Article

Not peer-reviewed version

Relative Growth Rates and Root Colonization of Mycorrhizal Inoculated *Corchorus olitorius* L. Cultivars as a Measure of Crop Productivity

Sunday Oni *, Kingsley Ayisi, Victoria Ayodele, Tlou Mogale

Posted Date: 12 March 2026

doi: 10.20944/preprints202603.0949.v1

Keywords: growth rates; arbuscular mycorrhiza fungi; Jew's mallow; root colonization; soil sources

 doi:10.20944/preprints202603.0949.v1

Article

Relative Growth Rates and Root Colonization of Mycorrhizal Inoculated *Corchorus olitorius* L. Cultivars as a Measure of Crop Productivity

Sunday Oni [1,2,*], Kingsley Ayisi [1,2], Victoria Ayodele [1] and Tlou Mogale [2]

[1] Department of Plant Production, Soil Science and Agricultural Engineering, University of Limpopo, Private Bag X 1106, Sovenga 0727, South Africa

[2] Centre for Global Change, University of Limpopo, Private Bag X 1106, Sovenga 0727, South Africa

* Correspondence: adesunoni@gmail.com; Tel: +234-802-082-9620

Abstract

Mycorrhizae utilization is an integral part of adaptation strategy to climate change in semiarid regions. A controlled experiment was conducted at the University of Limpopo, South Africa, to determine the effects of arbuscular mycorrhiza fungi (AMF) and soil type on crop growth and root colonization of Jew's mallow. Treatments comprised two levels of mycorrhiza (with and without), two soil sources (Ofcolaco and Syferkuil), and three Jew's mallow cultivars ("*Amugbadu*", "*Oniyaya*", and a Landrace). The results revealed that the *Amugbadu* cultivar produced the highest Mycorrhizal growth response (MGR) in Syferkuil soil, whereas *Oniyaya* cultivar was the highest in Ofcolaco soil. MGR in Ofcolaco soil was six times higher than in Syferkuil soil. AMF-inoculated *Amugbadu* consistently resulted in the highest crop growth rate (CGR) in Ofcolaco soil. Inoculated Landrace was superior in CGR, compared to uninoculated Landrace in Syferkuil soil. Approximately 71.23-75.86% of the Jew's mallow roots were colonized by AMF in both soil sources, with inoculated "*Amugbadu*" producing the highest root colonization. The landrace root colonization was inferior in both soil sources. Our results indicate that incorporating AMF in Jew's mallow could improve root colonization, growth rate, and productivity in the semi-arid regions of South Africa, depending on soil type.

Keywords: growth rates; arbuscular mycorrhiza fungi; Jew's mallow; root colonization; soil sources

1. Introduction

Jew's mallow (*Corchorus olitorius* L.) is a very useful and important green leafy vegetable widely eaten as a viscous soup for starch supplements in Southern Africa and other parts of the world. It is also cultivated in 0intercropping systems with various crops for its high vitamins and mineral content, and as a pot herb and purgative in many African countries [1,2]. The crop's yield is compromised due to factors including shading from companion bushy or creeping crops, poor cropping, and agronomic practices such as overpopulation and weed pressure. Yields in Jew's mallow in most cases are determined by leaf biomass accumulation (marketable yield), which is a function of growth of the crop. Understanding the relative growth rates of the plant is therefore crucial. Growth is a function of environmental (biotic and abiotic), genetic make-up (heritability), which is influenced by cultivar variability, soil inputs, management factors, and many more. The use of plant growth analysis as indices of production has become a useful tool for comparing growth differences that arise from experimental treatments [3]. Its applications have been found in many fields of research, with ultimate success recorded in forest research as one of the most ecologically significant and useful indices of plant growth [3] for determining productivity in plants. It is also an applicable instrument in plant breeding, plant physiology, and plant ecology for comparing the growth of seedlings with varying initial sizes under different fertilizer levels [4]. Advantages of

 doi:10.20944/preprints202603.0949.v1

growth analysis application in plant studies has been widely reported, including weed control programmes, shading experiments [5], soil moisture studies and carbon dioxide research [6,7]. Analysis of plant growth is necessary as it eliminates growth differences between plants caused by initial size differences [8–10]. The importance of mycorrhiza in influencing growth of plants has long been reported [11,12]. More than 90% of flowering plants depend on mycorrhizae (plant root-fungi symbiosis) for their growth and yields through the influence of root zone nutrient availability. Mycorrhizal dependency is a plant property which refers to the degree of its responsiveness to mycorrhizal colonization. It can be measured by quantifying the growth improvement in response to the mycorrhizal performance, such as the relative non-mycorrhizal contribution compared with mycorrhiza-mediated nutrient uptake [13]. Mycorrhizal dependency has often been quantified by calculating the yield ratio between mycorrhizal-inoculated plants and uninoculated control plants grown in a particular soil at a single soil phosphorus (P) level [14]. Any measure of the benefit provided by mycorrhizae depends on the relative contribution of root and mycorrhiza-mediated nutrient uptake to plants. Mycorrhizal dependency is the result of morphological and physiological plant traits which can vary greatly from one plant species to another and even between cultivars or ecotypes within a single species [11,12,15].

