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Soil Physic-Chemical Properties, Arbuscular Mycorrhizal Fungi (AMF) Diversity and Abundance in the Rhizosphere of *Calotropis procera* in two Wild Locations of Semi-Arid Eastern Kenya

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A B S T R A C T

Soil and root samples were randomly collected from the rhizosphere of *Calotropis procera* (Calotropis) growing in two wild locations of Makueni and Tharaka-Nithi Counties, Kenya. The root samples were assessed for arbuscular mycorrhizal fungi (AMF) colonization. The soil samples were analyzed for physic-chemical properties and screened for the presence, abundance and diversity of AMF. The AMF inoculum potentials of the soils were determined using the bioassay test. The results depicted significant variations between the sites in soil pH, % sand, % clay, % silt, available P and Mg concentrations. These soil parameters had, generally, marked effects on AMF colonization, spore numbers and genus richness from the correlation analysis results. The Makueni site registered slightly higher mean spore densities than the Tharaka-Nithi site. Tharaka-Nithi site recorded relatively higher genus richness and AMF root colonization percentage than the Makuenisite. Overall, 6 AMF genera were identified in the soils, of which 4 (*Dentiscutata*, *Scutellospora*, *Glomus* and *Acaulospora*) were discovered in both sites while 2 (*Gigaspora* and *Rhizaphagous*) occurred in Tharaka-Nithi site only. *Glomus* was comparatively dominant and ubiquitous in both sites. The genera *Dentiscutata*, *Scutellospora*, *Glomus* and *Acaulospora* could possibly be tested for suitability as mycorrhizal inoculants of Calotropis seedlings in the dry lands of Kenya.

Introduction

C. procera (Ait.) R. Br. (Calotropis) is an economically valuable species of the drylands. The species originates from tropical Africa and Asia (Payal and Sharma, 2015), and it’s widely distributed in the tropical and subtropical regions of the world (Moustafa and Sarah, 2017). Calotropis inhabits degraded sites such as overgrazed rangelands, abandoned farmlands, disturbed urban

regions, and mined areas in drylands (Verma, 2016), hence, it is used in ecological restoration. Besides, Calotropis has numerous other uses including textile manufacturing and soil fertility amelioration (Payal and Sharma, 2015). However, in Kenya, the species has not been naturalized due to a lack of knowledge on its cultivation requirements, yet collection from the wild is inadequate (Mutiso *et al.,* 2017).

Arbuscular mycorrhizal fungi (AMF) are an integral component of the soil microbiota, playing a significant role in plant growth and development. The association of plants with AMF has gained substantial attention in recent years because of their role in promoting plant growth and productivity. AMF form mutualistic associations with roots of terrestrial plants, aiding them in water uptake and mineral nutrition through efficient acquisition mechanisms. AMF also, improve the tolerance of plants to drought, root pathogens and toxic heavy metals, and promote soil aggregation which improves soil structure hence, contributing to proper plant growth and health (Gao *et al.,* 2020; Zhang *et al.,* 2019). Over 80% of tropical plants depend on AMF symbiosis for their growth and survival, especially in moisture deficient and low fertile soils (Bahadur *et al.,* 2019; Berruti *et al.,* 2016).

In diverse ecosystems, AMF have coevolved with certain plant taxa resulting in their adaptation to certain environments and efficacy for specific hosts (Gosling *et al.,* 2006). Thus, understanding the AMF community associated with specific plant species under natural settings and target regions is a prerequisite for the successful application of AMF biotechnology and conservation and management of AMF symbiosis.

AMF biotechnology has been extensively exploited in the production of major socio-economically important species (Bernaola *et al.,* 2018; Kim *et al.,* 2017)and in ecological restoration (Aggangan and Cortes, 2018; Asmelash *et al.,* 2016). Inoculating nursery seedlings with native AMF has shown promise in enhancing the growth and vigor of the seedlings, and their subsequent survival and growth in the field (Brundrett *et al.,* 2005; Pera *et al.,* 1999). The current research developments are targeted at selecting, testing and nurturing suitable indigenous AMF strains for use as inoculants in crop production and ecological restoration (Séry *et al.,* 2016). However, soil physic-chemical properties play a key role in determining AMF symbiosis hence they are key considerations in the management of AMF symbiosis and the application of AMF biotechnology (Mahmoudi *et al.,* 2019).

The present work explored the soil physic-chemical properties, AMF diversity and abundance, and percentage root colonization in the rhizosphere of Calotropis plants in two wild locations of Makueni and Tharaka-Nithi Counties, Kenya. The study, also, assessed the mycorrhizal inoculum potential (MIP) of the soils.

This work points attention to AMF biotechnology in Calotropis production and serves as a guiding tool toward selecting suitable AMF strains for use as mycorrhizal inoculants in Calotropis production. The knowledge of the natural Calotropis-AMF associations will aid in the optimal exploitation of the symbiosis with AMF that are naturally and evolutionary associated with Calotropis for maximum benefits.

Materials and Methods

Sampling locations and descriptions

Soil and root sampling were done attwo wild locations of Makueni and Tharaka-Nithi Counties in semi-arid eastern Kenya. The study sites were situated at an altitude of less than 600 m a.s.l. The Makueni site was situated in Masongaleni sub-Location, Kibwezi east sub-County, Makueni County (Fig. 1) while the Tharaka-Nithi site was sited in Kamahindi sub-Location, Igambang'ombe sub-County, Tharaka-Nithi County(Fig. 2).The study areas receive unreliable and poorly distributed rainfall of less than 500 mm per year and high

temperatures of up to 35.8°C (Makueni)and 40°C (Tharaka-Nithi). The rainfall pattern is bimodal with the long rains occurring between March and May and the short rains occurring between October and December (Makueni County, 2018; Tharaka-Nithi County, 2018).

Soil and root sampling

Sampling was done during the dry season in September 2017. Five sample plots (5 × 5 m) were randomly established at each site and marked by a Global Positioning System (GPS) coordinate at the center of each plot (Fig. 1 & 2). Soil cores were removed from the rhizosphere of three randomly selected trees at 0.5 m from the root collar (Pera *et al.,* 1999) and their contents were carefully transferred into tightly fitting disinfected 250-cm3 plastic pots (having perforations at the bottom) to maintain their structure. The pots were labeled, packed into a box (to avoid further disturbance), and kept for bioassay test.

Rhizosphere soil was excavated around the circumference of the selected trees (at 0.5 m from the stem base) to a depth of 20 cm using a hole and a shovel. At each sampling point, the top 'O' layer (horizon) was scraped off to remove litter before sampling. Roots were carefully traced from the tree trunks and hand-excavated.

Soil and root samples collected were homogenized to form composite samples per plot, resulting in 5 composite samples (of soil and roots) per site. 5 root sub-samples were randomly selected from each composite sample and preserved under 50% ethanol in well-labeled plastic containers for the assessment of AMF root colonization intensity. Then, 5 sub-samples of soil amounting to 1 kg each were pooled from each soil composite sample and placed in well-labeled foil carriers for physic-chemical analyses.

