Bars represent SE. Different letters are significantly different ($P < 0.05$) among AMF/cellulolytic fungi, small letter for 45 DAS while capital letter for 90 DAS.

That the effect of AMF on mycorrhizal colonization was independent of the native cellulolytic fungi except for *Gi.*cf.*gigantea* at 90 DAS. These results were consistent with those of Adnan and Al-Asbahi (2012) who reported that some cellulolytic have significant effects on mycorrhizal development and symbiosis in many investigated plant species. Volatile and soluble exudates produced by these fungi are involved in these effects (Fracchia *et al*., 1998).

Contrary to the native AMF, the effect of the native cellulolytic fungi on mycorrhizal colonization was depended on the AMF inoculants (Figure 1b). *Talaromyces pinophilus* strain MR107 had a better effect after combining with both AMF inoculants at 45 and 90 DAS while *T. pinophilus* isolate OK3SP103P showed their best influence as co-inoculation only with *Gi.*cf. *Gigantea* at 90 DAS. These results indicated that the effectivity of cellulolytic fungi as dual inoculant with AMF was the cellulolytic species-dependent.

This study also revealed that higher mycorrhizal colonization at uninoculated maize by both inoculant types (AMF and CF) was found at 45 DAS while dual inoculation was at 90 DAS. This result indicated that the native AMF and CF acted synergistically to enhance the fungal performance longer under water stress. Albrechtova et al. (2012) also reported the positive relationship between those microorganisms.

Soil Fungi Population

Fungi are component of soil quality that performs a range of important ecological functions. These microfloras play a vital role in the soil ecosystem functioning related to soil fertility and primary production such as decomposition, parasitism, pathogenesis, and symbiosis (Pankaj *et al*. 2017). This study showed that soil fungal population was significantly ($P < 0.01$) affected by AMF, cellulolytic fungi, and the interaction of both fungal inoculant groups at 45 and 90 DAS (Table 1). The interaction between both soil microorganisms was presented in Figure 2.

The effect of AMF on soil fungi varied according to the celluolytic species (Figure 2a). The highest population of soil fungi was significantly found after combined inoculation of *Gi.* cf *gigantea* and *T. pinophilus* isolate OK3SP103P at 45 DAS. Inoculation of *A. tuberculata* exhibited best effect after being combined with *T. pinophillus* strain MR107 at 45 DAS. Both AMF species had similar influence increasingly by combined inoculation with either *T. pinophillus* strain MR107 or *T. pinophilus* isolate OK3SP103P at 90 DAS. The increased fungal abundance may be related to the root exudates and

different response of the microbes to root exudates or rhizodepositions (Liu *et al*. 2019).

Similar to AMF, the cellulolytic fungi effect on soil fungi was also depended on AMF species (Figure 2b). *T. pinophillus* strain MR107 was more compatible with *A. Tuberculata* at 45 DAS and *Gi.* cf *gigantea* at 90 DAS while *T. pinophilus* isolate OK3SP103P was well-matched with *Gi.* cf *gigantea* at 45 and 90 DAS.

The application of both native AMF and cellulolytic fungi as single inoculation showed varying effects on the soil fungal population. Both AMF species revealed similar effects at 45 DAS but *Gi.* cf *gigantea* was superior at 90 DAS. Instead, the cellulolytic fungi exhibited better effect at 45 and 90 DAS, and *T. pinophilus* isolate OK3SP103P showed the best

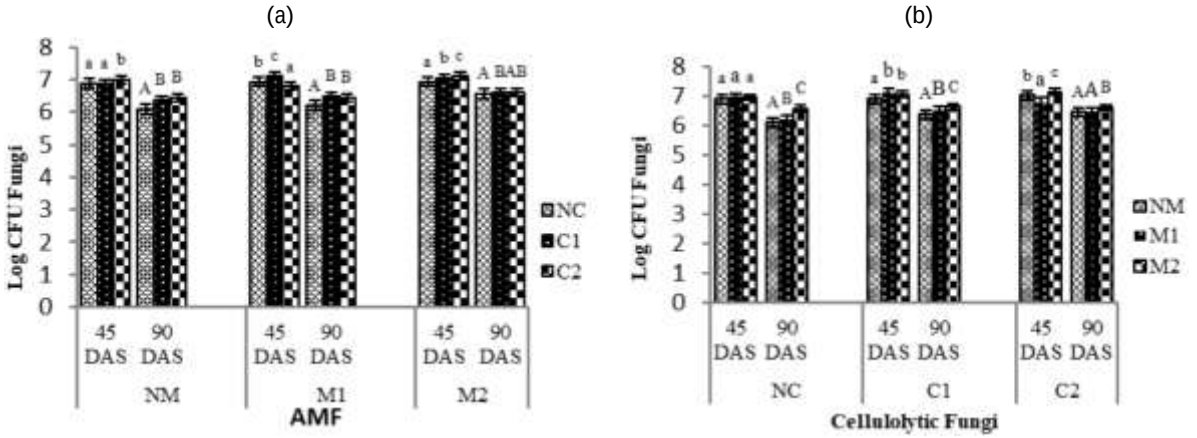

Fig. 2. Population of soil fungi as affected by different AMF (a) and cellulolytic fungi (b) under water stress. NM = no AMF; M1 = *Acaulospora tuberculata*; M2 = *Gigaspora* cf *gigantea*; NC = no cellulolytic fungi; C1 = *T. pinophillus* strain MR107; C2 = *T. pinophilus* isolate OK3SP103P. Bars represent SE. Different letters are significantly different (P < 0.05) among AMF/cellulolytic fungi, small letter for 45 DAS while upper case letter for 90 DAS.