Jew's mallow is a highly mycotrophic plant, but the AMF communities in its roots remain undocumented. This study was established to assess the effectiveness of mycorrhiza in two distinct Plinthic Oxisol soils, growth and root colonization of three Jew's mallow cultivars under controlled conditions.

2. Materials and Methods

2.1. Study Site

An experiment was established under controlled conditions at the Green Biotechnology Research Centre of Excellence (GBRCE), University of Limpopo located between 23°49◎S and 29°42◎E, South Africa. Two contrasting topsoil (0-30cm) from different agro-ecological zones of Limpopo, namely, the University of Limpopo Experimental Farm, Syferkuil and Ofcolaco Cooperative farmers' fields, were used for the experiment. Syferkuil is located in the Capricorn District Municipality, Limpopo Province (23°49◎S, 29°42◎E) with annual precipitation between 400-500 mm, and minimum and maximum temperatures of 4°C and 28°C, respectively. Ofcolaco is located at Sekororo village (24°06◎S, 30°23◎E) in the Mopani District Municipality, Limpopo Province, with an annual rainfall of 700 mm and minimum and maximum temperatures of 18°C and 31°C, respectively. The two fields are located approximately 115 km apart.

2.2. Soil Fertility Analysis

Soil samples were collected from the two experimental sites prior to planting at 0-30 cm depth (where most vegetable functioning root hairs and soil microorganisms, including AMF reside) using a random sampling method. The sampled soil samples were air-dried, crushed, sieved (< 2 mm sieve) and pulverized to form a composite sample. The processed samples were analyzed in the laboratory for some selected chemical and physical properties. The chloromolybdate blue method (Murphy and Riley Bray 1-P extraction) by [16] was adopted to determine available Phosphorus (Avail. P). Potassium (K) and calcium (Ca) were determined by the flame photometer and atomic absorption spectrophotometer (AAS), while magnesium (Mg) and zinc (Zn) were determined by the AAS. Manganese (Mn) and copper (Cu) were analyzed following the procedure of Mehlich-III multi-nutrient extraction method. Soil pH was determined in potassium chloride (KCl) [17]. Total nitrogen (N) was determined by the Kjeldahl method using a Block Digester [18]. Cation exchange capacity (CEC) following the procedure of [19]. [20], modified by [21] was employed in determining organic carbon (Org. C). Soil particle size was determined using the Hydrometer method [22], and textural class by the Textural Triangle chart. Soil exchangeable bases were determined by the flame spectrophotometric method as described by [17].

 doi:10.20944/preprints202603.0949.v1

2.3. Experimental Design

The experiment was laid out as a completely randomized design (CRD) in a 2 x 2 x 3 factorial arrangement, replicated four times in 25 cm diameter and 22 cm depth pots. Treatments consisted of a) two soil sources (Syferkuil and Ofcolaco); b) Commercial mycorrhiza inoculum of "MycoGROW" product applied at 2 levels of inoculation [″with″ (M+) and ″without″ (M-)]; and c) Three (3) Jew's mallow cultivars (*"Amugbadu", "Oniyaya"* and Landrace). The topsoil samples were not pasteurized in order to preserve the native inoculants used as control against the applied inoculum. Five grams of arbuscular mycorrhiza (AM) inoculum was placed at the 1/3 portion of the inoculated pots before transplanting each Jew's mallow seedling cultivar on top and covered with earth. No other treatments were introduced and all cultural practices of production were observed. The pots were irrigated with 250 ml of water at three days' intervals to keep the moisture content of the soils.

2.4. Data Collection, Harvesting and Plant Processing

The growth parameter data for plant growth analysis included the following: Plant height (cm) was measured using a meter rule from the base of the plant to the tip of the main stem. The number of leaves and branches was determined through physical counting. Stem diameter (mm) was measured using a Venire caliper, placed 3 cm from the base of each main plant stem. Leaf chlorophyll content (μg cm^{-2}) was measured with the aid of a Chlorophyll Meter (CCM-200 plus Opti-Sciences). Chlorophyll concentration on each leaf was recorded from two fully selected matured leaves of each plant per pot. The blades of the leaves were clipped with the meter to assess the chlorophyll concentration on the leaves. Plant vigour was determined using a hand-held green seeker crop sensor to assess the health status or vigour of the crop. Soil pH and temperature were determined using soil pH Meter Jenway (370 pH meter model) by inserting the probe vertically into the central portion of the soil to a depth of 15 cm in each pot before recording the readings. Growth parameters were measured from 3 to 9 weeks after establishment (WAE) at every two weeks' interval. Leaf area (LA) was determined in situ using a destructive method by scanning the leaf blade with the aid of a Leaf Area Meter (Area Meter AM 350). Recording was taken between the hours of 10H00 to 13H00 fortnightly on five (5) selected tagged leaves per plant per pot, that were fully expanded and well-illuminated. Hence, LA was computed as length (L) x width (W) in cm. Leaf area index (LAI) was measured using the LAI Ceptometer (Leaf Area Index PAR/LAI Ceptometer, LP-80 AccuPAR Decagon Devices, Inc, Pullman, WA, USA). The Ceptometer probe was inserted into the middle of the canopy of each plant per pot to capture both the leaf canopy and the ground area covered by the entire canopy of the plant. The LAI per plant was computed as postulated by [8]: LAI = LA/GA (where GA = area of pot surface occupied by the plant's canopy). Total canopy chlorophyll content (TCCC) was determined as chlorophyll content x LAI of each plant per pot. These agronomic parameters were determined at harvest intervals using a non-destructive method. The following growth indices equations were computed as postulated by [8,23,24]:

Crop Growth Rate: CGR (g m^{-2} day^{-1}) = $\frac{W_2 - W_1}{t_2 - t_1}$

where: W_1 = Dry weight per unit area at t_1; W_2 = Dry weight per unit area at t_2

t_1 = first harvesting (CUT 1); t_2 = second harvesting (CUT 2)

t_1 and t_2 are time intervals in days.

Relative Growth Rate: RGR (g m^{-2} day^{-1}) = $\frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}$

where: $\log_e W_1$ = Natural logarithm of dry weight per unit area at t_1; $\log_e W_2$ = Natural logarithm of dry weight per unit area at t_2.

Net Assimilation Rate: NAR (g m^{-2} day^{-1}) = $\frac{(W_2 - W_1)(\log_e LA_2 - \log_e LA_1)}{(t_2 - t_1)(LA_2 - LA_1)}$

 doi:10.20944/preprints202603.0949.v1

where: $\log_e LA_1$= Natural logarithm of leaf area per unit area at t_1; $\log_e LA_2$ = Natural logarithm of leaf area per unit area at t_2; LA_1 = leaf area at first harvesting (CUT 1); LA_2 = leaf area at second harvesting (CUT 2).

Leaf Area Ratio: LAR ($m^2\ g^{-1}$) = $LA_2 + LA_1$
$W_2\ W_1$ 2
Leaf Area Index: LAI = LA
GA

where: GA = ground area

The interrelationship between the equations are:

$$RGR = LAR \times NAR$$

$$CGR = NAR \times LAI$$

where: LAI (Leaf Area Index = ratio of leaf to the ground area), LAR (Leaf area ratio = ratio of leaf area to the plant dry weight), NAR (Net Assimilation Rate = rate of increase in dry matter per unit leaf area), RGR (Relative Growth rate = rate of increase in dry matter per unit dry matter) and CGR (Crop Growth Rate = rate of increase in dry matter per unit ground area).

Mycorrhizal Growth Responses (MGR) of Jew's Mallow

Mycorrhizal growth response of the three cultivars was assessed to determine the effectiveness of the mycorrhiza inoculation of the cultivars between 3-9 weeks after establishment (WAE) on the two soils used. The harvest method was cut-and re-growth, where only aboveground samples of the initial and regrowth plants were collected at each harvest in each treatment until the final harvest. The final harvest of the crops was collected at 9 WAE by uprooting the entire plants from the soil. Fresh and dry mass at each and final harvests were determined. Above-ground biomass was oven-dried at 65°C for 72 hours till constant weight was attained. Dried samples were stored in brown envelopes and kept for further analyses while the roots were weighed and preserved in Formalin Acetic Acid (FAA) for assessment of arbuscular mycorrhizal fungi (AMF) root colonization. The plants had no fruits for evaluation. MGR were computed according to [25]:

$$\textbf{If } \overline{NM} < \textbf{AMF, then MGR (\%)} = \left(1 - \left(\frac{\overline{NM}}{AMF}\right)\right) \bullet 100 \quad (1)$$

$$\textbf{If } \overline{NM} > \textbf{AMF, then MGR (\%)} = \left(-1 + \left(\frac{AMF}{\overline{NM}}\right)\right) \bullet 100 \quad (2)$$

where: $\overline{NM}$ is the mean total biomass of control or No-mycorrhizal (NM) Jew's mallow plants, and AMF is the total biomass of the treated or mycorrhizal plants per cultivar. A positive MGR suggests a benefit of the plant from introduced AMF whereas a negative MGR indicates the plant is suppressed by AMF.