Then, approximately 500 g of soil was sampled from each composite soil sample for extraction and characterization of AMF spores. The remaining composite soil samples were separately packed in well-labeled alcohol sterilized bags for trap culturing. Soil and root sub-samples were kept at 40°C until analyses.

Soil physic-chemical analysis

The physic-chemical analyses of soil were done at the Soil Laboratory in the Kenya Forestry Research Institute (KEFRI), Nairobi. The soils were air dried at room temperature, ground to pass through a 2 mm sieve, and analyzed following the standard protocols: Soil texture by hydrometer method (Black *et al.,* 1965), available phosphorus (P) by the Olsen's method (Olsen and Dean, 1965)and determined calorimetrically by the ascorbic acid-molybdate blue method (Watanabe and Olsen, 1965), pH and electrical conductivity (EC) by potentiometric methods (1:2.5 soil:water ratio) using conductivity meter and pH meter, respectively (Page and Keeney, 1982), organic carbon (SOC) by the Walkley-Black oxidation (Loss on Ignition) method (Page *et al.,* 1982), total nitrogen (N) by the Kjeldahl distillation method (Bremner, 1996), and exchangeable bases (magnesium [Mg2+] and potassium [K+]) by extraction in 1M ammonium acetate at pH 7 and measuring using a flame photometer (K+) and atomic absorption spectrophotometer (Mg2+) (Chapman, 1965).

Trap cultures

Pot trap cultures of field soil from the two sampling sites were set up in a greenhouse at the National Museums of Kenya (NMK) in a split-plot design. Soil sub-samples containing root fragments from each sampling site were thoroughly homogenized (1:1, v/v) with coarse sand and sterilized twice using an autoclave at121ºC for 1 hour, with intervals of 24 h between autoclaving. Disinfected 250-cm^3 plastic pots (having drainage perforations at the bottom) were filled ¾ full with the autoclaved soil/sand mixture. 200 g of the rhizosphere field soil (containing root fragments from each sampling site) was added to the pots and topped up with a sterilized soil mixture, forming a layer of field soil 2

cm below the surface. The growing medium in each pot was wetted and sawn with seeds of the mycotrophic species, *Sorghum bicolor* (Sorghum). Sowing was done at 2 cm depth and the seeds were covered with a sterilized mixture. Seeds were surface sterilized by immersing in a 0.5% sodium hypochlorite solution for 15 minutes and rinsed with distilled water before sowing (Vestberg and Assefa, 2015). Three seeds were sawn per pot and watered to field capacity using tap water. After emergence, the pots were thinned to two seedlings per pot. The experiment was maintained for 3 months after which watering was minimized to allow the pots to dry slowly in order to stimulate sporulation by AMF. At the end of the 4th month, the plants were cut near the base, and the cultures left to dry undisturbed. 100 g of soil was sampled from each pot using a soil core at a depth of 0–15 cm for AMF spore extraction and characterization.

Spore extraction and identification

AMF spore extraction and identification were performed at the NMK Mycology Laboratory. AMF spores were extracted from100g of field and trap culture soil sub-samples using the wet-sieving and decanting method (Gerdemann and Nicolson, 1963). The extracted spores were washed with running tap water, transferred into a petri dish and counted under a dissecting microscope (4.0x). Representatives of each spore morphotype (as differentiated by color or size) were mounted on glass slides in polyvinyl alcohol-Lacto glycerol (PVLG) or a mixture of PVLG and Melzer's reagent (1:1; v:v) (Koske and Gemma, 1989), crushed with cover slides(to expose internal features) and examined under a compound microscope (40x).

The spores were identified up to the genus level based on their morphological features observed, with reference to standard descriptions by Schenck and Perez (1990).The following indices were computed as indicators of AMF diversity and abundance in each soil sample(100 g of dry soil) and expressed as mean values per site:(1)spore density (the number of spores per 100 g of dry soil), (2) frequency of occurrence (the percentage of the total samples in which a particular genus was observed), (3) genus richness (the number of genus observed per sample), (4) relative abundance (the percentage of the total number of spores in a sample belonging to a particular genus) and (5) the Shannon–Wiener diversity index (*H*) (Shannon and Weaver, 1949):

$$H = -\sum_{i=1}^{n} Pi \ln Pi$$

Where, *H*= Shannon–Wiener diversity index, ∑ = summation, *n* = genus richness, P_i= the proportion of total sample represented by genus *i*, $\ln Pi$= natural logarithm of P_i.

Bioassay test

A bioassay test was set up in a greenhouse at the NMK to assess the AMF inoculum potential of the soils. The experimental design used for the bioassay test was a split plot. The plastic pots containing field soil from the two sampling locations were seeded with mycotrophic host, *S. bicolor* (Sorghum) and watered to field capacity using tap water.

Seeds were surface sterilized by immersing in a 0.5% sodium hypochlorite solution for 15 minutes and rinsed with distilled water before sowing (Vestberg and Assefa, 2015). Three seeds were sawn per pot and thinned to one seedling per pot after emergence. Subsequent watering was regulated based on the soil drainage characteristics and weather conditions. Seedlings were destructively harvested after eight weeks.

The roots were cleaned by immersing them in a tub of water overnight and washing over a 1 mm sieve with a 800µm sieve beneath to collect fine roots that got detached from the main root system. Clean roots were preserved under 50% ethanol in well-labeled plastic containers until further processing and analysis.

Assessment of AMF colonization on roots

AMF root colonization assessment was performed on the field-collected (Calotropis) and bioassay (sorghum) root sub-samples at the NMK Mycology Laboratory following the procedures outlined by Phillips and Hayman (1970) and Giovannetti and Mosse (1980) as modified by Ingleby (2007). The percentage root colonization was calculated using the following formula.

$$\text{AMF root colonization (\%)} = \frac{\text{Number of AMF positive root segments}}{\text{Total number of root segments observed}} \times 100$$

Statistical analysis

Data on the soil physic-chemical properties and AMF parameters were compiled in Microsoft excel. AMF spore density, genus richness, relative abundance, isolation frequency and Shannon–Wiener diversity index were computed per site in Microsoft excel. Statistical differences between the sampling sites were determined using a *t*-test at $P < 0.05$.Pearson's correlation analysis was done to determine the relationship between soil physic-chemical properties and mycorrhizal parameters and between AMF root colonization intensity and soil AMF parameters.

Results and Discussion

Soil physic-chemical properties

Soils obtained from the rhizosphere of *C. procera* in Makueni and Tharaka-Nithi sampling locations were distinctively varied in terms of their physic-chemical properties (Table 1). Makueni soil was moderately acidic and sandy clay loam in texture whereas Tharaka-Nithi soil was slightly alkaline and sandy loam in texture.

The two soil types also differed significantly in pH ($p = 0.001$), available P ($p = 0.026$) and Mg concentrations ($p = 0.0002$) (Table 1). According to Duniway *et al.,* (2010), soil properties vary from region to region due to the differences in their forming processes and the variations in the chemical constituents of the parent materials from which they developed. Moreover, soil properties differ depending on the prevailing topography and biological, physical, and chemical processes (Panday *et al.,* 2019).