Performance. These results indicated that the existence of soil fungi was influenced by a single inoculation of cellulolytic fungi more than AMF.

Overall, the population of soil fungi was decreasing with increasing plant age either as a single or combined application. It indicated that soil fungi were sensitive to long-term drought. Our results are in agreement with Kaisermann *et al*. (2015) but contrary to Preece *et al*. (2019), who observed that drought had no strong impact on the fungal community.

Soil respiration activity

Soil respiration is a well-established bioindicator of soil quality (Askari and Holden. 2015; Jansa *et al*., 2016). this activity is considered a robust estimate of potential soil biological activity (Bakhshandeh *et al*., 2019). This study showed that soil respiration was significantly affected by AMF and the interaction treatments at 45 DAS (P < 0.05) and 90 DAS (P < 0.01), but cellulolytic fungi influenced significantly (P<0.01) at 45 and 90 DAS (Table 1). The interaction between both soil microorganisms was presented in Figure 3.

This study indicated that native AMF and cellulolytic fungi as single affected soil respiration increasingly and combine inoculation. *Gigaspora* cf *gigantea* was a potential AMF species as a single factor and dual inoculation with *T. pinophillus* strain MR107 at

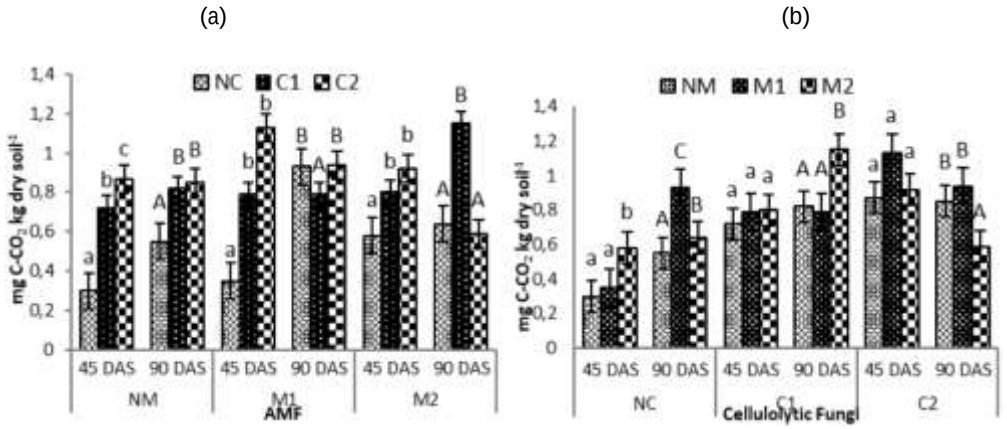

Fig 3. Effect of different native AM (a) and cellulolytic fungi (b) on soil respiration in maize rhizosphere at45 and 90 DAS.

Different letters above the bars (represent SE) represent significant differences beween diferent treatments (P< 0.05; Duncan test). NM= no AMF; M1= *A. tuberculata*; M2= *Gi.* cf *gigantea*; NC= no CF; C1= *T. pinophillus* strain MR107; C2= *T. pinophilus* isolate OK3SP103P 90 DAS while *A. tuberculata* showed similar encouragement after combining with either *T. pinophillus* strain MR107 or *T. pinophilus* isolate OK3SP103P at 45 DAS. These results are consistent with some previous studies (Cavagnaro *et al*., 2012; Liu *et al*., 2018) but inconsistent with Del-Saz *et al*. (2017) who showed AMF colonization decreases the rate of root respiration.

However, it is difficult to generalize across other systems, and there is some evidence indicating that the effect of both inoculants (AMF and cellulolytic fungi) depended on the type and species of the inoculants and the plant age. Generally, the inoculants significantly increased soil respiration under water stress compared to control but dual inoculation of *A. tuberculata* and *T. pinophillus* strain MR107 or *Gi.* cf *gigantea* and *T. pinophilus* isolate OK3SP103P at 90 DAS. Liu *et al*. (2018) showed that microbial community composition explained a unique portion of the variation in soil respiration.

Phosphatase activity

Soil phosphatase activity reflects the activity of enzymes associated with soil colloids and humic substances and free phosphates in the soil solution and with living and dead cells, plants, and microorganisms (Galązka *et al*., 2019). This enzyme participates in the phosphorus cycle, which catalyzes the hydrolysis of organic phosphorus into inorganic phosphorus for plants and microorganisms and is considered to be a sensitive bioindicator of soil quality (Vinhal-Freitas *et al*., 2013).