2.5. Plant Processing for AM Colonization

Fresh roots of the harvested plant samples were carefully extracted from the soil and rinsed thoroughly under running tap water over a wire sieve to remove the adhered soil particles and other particulates. Root samples containing fine root hairs and terminal finer feeder (where absorption of nutrients and water takes place) were selected per treatment and preserved in freshly prepared Formalin Acetic Acid (FAA) until clearing and staining [26]. The FAA consisted of 85 ml ethanol absolute, 10 ml of 40% Formaldehyde and 5 ml acetic acid glacial solution.

 doi:10.20944/preprints202603.0949.v1

2.5.1. Staining of AM Fungal Roots

The modified simple technique for staining AMF structures in roots using Ink and Vinegar as described by [27] was adopted. The preserved root samples were chopped into small fragments of approximately 1 cm and mixed thoroughly to ensure uniform staining. The roots were cleared by completely covering them with prepared 10% KOH and heated at 90°C for 5 minutes in a water bath using an autoclave. The KOH solution was rinsed off from the roots, and the roots were thoroughly rinsed with tap water acidified with a few drops of vinegar 5% Acetic Acid (AA) for a minimum of 20 minutes, or until the rinse water was no longer brown. Cleaned roots were boiled in 5% freshly prepared ink-vinegar solution (Shaeffer black ink) with pure white household vinegar (5% acetic acid). Roots were removed from the autoclave after 3 minutes when the solution began to boil and distained by rinsing in 4 to 5 changes of tap water acidified with few drops of vinegar. After distaining, root tissues were pale blue in contrast. The stained roots were then stored in tap water acidified with a few drops of vinegar at 4°C in the refrigerator for 2 weeks. It allowed excess stain to leach out from the roots, obtaining more contrast between fungal tissue and background plant cells. Stained samples were examined under a dissecting stereo-binocular microscope for percentage root colonization.

2.5.2. Assessments of AMF Root Colonization Using a Grid-Line Intersection Method

The simplified gridline intersection technique proposed by [28] was employed to determine the level of infection and estimation of AMF root colonization. For each sample, 30 pieces of stained root segments were randomly selected and spread out evenly on a 9 cm x 9 cm petri dish with grid lines marked on the bottom of the dish to form 0.5-inch x 0.5-inch squares. Observation was made under a visible light dissecting stereo-binocular microscope at 40 X magnification power (ZEN lite 2012 computer software package of ZEISS –Discover, V12 Release 2.0) to estimate AMF colonization. Vertical and horizontal gridlines were scanned and the presence or absence of infection were recorded at each point where the roots intersected a line.

Percentage root colonization (%) = No. of mycorrhizal root segment x 100
Total No. of root segment

2.6. Statistical Analysis

In this experiment, a standard procedure of variance analysis was used (Completely Randomized Design in Factorial Model-ANOVA). AMF% infection values were transformed using arcsine square root to satisfy normal distribution and homogeneity of variance assumptions for three-way ANOVA. Multiple comparisons of means were performed by the Tukey LSD test ($P \leq 0.05$). The STATISTIX (version 10.0) package was used for statistical analysis.

3. Results

Results of the initial soil analysis from the two sites (Table 1) were generally very low in N, medium in P, and high in K compared to the amounts of 2.00 g kg^{-1}; 20.00 mg kg^{-1} and 250.00 mg kg^{-1}, respectively, required by plants to complete their life-cycles [29]. The pH of soil from Syferkuil was neutral and that of Ofcolaco was slightly acidic range (7.02 and 5.97, respectively). The results further indicated that the soil from Syferkuil has a high organic carbon of 1.25% compared to Ofcolaco with 0.95% organic carbon. The Ofcolaco and Syferkuil soils are contextually classified as clayey loam with 29.51% clay and Sandy-clay loam with 38.62% clay, respectively.

Table 1. Pre-planting physical and chemical characteristics of the study sites.

Properties	Values	
	Ofcolaco	Syferkuil
pH (H2O)	7.02	5.97
Total N (%)	<0.05	<0.05

 doi:10.20944/preprints202603.0949.v1

Available P (mg. L^{-1})	24.00	52.75
Organic Carbon estimate (%)	0.95	1.25
Electrical Conductivity (dS m^{-1})	0.58	1.08
K (mg. L^{-1})	288.75	153.75
Ca (mg. L^{-1})	752.50	568.00
Mg (mg. L^{-1})	543.00	179.50
Total cations (cmol. L^{-1})	9.09	4.78
Acid saturation (%)	1.25	1.50
Base saturation (%)	99.22	99.26
Mn (mg. L^{-1})	21.75	40.25
Zn (mg. L^{-1})	1.33	5.83
Cu (mg. L^{-1})	2.70	4.73
Clay estimate (%)	38.62	29.51
Textural class	Clayey sand	Sandy-clayey loam

Although, not statistically different from each other, Jew's mallow plants grown in Ofcolaco and Syferkuil soil types showed a tendency for a more positive response to soils amended with mycorrhiza than non-amended (control) soils (Table 2). The percentage MGR in the Ofcolaco soil type was six (6) times greater than the %MGR in the Syferkuil soil type. Significant differences in cultivar response were observed in plants grown in the Syferkuil soils and not in the Ofcolaco soil type (Table 2). In the Syferkuil soils, growth response ranged from 39.23% to 57.64, with the "*Amugbadu*" cultivar exhibiting an 18.41 percentage point increase in response compared to the landrace. No difference in response was observed between the "*Oniyaya*" cultivar and the landrace.