AMF spore abundance and diversity

The soil samples collected from the rhizosphere of Calotropis in both Makueni and Tharaka-Nithi sampling locations harbored AMF. Trap cultures depicted higher AMF spore numbers and greater AMF genus richness than field soils (Table 2). However, there were no significant differences in AMF spore density and genus richness between the sites.

Makueni site had slightly higher mean spore densities in both field and trap culture of 15.60±2.78 (SE) spores per 100 g of soil and 105.20±23.20 (SE) spores per 100 g of soil, respectively than the Tharaka-Nithi site which had mean spore densities of 14.80±3.04 (SE) spores per 100 g of soil and 96.60±14.46 (SE) spores per 100 g of soil in field and trap culture, respectively (Table 2). On the other hand, the Tharaka-Nithi site had relatively higher genus richness in both field and trap culture of 2.00±0.00 genera per 100 g of soil and 2.40±0.24 genera per 100 g of soil, respectively than the Makueni site which had mean spore densities of1.80±0.20 genera per 100 g of soil and 1.80±0.37 genera per 100 g of soil, in field and trap culture, respectively(Table 2).The Shannon–Wiener diversity index value of the trap culture isolates was higher in the Tharaka-Nithi site (1.40) than in the Makueni site (1.26).

The observed variations in the AMF spore abundance and diversity between the study sites were associated with the dissimilarities in their edaphic properties as depicted by the correlation analysis results (Table 3). The soil pH ($p = 0.001$),

% sand ($p = 0.001$), % silt ($p < 0.001$), % clay ($p < 0.001$) and concentrations of available P ($p = 0.026$) and Mg ($p = 0.0002$) were significantly varied between the regions (Table 1). These soil parameters had marked effects on AMF spore density and genus richness (Table 3). Similarly, other workers have documented variations in AMF abundance and diversity between sampling locations due to contrasting edaphic properties (Lauber *et al.,* 2008; Reyes *et al.,* 2019; Sivakumar, 2013).

Overall, six (6) AMF genera (*Dentiscutata*, *Scutellospora*, *Gigaspora*, *Glomus*, *Rhizaphagous,* and *Acaulospora*) were detected in the rhizosphere of Calotropis in both Makueni and Tharaka-Nithi sampling locations (Table 4, Plates 1 and 2). The genera *Scutellospora*, *Glomus,* and*Acaulospora* were also found associated with Calotropis in the dryland localities of India (Prasanthi *et al.,* 2016), suggesting their adaptation to different edaphic and climatic factors. Hence, members of these genera could be explored for exploitation as mycorrhizal inoculants for *C. procera*. However, further research is needed to test the compatibility of specific strains and their persistence in the field.

Tharaka-Nithi field soil harbored four (4) AMF genera (*Glomus*, *Rhizophagous*, *Acaulospora,* and *Gigaspora*) whereas Makueni field soil had three (3) AMF genera (*Dentiscutata*, *Scutellospora,* and *Glomus*) (Table 4). On the other hand, the Tharaka-Nithi trap culture depicted six (6) AMF genera (*Dentiscutata*, *Scutellospora*, *Gigaspora*, *Glomus*, *Rhizaphagous,* and *Acaulospora*) (Table 4, Plate 1) while the Makueni trap culture revealed four (*Dentiscutata*, *Scutellospora*, *Glomus* and *Acaulospora*) (Table 4, Plate 2). Hence, trap culture revealed two (2) more AMF genera (*Dentiscutata* and *Scutellospora*) in the Tharaka-Nithi site and 1 more AMF genus (*Acaulospora*) in the Makueni site than observation of field soil.

The observed variations in AMF spore numbers and diversity between field soil samples and trap culture samples could be explained by the differential sporulation potential of AMF under different conditions of field and trap culture (Husband *et al.,* 2002; Muthukumar and Udaiyan, 2002), and the variations in the processes of spore germination and degradation among AMF genera (Smith, 1980). Trap culture stimulates sporulation of strains that are in vegetative forms (hyphae and infected root fragments) hence revealing AMF genera that are not observable in field soil at the point of sampling (Songachan and Kayang, 2013). This is because trap culture provides more standardized and constant environments that enable the rapid proliferation of fungi thus providing large quantities of fresh spores (Stutz and Morton, 1996). So, fungal surveys based merely on spore observations from field-collected soils may be underestimated while combining both methods may yield more accurate results. The findings of the present investigation are consistent with the results of several authors who found higher AMF spore abundance and diversity in trap cultures than field soils (Costa *et al.,* 2016; Muthukumar and Udaiyan, 2002; Rodríguez-Morelos *et al.,* 2014; Songachan and Kayang, 2013). Ezawa *et al.,* (2000) underlined the importance of trap culturing than observation of field-collected spores for the analysis of fungal communities because field spores are often damaged or infected by other soil microbes hence they may not be easily identified.

With regard to the relative abundance of spores, *Glomus* was the dominant genus in field soils, occurring in 70.51% and 52.70% of Tharaka-Nithi and Makueni spore isolates, respectively (Table 4). *Glomus* was also dominant in trap cultures, appearing in 43.48% and 45.44% of Tharaka-Nithi and Makueni spore isolates, respectively. In terms of the isolation frequency of spores, *Glomus* was the dominant genus in field soils, occurring in all Tharaka-Nithi field samples and 4 out of 5 (80%) of the Makueni field samples (Table 4). On the other hand, *Glomus* was the dominant genus in the Tharaka-Nithi trap culture, occurring in 4 out of 5 (80%) of the Tharaka-Nithi trap culture samples while *Glomus* and *Scutellospora* were the dominant genera in Makueni trap culture samples, both

appearingin 3 out of 5 (60%) of the Makueni trap culture samples (Table 4). The dominance of *Glomus*in both study are as indicated the adaptability of the genus to varied edaphic conditions. Prasanthi *et al.,* (2016) had a similar observation where they reported the dominance of *Glomus* in the rhizosphere of *C. procera* in India.

Members of *Glomus* have also been reported dominating the rhizosphere of other medicinal plants around the globe (Kokni *et al.,*; Kumar *et al.,* 2017; Mangla *et al.,*) and other ecosystems in different habitats and land use types (Alguacil *et al.,* 2009; Hijri *et al.,* 2006; Husband *et al.,* 2002; Jefwa *et al.,* 2009; Jefwa *et al.,* 2012; Muneer *et al.,* 2019), demonstrating their adaptability to varied ecological conditions.

The dominance of *Glomus* has been related to their ability to sporulate faster and produce large amounts of hyphae fragments than most genera (Bever *et al.,* 1996; Oehl *et al.,* 2009). Furthermore, *Glomus* is regarded as the most adapted to semi-arid conditions (Mosbah, Philippe, & Mohamed, 2018; Zhao *et al.,* 2017)and resistant to disturbances (Welemariam *et al.,* 2018).

Notably, Tharaka-Nithi soil, which was slightly alkaline, harbored more *Glomus* spores than Makueni soil which was moderately acidic. This result conforms with the observation of *Mukerji et al.,* (2002) that members of this genus thrive under slightly alkaline environments, and nearly agrees with the assertion of Al-Arequi *et al.,* (2013) and Mosbah *et al.,* (2018) that alkaline pH favors the development of Glomus.