Table 1 showed that soil phosphatase activity was significantly (P < 0.01) affected by the inoculation of AMF, cellulolytic fungi, and their interaction at 45 DAS but not at 90 DAS. The activity of the enzyme under water stress fluctuated between 8.75 and 40.91 ppm (Table 2). The highest soil enzyme activity was found by *Gi.* cf *gigantea* and *T. pinophilus* strain MR107 as dual inoculation. This result indicated that both native inoculants acted synergistically to improve soil phosphatase activity. Besides, this AMF species also better as a single inoculation than as dual inoculation with other cellulolytic fungi (*T. pinophilus* isolate OK3SP103P).

This study revealed that Gi. cf *gigantea* was the best AMF inoculant as a single and dual inoculation with *T. pinophilus* strain MR107 in improving soil phosphatase as a soil quality indicator. Furthermore, those inoculants could be developed as native biofertilizers especially to improved P-deficient soils.

The phosphatase activities in the soil affected those inoculants were decreased with the length of water stress, as presented in Figure 4. *Gigaspora* cf *gigantea* and *T. pinophilus* strain MR107 were the mycorrhizal and cellulolytic species, respectively, that showed the highest effect in decreasing the enzyme activity. It may relate to the higher phosphor in the soil (data not shown). The inverse relationship between phosphatase activity and soil phosphorus concentration has been shown in several studies (Sakurai *et al*., 2008; Yang *et al*., 2019). The production of phosphatase is mainly regulated by the demand for P by organisms and environmental P availability (Adnan and Al-Asbahi, 2012; Margalef *et al*., 2017).

Table 2. The combine effect of indigenous AMF and cellulolytic fungi on soil phosphatase (ppm) at 45 DAS

Treatments	No-cellulolytic			*T. pinophilus* strain MR107			*T. pinophilus* isolate OK3SP103P		
No-AMF	8.75	a	A	18.60	b	A	18.65	b	A
A. tuberculata	18.09	a	B	16.72	a	A	15.63	a	A
*Gi.*cf.*gigantea*	31.67	ab	C	40.91	b	B	26.10	a	A

Note: Means followed by different letters (lowercase letter for row and capital letter for coloum) above the numbers indicate a significant difference at < 0.05 by DNMRT test.

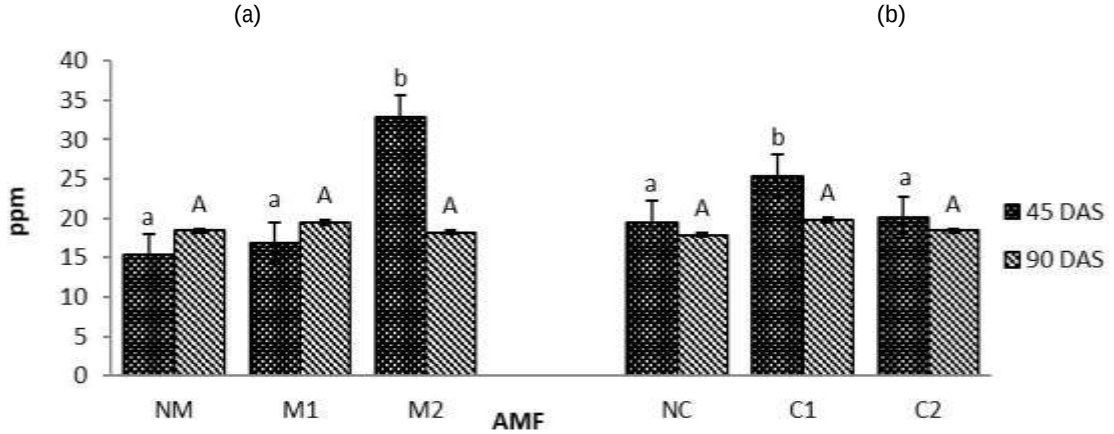

Fig. 4. Effect of different native AM (a) and cellulolytic fungi (b) on soil phosphatase in maize rhizosphere at45 and 90 DAS. Different letters above the bars (represent standard error) represent significant differences beween diferent treatments (P< 0.05; Duncan test). M1 = *A. tuberculata*; M2 = *Gi.* cf *gigantea*; C1 = *T. pinophillus* strain MR107; C2 = *T. pinophilus* isolate OK3SP103P

CONCLUSION

Both native AMF and CF species significantly improved soil biological quality by increasing mycorrhizal colonization, the fungal population, soil respiration, and soil phosphatase at 45 and 90 days after sowing (DAS). *Gi.* cf. *gigantea* was a native AMF spore superior to *A. tuberculata* in promoting soil biological quality under water deficit. Both native CF had similar effects on the fungal population, soil respiration, and soil phosphatase under water stress. *A. tuberculata* was more suitable to *T. pinophilus* strain MR107 while *Gi.* cf. *gigantea* well combined to either *T. pinophilus* strain MR107 or *T. pinophilus* isolate OK3SP103P. This study could provide more insight into the sustainability of soil biological quality under drought by native AMF and cellulolytic fungi.

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