Table 2. Mycorrhizal Growth Response (%) of Jew's mallow cultivars grown on two different soils under controlled conditions.

Treatment	Soil Source	Mycorrhizal Growth Response (%)	Soil Source	Mycorrhizal Growth Response (%)
Mycorrhiza				
M+	Ofcolaco	52.91[a]	Syferkuil	7.26[a]
M-	Ofcolaco	52.62[a]	Syferkuil	5.68[a]
Tukey LSD (0.05)		ns		ns
SE±		4.66		6.29
Cultivar				
"Amugbadu"	Ofcolaco	51.80[a]	Syferkuil	57.64[a]
"Oniyaya"	Ofcolaco	57.10[a]	Syferkuil	46.60[ab]
Landrace	Ofcolaco	49.40[a]	Syferkuil	39.23[b]
Tukey LSD (0.05)		ns		*
SE±		5.59		7.12

Means within columns followed by the same letter are not significantly different ($P \leq 0.05$); *significantly different at ($P \leq 0.05$); **significantly different at ($P \leq 0.01$); ns=Not significant; M+ = With Mycorrhiza; M- = Without Mycorrhiza.

3.1. Relative Growth Rate

The effects of mycorrhiza inoculation on the leafiness (Leaf Area Ratio - LAR) and assimilatory capacities (Net Assimilatory Rate - NAR) assessed through relative growth rate (RGR) and crop growth rate (CGR) of Jew's mallow cultivars grown in Ofcolaco and Syferkuil soils are presented in Figures 1 and 2. There was no mycorrhiza effect on the RGR of Jew's mallow plants grown in the two soil sources. However, an effect of mycorrhizal application on CGR was observed in plants grown on Syferkuil soil but not in those of Ofcolaco soil. At Syferkuil, CGR of uninoculated plants was only 35.8% that of the inoculated plants (Figure 2).

 doi:10.20944/preprints202603.0949.v1

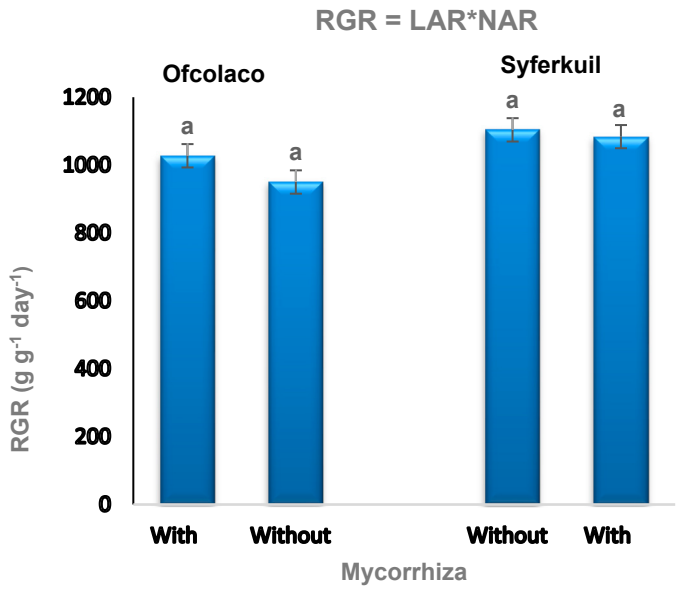

Figure 1. Effect of arbuscular mycorrhiza (AM) on relative growth rate (RGR) of inoculated and non-inoculated Jew's mallow plants grown on Ofcolaco and Syferkuil soils under controlled conditions.

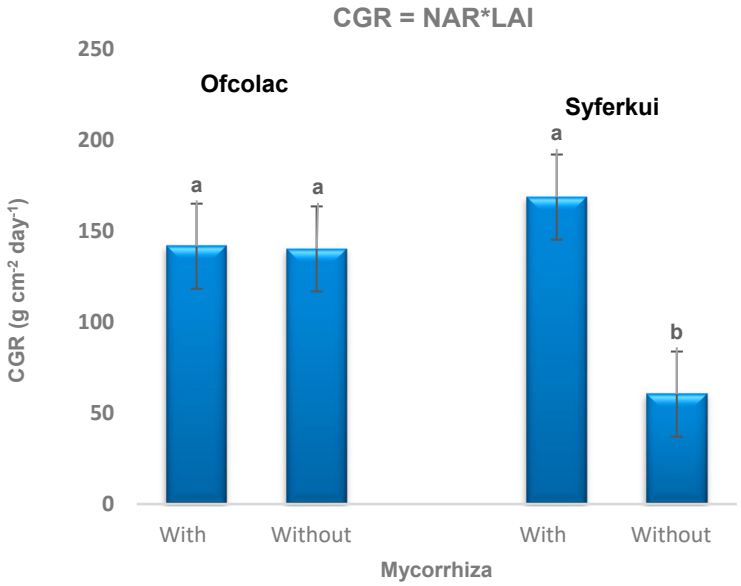

Figure 2. Crop growth rate (CGR) of different Jew's mallow cultivars as influenced by arbuscular mycorrhiza (AM) inoculation on Ofcolaco and Syferkuil soils under controlled conditions.