In this study, spore density had a positive correlation with soil P (r = 0.06) (Table 3). This result collaborates the finding of Muleta *et al.,* (2007) who observed a positive correlation between spore density and available P in soils collected from a natural coffee forest in Ethiopia. He et al., (2016), also, had the same observation in the rhizosphere soil of *Robinia pseudoacacia* in the semi-arid Loess Plateau of northwest China. The afore mentioned results portrayed the need for soil P amelioration to induce more sporulation by AMF in the study areas. On the other hand, in the present investigation, soil % clay had an inverse relationship with genus richness and spore density (r = -0.1776, r = -0.0341) (Table 3). This observation corroborates the finding of Belay *et al.,* (2013) and Carrenho *et al.,* (2007) that high levels of clay in soil inhibited mycorrhizal development.

AMF root colonization intensity

All the root samples collected from the rhizosphere of Calotropis plants in Tharaka-Nithi and Makueni sampling locations were positive for AMF colonization (Table 5). AMF, also, colonized the sorghum roots in the bioassay test established using soils collected from the rhizosphere of *C. procera* in both sites (Table 6). This confirmed that *C. procera* is a symbiont with AMF as previously determined by Prasanthi *et al.,* (2016). However, there were no statistical differences between the study areas with regard to the extent of AMF colonization of both field-collected and bioassay test roots (Tables 5 and 6).

AMF colonization was characterized by the presence of typical AMF structures viz. arbuscules, vesicles, hyphae, intraradicale coils, and appressoria in the roots of the hosts (Tables5 and 6, Plates 3 and 4). Prasanthi *et al.,* (2016) had the same observation in the dryland localities of India, where they observed hyphae, arbuscules, and vesicles in the roots of *C. procera* growing in the field.

Tharaka-Nithi site recorded a slightly higher percentage AMF colonization in both field and bioassay test of 82.33±3.83% and 64.3±3.49%, respectively than the Makueni site which registered 69.99±10.49% and 61.0±9.29% RC in the field and bioassay test, respectively (Tables 5 and 6). The statistical similarities in the extent of AMF colonization between the sites were associated with the uniformity in climate and altitude of the regions (Makueni County, 2018; Tharaka-Nithi County, 2018).

Table.1 Physical and chemical properties of *C. procera* rhizosphere soil obtained from Makueni and Tharaka-Nithi County sampling sites, Kenya

	pH	EC (mS/cm)	% TN	AP (ppm)	K^+ (ppm)	Mg^{+2} (ppm)	% SOC	% Sand	% Clay	% Silt	Soil class
Makueni	6.19±0.19	0.09±0.02	0.17±0.10	4.47±0.27	505.60±52.22	147.0±27.59	4.29±0.99	60.0±0.00	30.0±0.0	10.0±0.00	sandy clay loam
Tharaka-Nithi	7.45±0.12	0.03±0.01	0.05±0.02	12.83±3.05	100.84±18.91	354.8±17.53	2.89±0.36	68.40±1.72	16.40±1.47	16.0±0.63	sandy loam
t-test	-5.53*	2.12 ns	1.16 ns	-2.73*	7.29 ns	-6.36*	1.32 ns	-4.88*	9.25*	-9.49*	
P-value	0.001	0.067	0.280	0.026	8.495-e-05	0.0002	0.224	0.001	<0.001	<0.001	

EC = Electrical conductivity; TN = Total N; AP = Available P; SOC = Soil organic carbon; ns Non significant; *Significant at P < 0.05; Mean ± SE

Table.2 AMF spore density and genus richness of Tharaka-Nithi and Makueni field soils and trap cultures

Sampling site	Spore density (Number of spores per 100 g of soil)		Genus richness (Number of genera per 100 g of soil)	
	Field soil	Trap culture	Field soil	Trap culture
Tharaka-Nithi	14.80±3.04	96.60±14.46	2.00±0.00	2.40±0.24
Makueni	15.60±2.78	105.20±23.20	1.80±0.20	1.80±0.37
***t*-test**	0.19	0.32	-0.53	-1.90
P-value	0.085	0.076	0.61	0.09

Table3 Pearson's correlation coefficients for physic-chemical properties versus AMF genus richness and spore density of soil collected at the rhizosphere of Calotorpis plants growing in the field at Makueni and Tharaka-Nithi Counties

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	-																	
2	0.75	-																
3	-0.97	-0.86	-															
4	0.09	0.10	-0.14	-														
5	-0.51	-0.38	0.49	-0.37	-													
6	-0.51	-0.38	0.49	-0.37	1.00	-												
7	-0.36	-0.32	0.39	-0.33	0.79	0.79	-											
8	0.38	0.33	-0.40	-0.02	0.09	0.09	0.34	-										
9	0.36	0.75	-0.51	-0.22	-0.11	-0.11	-0.13	0.14	-									
10	0.25	0.17	-0.17	0.10	0.01	0.01	0.22	-0.04	-0.31	-								
11	0.32	0.61	-0.42	-0.58	-0.18	-0.18	-0.27	-0.05	0.79	-0.15	-							
12	0.21	0.56	-0.39	-0.24	-0.01	-0.01	-0.24	0.06	0.70	-0.25	0.77	-						
13	-0.49	-0.62	0.56	-0.23	0.17	0.17	-0.24	-0.54	-0.47	-0.20	-0.16	-0.26	-					
14	0.16	0.18	-0.18	0.06	-0.65	-0.65	-0.39	0.01	0.09	0.10	0.24	0.11	-0.04	-				
15	-0.84	-0.88	0.91	-0.15	0.55	0.55	0.30	-0.42	-0.63	-0.14	-0.49	-0.48	0.77	-0.36	-			
16	0.75	0.91	-0.85	0.16	-0.42	-0.42	-0.57	0.21	0.59	0.09	0.55	0.57	-0.30	0.16	-0.74	-		
17	-0.09	0.05	-0.03	0.34	-0.44	-0.44	-0.43	-0.07	0.06	-0.23	0.10	0.47	-0.13	0.61	-0.25	0.10	-	
18	0.85	0.80	-0.91	0.18	-0.61	-0.61	-0.52	0.53	0.46	-0.04	0.41	0.45	-0.50	0.36	-0.88	0.83	0.28	-

1 = %Sand; 2 = %Silt; 3 = %Clay; 4 = %MC; 5 = %OC; 6 = %SOM; 7 = %Total N; 8 = %RC; 9 = Available P; 10 = Bulk density; 11 = Ca^{+2}; 12 = Cu^{+2}; 13 = EC; 14 = Genus richness; 15 = K^{+}; 16 = Mg^{+2}; 17 = Spore density; 18 = pH.

Table.4 AMF spore density, relative abundance and isolation frequency per genera for Tharaka-Nithi and Makueni field soil and trap cultures

AMF Genus	AMF Spore density (Number of spores per 100 g of soil)				Relative abundance (%)				Isolation frequency (%)			
	Field soil		Trap culture		Field soil		Trap culture		Field soil		Trap culture	
	Tharaka-Nithi	Makueni	Tharaka-Nithi	Makueni	Tharaka-Nithi	Makueni	Tharaka-Nithi	Makueni	Tharaka-Nithi	Makueni	Tharaka-Nithi	Makueni
Dentiscutata	-	2.80	2.00	13.60	-	18.92	2.07	12.93	-	40.00	20.00	40.00
Scutellospora	-	4.20	1.60	27.20	-	28.38	1.66	25.86	-	60.00	40.00	60.00
Gigaspora	1.00	-	13.60	-	6.41	-	14.08	0.00	40.00	-	60.00	0.00
Glomus	11.00	7.80	42.00	47.80	70.51	52.70	43.48	45.44	100.00	80.00	80.00	60.00
Rhizophagous	0.60	-	13.00	-	3.85	-	13.46	0.00	20.00	-	20.00	0.00
Acaulospora	3.00	-	24.40	16.60	19.23	-	25.26	15.78	40.00	-	20.00	20.00

Table.5 Arbuscular mycorrhizal fungal (AMF) colonization in the roots of *C. procera* growing in Tharaka-Nithi and Makueni County sampling sites, Kenya

Region	% Arbuscules	% Vesicles	% Hyphae	% Intraradicale coils	% Appressoria	% AMF colonization
Tharaka-Nithi	14.58±6.22	70.66±6.95	93.86±1.90	22.68±4.15	36.66±8.23	82.33±3.83
Makueni	28.00±6.54	65.32±14.17	74.00±9.15	21.98±7.57	12.00±6.20	69.99±10.49
***t*-test**	1.49	-0.34	-2.12	-0.08	-2.39	-1.11
P-value	0.175	0.744	0.095	0.937	0.044	0.301

Table.6 Arbuscular mycorrhizal fungal (AMF) root colonization in the roots of sorghum 8 WAE in soil collected from the rhizosphere of *C. procera* growing in the sampling locations of Tharaka-Nithi and Makueni Counties, Kenya

Region	% Arbuscules	% Vesicles	% Hyphae	% Intraradicale coils	% Appressoria	% AMF colonization
Tharaka-Nithi	7.12±2.52	38.60±3.61	90.00±3.50	3.88±1.59	5.26±2.72	64.30±3.49
Kibwezi	9.74±3.72	47.98±13.20	74.02±6.28	0.00	1.20±1.20	61.00±9.29
***t*-test**	0.58	0.69	-2.22	-2.44	-1.37	-0.33
P-value	0.576	0.526	0.057	0.04	0.209	0.748

Fig.1 Location of the sampling points in Makueni County, Kenya.

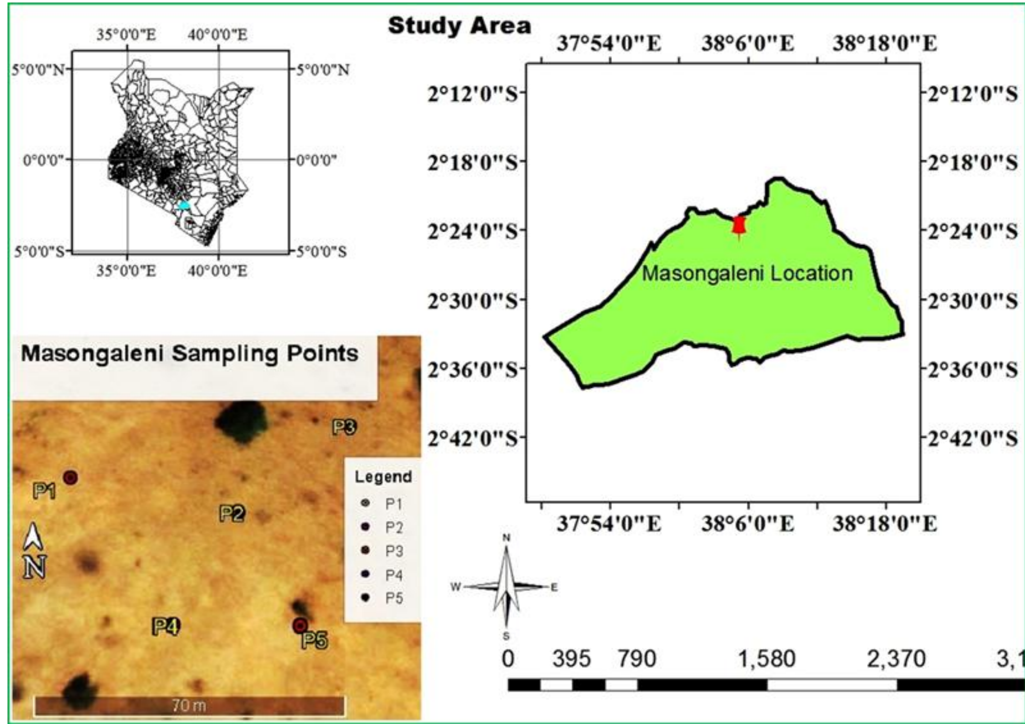


Fig.2 Location of the sampling points in Tharaka-Nithi County, Kenya.

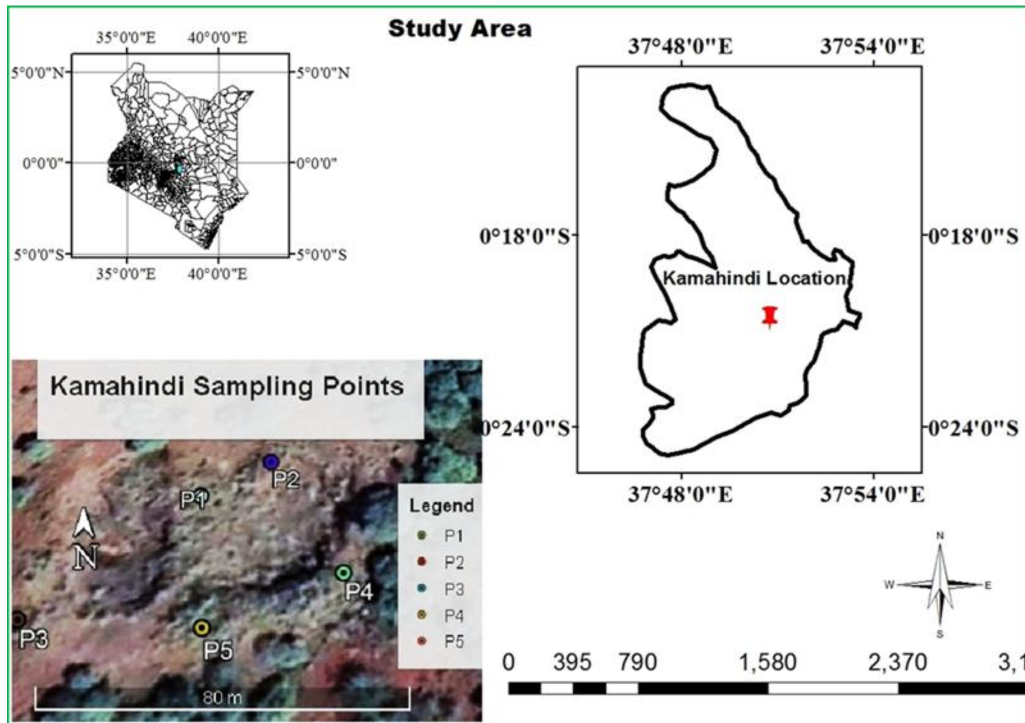


Plate.1 AMF genera in the rhizospheric soil of *C. procera* at Tharaka-Nithi natural site (trap culture): a - *Acaulospora*: Germination orb, mucilaginous wall layer; b - *Gigaspora*: 2 wall layers, globose, spore color-ochraceous; c - *Scutellospora*: Globose, germination shield, germination orb, substending hyphae; d - *Glomus*: 2 wall layers, color; sienna rust, smooth outer layer, e - *Rhizophagous*: 2 wall layers, spore shape sub globose, germination orb, spore color; yellow cream, mucilaginous outer layer; f - *Dentiscutata*: 2 wall layers, spore color; pale ochraceouse, germination orb, ornamented outer layer, cicatrix.