In Table 3, cultivar differences in RGR and CGR due to mycorrhiza inoculation were observed in Ofcolaco soil, whereas responses of CGR and AGR to inoculation were recorded from plants grown in Syferkuil soil. The RGR of the inoculated "*Amugbadu*" cultivar grown on Ofcolaco soil was 49.41% and 12.99% greater than that of the inoculated "*Oniyaya*" cultivar and Landrace, respectively (Table 3). Crop growth rate, defined as the rate of increase in dry matter per unit ground area, showed that "*Amugbadu*" cultivar treated with AMF was again consistently 57.70% and 39.59% greater than "*Oniyaya*" cultivar and Landrace, respectively, on the Ofcolaco soil. On Syferkuil soil, inoculated Landrace was superior in CGR (233.29 g cm^{-1} day^{-1}) compared to other cultivars. The lowest value (24.82 g cm^{-1} day^{-1}) was obtained from the same cultivar, but, without AM inoculation (Table 3). Although there were no significant differences among the three Jew's mallow cultivars grown either

 doi:10.20944/preprints202603.0949.v1

with or without AM applications, the interaction effect between AMF and Landrace was significant, with the highest AGR (0.13 g day^{-1}) observed in Ofcolaco soil and inoculated "*Oniyaya*" cultivar having the lowest value (0.07 g day^{-1}). Similarly, inoculated "*Amugbadu*" outperformed all other cultivars with or without AM treatment application relative to the least value (0.04 g day^{-1}) obtained from uninoculated Landrace cultivated on Syferkuil soil (Table 3).

Table 3. Interactive effects of mycorrhiza and cultivars of Jew's mallow on relative growth ratio, crop growth rate and average growth rate on Ofcolaco and Syferkuil soils under controlled conditions.

Mycorrhiza (M)	Cultivar	RGR (g g^{-1} day^{-1})	CGR (g cm^{-1} day^{-1})	AGR (g day^{-1})
Ofcolaco soil				
M+	*"Amugbadu"*	1297.20^{a}	209.75^{a}	0.09^{a}
M+	*"Oniyaya"*	656.20^{b}	88.72^{b}	0.07^{a}
M+	Landrace	1128.60^{ab}	126.71^{a}	0.13^{a}
M-	*"Amugbadu"*	1120.10^{ab}	118.22^{a}	0.10^{a}
M-	*"Oniyaya"*	901.40^{ab}	141.45^{a}	0.12^{a}
M-	Landrace	829.30^{ab}	161.27^{a}	0.11^{a}
Tukey LSD (0.05)		*	ns	ns
SE±		236.49	72.67	0.04
Syferkuil soil				
M+	*"Amugbadu"*	1104.70^{a}	158.63^{ab}	0.17^{a}
M+	*"Oniyaya"*	1099.60^{a}	114.48^{ab}	0.14^{a}
M+	Landrace	1047.20^{a}	233.29^{a}	0.10^{ab}
M-	*"Amugbadu"*	955.40^{a}	102.81^{ab}	0.13^{a}
M-	*"Oniyaya"*	1029.50^{a}	53.72^{b}	0.07^{ab}
M-	Landrace	1326.40^{a}	24.82^{b}	0.04^{b}
Tukey LSD (0.05)		ns	*	*
SE±		245.92	69.17	0.04

Means within columns followed by the same letter are not significantly different ($P \leq 0.05$); *significantly different at ($P \leq 0.05$); **significantly different at ($P \leq 0.01$); ns=Not significant; M+ = With Mycorrhiza; M- = Without Mycorrhiza.

3.2. Root Colonization

Our results showed that approximately 71.23% and 75.86% of the studied Jew's mallow plant roots grown in Ofcolaco and Syferkuil soil types, respectively, were colonized by AM fungi (Tables 4 and 5). No statistical differences in AM root colonization with respect to plant vigour of the inoculated plants relative to control plants were observed in both soil types. Among the three cultivars cultivated on the two soil sources, "*Amugbadu*" cultivar consistently showed a significantly higher AMF root colonization compared to others under the same experimental conditions, whereas the landrace was consistently the lowest (Tables 4 and 5).

Results from the Ofcolaco soil type revealed that the "*Amugbadu*" cultivar outperformed "*Oniyaya*" and Landrace cultivars regarding the plant vigour and soil pH, whereas the Landrace cultivar outperformed the other two cultivars on the same parameters on Syferkuil soil type (Tables 4 and 5). The results also indicated a non-responsive variation between Ofcolaco and Syferkuil soil types.