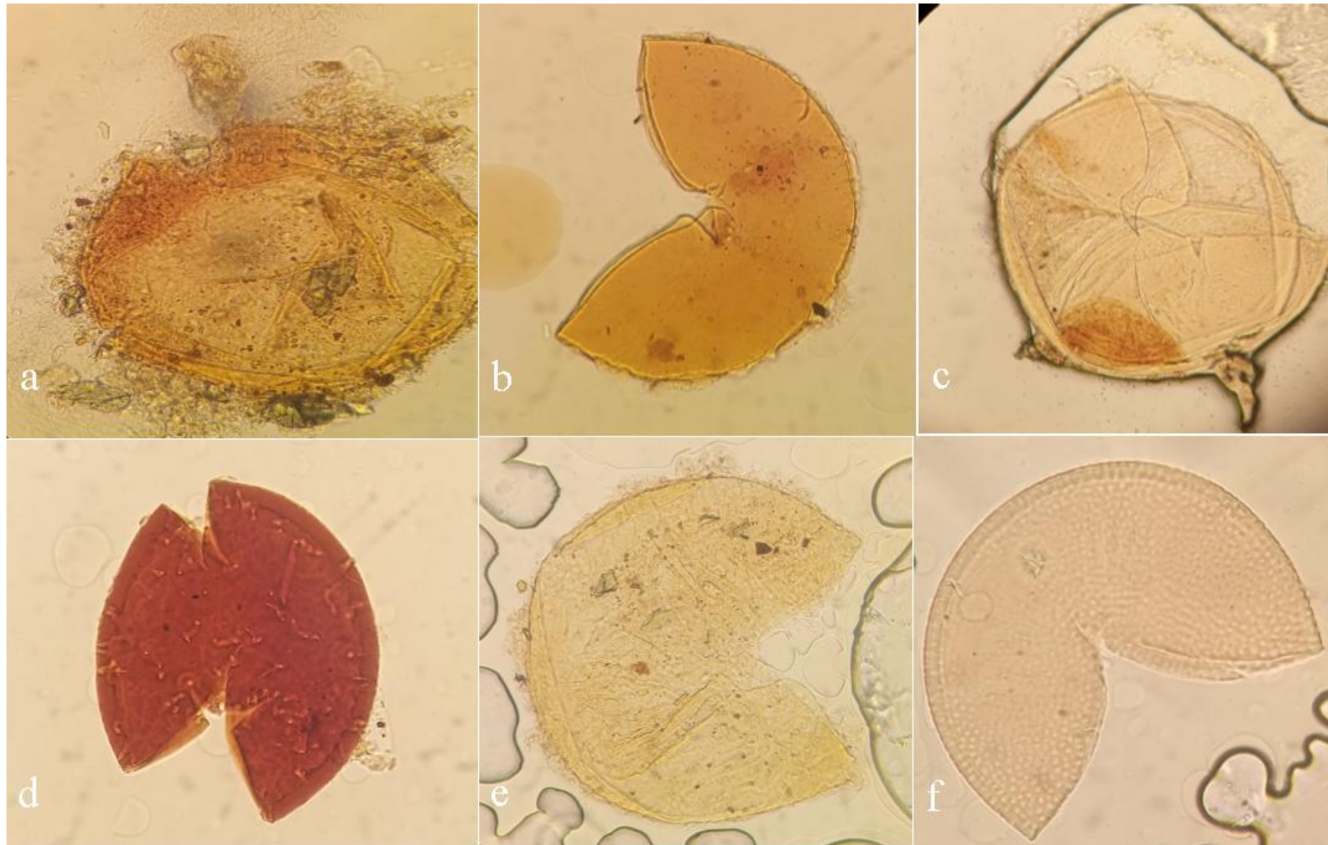


Plate.2 AMF genera in the rhizospheric soil of *C. procera* at Makueni natural site (trap culture): a – *Glomus*: 2 wall layers, spore color; ochraceouse; b - *Scutelospora*: 3 wall layers, spore color; hyaline, germinal wall; 3 bilayares, sporogenous cell, subtending hyphae, germination shield, smooth outer wall layer; c - *Dentiscutata*: two wall layers, spore color; pale ochraceouse, germination orb, ornamented outer layer; d - *Acaulospora*: 3 wall layers; spore color; yellow cream, germination orb, globose-sub globose, subtending hyphae, mucilaginous outer wall layer.