The interaction effects of inoculating "*Amugbadu*" cultivar with AMF consistently resulted in significantly higher values of root colonization on the two soil sources compared to other treatment combinations. Although statistically similar to other counterparts, the uninoculated "*Amugbadu*" cultivar also showed a tendency of high percentage plant vigour on the two soil sources. The results from Ofcolaco were similar to Syferkuil soils where soil pH and temperature on Ofcolaco and Syferkuil soils under the same experimental conditions were highest due to synergistic interactions between Landrace and AMF (Tables 4 and 5). The correlations between different growth

 doi:10.20944/preprints202603.0949.v1

characteristics and soil parameters at different growth stages in Ofcolaco and Syferkuil soil sources are shown in Tables 6 and 7. The results revealed that plant height positively correlated ($P \leq 0.01$) with the stem diameter at r = 0.53** and 0.63*** and chlorophyll contents at r = 0.52** and 0.41** on Ofcolaco and Syferkuil soils, respectively.

Table 4. AM inoculation, Jew's mallow cultivars and their interactions with selected soil factors and root colonization on Ofcolaco soil under a controlled environment.

Treatment		Colonization (%)	PV (%)	Soil pH	Soil Temp (°C)
Mycorrhiza (M)					
M+		26.87^{a}	2.78^{a}	6.97^{a}	26.85^{a}
M-		7.73^{b}	2.72^{a}	6.87^{a}	26.73^{a}
LSD$_{(0.05)}$		**	ns	ns	ns
Cultivar					
"Amugbadu"		27.99^{a}	3.76^{a}	6.89^{a}	26.78^{ab}
"Oniyaya"		13.67^{ab}	3.70^{a}	6.87^{a}	26.67^{b}
Landrace		10.24^{b}	3.72^{a}	6.88^{a}	26.91^{a}
LSD$_{(0.05)}$		*	ns	ns	*
Mycorrhiza (M)	**Cultivar**				
M+	*"Amugbadu"*	43.61^{a}	3.72^{a}	6.87^{ab}	26.85^{ab}
M+	*"Oniyaya"*	21.56^{ab}	3.71^{a}	6.87^{ab}	26.67^{b}
M+	Landrace	15.44^{b}	0.76^{a}	6.94^{a}	27.03^{a}
M-	*"Amugbadu"*	12.37^{b}	3.79^{a}	6.85^{b}	26.71^{b}
M-	*"Oniyaya"*	5.78^{b}	3.69^{a}	6.88^{ab}	26.68^{b}
M-	Landrace	5.05^{b}	0.71^{a}	6.87^{ab}	26.79^{ab}
LSD$_{(0.05)}$		**	ns	*	*
F-values					
Mycorrhiza		*	ns	ns	ns
Cultivar		*	ns	ns	*
Mycorrhiza*Cultivar		*	ns	*	*

Means within columns followed by the same letter are not significantly different ($P \leq 0.05$); *significantly different at ($P \leq 0.05$); **significantly different at ($P \leq 0.01$); ns=Not significant; M+ = With Mycorrhiza; M- = Without Mycorrhiza, PV=Plant Vigour, Soil Temp.=Soil temperature.

Table 5. AM inoculation, Jew's mallow cultivars and their interactions on some selected soil factors and percentage root colonization on Syferkuil soil under a controlled environment.

Treatment	Colonization (%)	PV (%)	Soil pH	Soil Temp (°C)
Mycorrhiza (M)				
M+	24.02^{a}	3.45^{a}	6.92^{a}	26.88^{a}
M-	5.04^{b}	2.44^{a}	6.88^{a}	26.83^{a}
LSD$_{(0.05)}$	***	ns	ns	ns
Cultivar				
"Amugbadu"	18.31^{a}	2.35^{a}	6.88^{a}	26.80^{a}
"Oniyaya"	14.97^{a}	2.91^{a}	6.89^{a}	26.77^{a}
Landrace	10.32^{a}	3.58^{a}	6.94^{a}	26.89^{a}
LSD$_{(0.05)}$	ns	ns	ns	ns

 doi:10.20944/preprints202603.0949.v1

Mycorrhiza (M)	Cultivar				
M+	*"Amugbadu"*	33.17[a]	0.74[a]	6.89[a]	26.77[a]
M+	*"Oniyaya"*	25.31[ab]	2.71[a]	6.88[a]	26.72[a]
M+	Landrace	13.58[bc]	3.87[a]	7.00[a]	26.94[a]
M-	*"Amugbadu"*	3.44[c]	3.95[a]	6.88[a]	26.83[a]
M-	*"Oniyaya"*	7.06[c]	3.12[a]	6.89[a]	26.82[a]
M-	Landrace	4.63[c]	3.29[a]	6.87[a]	26.85[a]
$LSD_{(0.05)}$		*	ns	ns	ns
F-values					
Mycorrhiza		**	ns	ns	ns
Cultivar		ns	ns	ns	ns
Mycorrhiza*Cultivar		*	ns	ns	ns

Means within columns followed by the same letter are not significantly different ($P \leq 0.05$); *significantly different at ($P \leq 0.05$); **significantly different at ($P \leq 0.01$); ns=Not significant; M+ = With Mycorrhiza; M- = Without Mycorrhiza, PV=Plant Vigour, Soil Temp.=Soil temperature.