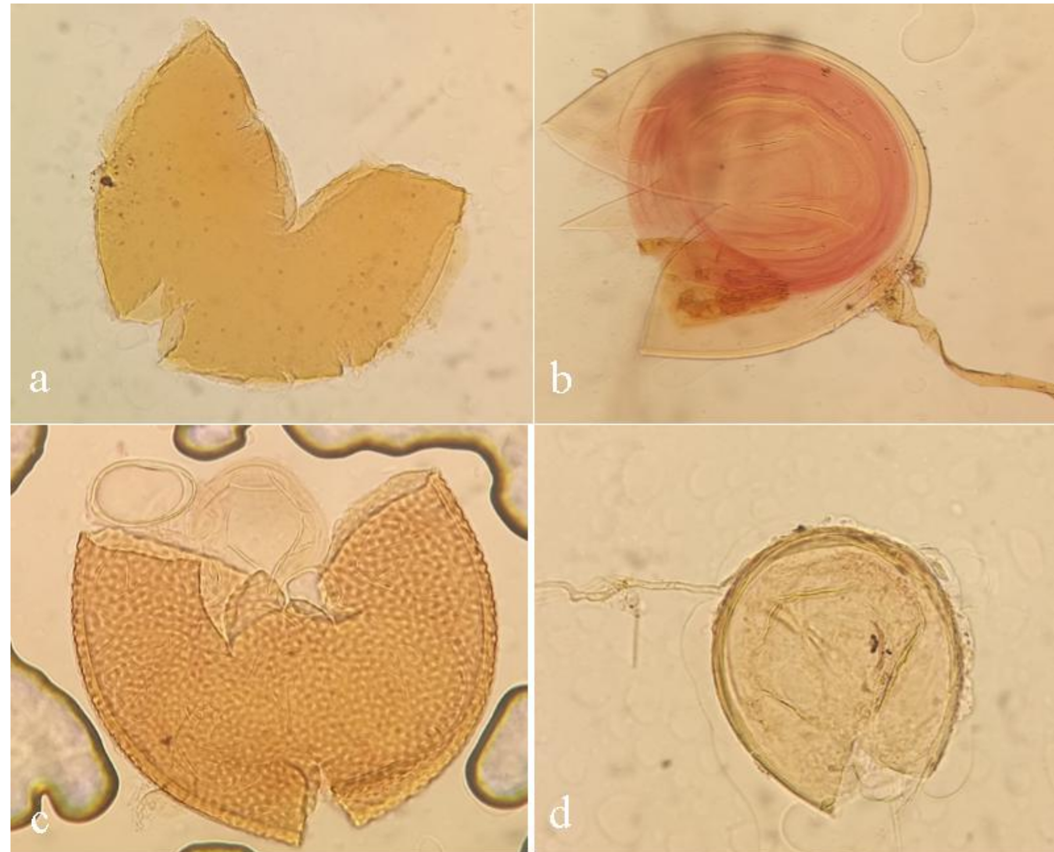


Plate.3 AMF structures (a – Arbuscule, b – Appressoria, c - Intraradicale coil, d – Vesicle, e - Hypha) in the roots of *C. procera* in the natural site of Makueni County.

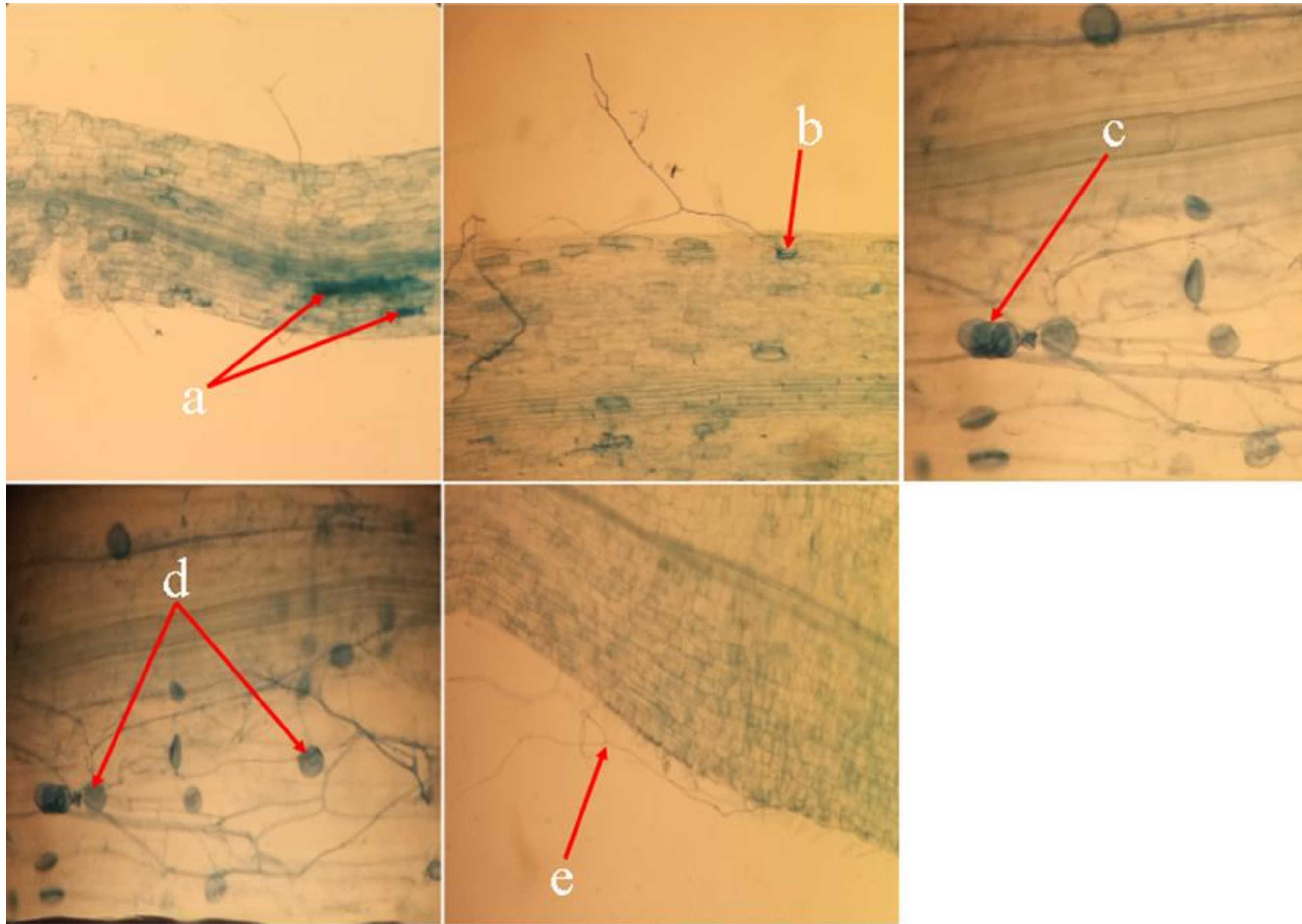
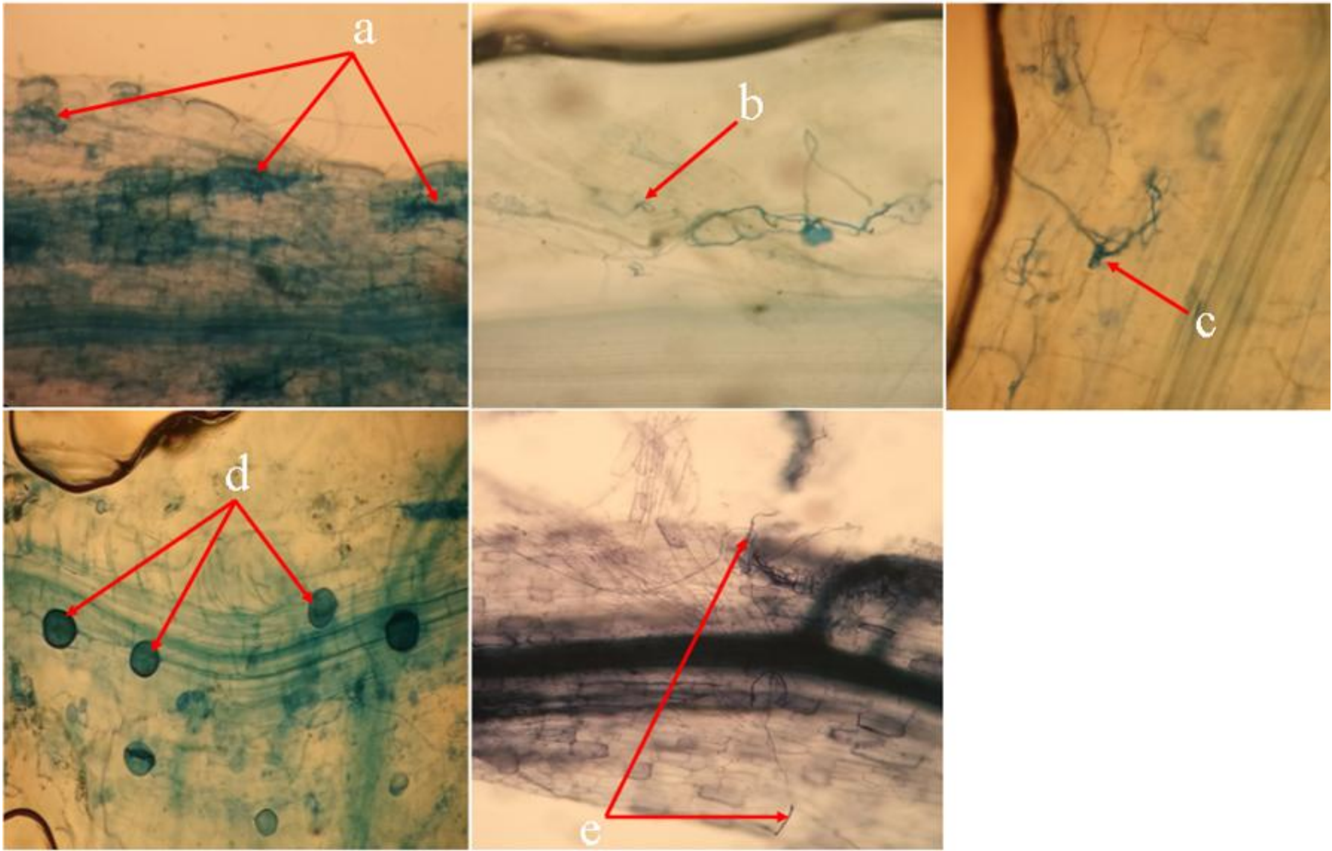


Plate.4 AMF structures (a – Arbuscule, b – Appressoria, c - Intraradicale coil, d – Vesicle, e - Hypha) in the roots of *C. procera* in the natural site of Tharaka-Nithi County.



However, the slight variations observed in the levels of AMF infectivity between the sites may be attributed to the variations in their edaphic properties (Table 1) and the AMF inoculum potentials (Table 6) (Kim *et al.,* 2017). Tharaka-Nithi soil had significantly higher pH ($p = 0.001$), % sand ($p = 0.001$), % silt ($p < 0.001$), available P ($p = 0.026$) and Mg concentrations ($p = 0.0002$) than Makueni soil whereas Makueni soil was significantly higher in % clay ($p < 0.001$) than Tharaka-Nithi soil (Table 1).

These soil parameters had generally marked, though, insignificant effects on percentage AMF colonization (Table 3). On the other hand, Tharaka-Nithi soil had higher AMF inoculum potential than Makueni soil (Table 6).

The variations in AMF colonization between the sites could also be explained by the differences in their AMF genus richness (Table 2) (del Mar Alguacil *et al.,* 2009). This claim is supported by the correlation analysis results (Table 3) which depicted a positive relationship ($r = 0.013$) between AMF colonization and genus richness, suggesting that AMF diversity was a key determinant of AMF colonization in the study areas. A similar observation was made by del Mar Alguacil *et al.,* (2009) in a semi-arid locality in Mediterranean Southeastern Spain. Rurangwa (2019), also, had the same observation where he recorded higher AMF root colonization intensity in banana plantlets inoculated with mixed AMF inoculants compared to plantlets inoculated with a single AMF species inoculant.

The correlation analysis results (Table 3) portrayed a negative correlation between AMF colonization and spore density (r = -0.067). Thus, an increase in AMF spore numbers was not reflected in an increase in AMF colonization. This finding is consistent with the result of He *et al.,* (2016), who observed a negative correlation between spore density and mycorrhizal colonization in a semi-arid ecosystem in northwest China. Becerra *et al.,* (2009) and Dobo *et al.,* (2018) found no association between AMF spore density and root colonization intensity. Hence, spore numbers do not necessarily reflect the level of root colonization.

AMF spores may sometimes remain dormant or may not have reached maturity enough to colonize the host, or maybe parasitized. Furthermore, AMF infectivity is determined by other infective AMF propagules in the soil such as mycelia and infected root fragments, and the host root properties, soil biota and the level of aggressiveness of AMF species to colonize the host (Berg and Smalla, 2009).

In line with this study, Prasanthi *et al.,* (2016) recorded high AMF root colonization (76%) on *C. procera* in the dryland localities of India during summer. These results may suggest that *C. procera* is a mycotrophic species. However, further studies are needed to validate this claim. Other workers have also documented high mycorrhizal dependency of invasive species (Majewska *et al.,* 2017; Moyano *et al.,* 2021) while Majewska *et al.,* (2017) asserted that invasive species highly depend on AMF symbiosis for their growth.

High mycorrhizal colonization of plants growing in the field has also been reported during dry seasons (Sivakumar, 2013; Su *et al.,* 2011). This is attributed to nutrients deficiency and hydric stresses on the plants in the growing sites (Mahmoudi *et al.,* 2019).

In such harsh environments, plants develop distinctive mechanisms to cope with stress (Gull *et al.,* 2019). AMF symbiosis is one of the fundamental mechanisms by which plants survive under conditions of low soil fertility and drought (Powers *et al.,* 2005; Yang *et al.,* 2014).

The low soil moisture, coupled with the inherent soil nutrient deficiency in the dry land areas (Pratt *et al.,* 1977) trigger high positive biotic interactions such as mycorrhizal symbiosis to enhance the survival of plants during harsh periods (Barrow and Aaltonen, 2001; Mahmoudi *et al.,* 2019).

Furthermore, the concentrations of P in the soils sampled in this study were inadequate i.e. 12.83±3.05 ppm (Tharaka-Nithi) and 4.47±0.27 ppm (Makueni) (Table 1) (Horneck *et al.,* 2011; Marks *et al.,* 1999; Okalebo *et al.,* 2002). Hence, P was an important limiting factor for the growth of plants in the study areas.

P has very low mobility and it is easily fixed in soil which restricts its absorption by plants (Shen *et al.,* 2011). AMF promote plant growth by increasing the uptake of P from the soil. The concentration of P in soil, therefore, affects AMF symbiosis(De Miranda and Harris, 1994). Under conditions of inadequate soil P, plants, naturally tend to associate more with AMF to aid in their uptake of P from the soil.

The present work revealed the natural association of AMF with *C. procera* in the dryland localities of Tharaka-Nithi and Makueni Counties, Kenya. Tharaka-Nithi site recorded a slightly higher percentage AMF root colonization, genus richness and diversity than the Makueni site. The natural association of AMF with *C. procera* in the field indicated that AMF symbiosis could potentially be used to improve Calotorpis production in the study areas.

The study also demonstrated the influence of soil physic-chemical properties on AMF diversity and abundance in the rhizosphere of *C. procera* and AMF colonization in the two locations, which could have an important practical implication in the management of the symbiosis. The genera *Dentiscutata*, *Acaulospora*, *Glomus* and

Scutellospora were commonly associated with Calotropis in the study areas, with Glomus being dominant hence, strains of these genera could be isolated and tested for possible incorporation as mycorrhizal inoculants of Calotropis seedlings for better establishment and growth in the field.

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