Significant positive correlations were also observed between the number of leaves and the number of branches at r = 0.83*** and 0.81*** on Ofcolaco and Syferkuil soils, respectively, and stem diameter with r = 0.56** on Syferkuil soil. The number of leaves also correlated positively with leaf area at r = 0.49**, leaf area index at r = 0.49** and total chlorophyll canopy content at r = 0.43**. Significant negative correlations were observed between stem diameter and soil pH at r = -0.58** and -0.51** on Ofcolaco and Syferkuil soils, respectively, and partially with plant vigour at r = 0.43** on Syferkuil soil type. However, leaf chlorophyll content was positively related to the total chlorophyll canopy content at r = 0.56**. Soil pH was partially positively correlated with leaf area at r = 0.42** and 0.48**, leaf area index at r = 0.42** and 0.48**, and leaf area index as well as total chlorophyll canopy content at r = 0.40** and 0.46** on Ofcolaco and Syferkuil soil type, respectively. On Syferkuil soil, soil temperature showed a negative correlation with leaf area and leaf area index. The relationship between leaf area and leaf area index was perfectly correlated at r = 1.00*** on both soil sources and with total canopy chlorophyll contents at r = 0.82*** and 0.88*** on Ofcolaco and Syferkuil soil types, respectively. However, leaf area index was significantly positively correlated ($P \leq 0.05$) with total canopy chlorophyll contents at 'r' values 0.82*** and 0.88*** on Ofcolaco and Syferkuil soils respectively (Tables 6 and 7).

Table 6. Correlation coefficient relationships between plant growth parameters and soil factors on Ofcolaco soil.

	PH	NoL	NoB	SD	Chl	PV	Soil pH	Soil Temp	LA	LAI	TCCC
NoL	-0.10[ns]										
BoB	-0.22[ns]	0.83***									
SD	0.53**	0.18[ns]	-0.05[ns]								
Chl	0.52**	-0.07[ns]	-0.16[ns]	0.10[ns]							
PV	0.14[ns]	0.03[ns]	-0.02[ns]	0.13[ns]	0.08[ns]						
Soil pH	-0.10[ns]	-0.29*	0.08[ns]	-0.58**	0.04[ns]	-0.21[ns]					
Soil Temp	0.32*	-0.01[ns]	-0.07[ns]	0.31*	0.01[ns]	0.36*	-0.31*				
LA	-0.17[ns]	-0.16[ns]	0.12[ns]	-0.19[ns]	-0.23[ns]	0.00ns	0.42**	-0.17[ns]			
LAI	-0.17[ns]	-0.16[ns]	0.12[ns]	-0.19[ns]	-0.23[ns]	0.00[ns]	0.42**	-0.17[ns]	1.00***		
TCC	0.15[ns]	-0.19[ns]	0.03[ns]	-0.08[ns]	0.29*	0.08[ns]	0.40**	-0.15[ns]	0.82***	0.82***	

Significance levels: * P ≤ 0.05, ** P ≤ 0.01, *** P≤ 0.001, ns= not significant. PH=Plant Height, NoL=Number of leaves, NoB=Number of branches, SD=Stem diameter, Chl=Chlorophyll content, PV=Plant vigour, Soil Temp=Soil Temperature, LA=Leaf Area, LAI=Leaf Area Index, TCCC=Total Canopy Chlorophyll Content.

Table 7. Pearson correlation among plant growth characteristics and soil conditions on Syferkuil soil.

 doi:10.20944/preprints202603.0949.v1

	PH	NoL	NoB	SD	Chl	PV	Soil pH	Soil Temp	LA	LAI	TCCC
NoL	0.16^{ns}										
NoB	-0.21^{ns}	0.81***									
SD	0.63**	0.56**	0.18^{ns}								
Chl	0.41**	0.23^{ns}	0.14^{ns}	0.30*							
PV	0.32*	0.39*	0.27^{ns}	0.43**	0.33*						
Soil pH	-0.27^{ns}	-0.17^{ns}	0.25^{ns}	-0.51**	0.14^{ns}	-0.04^{ns}					
Soil Temp	0.40*	-0.00^{ns}	-0.23^{ns}	0.27^{ns}	0.03^{ns}	0.38*	-0.30*				
LA	-0.01^{ns}	0.29*	0.49**	0.15^{ns}	0.14^{ns}	0.18^{ns}	0.48**	-0.44**			
LAI	-0.01^{ns}	0.29*	0.49**	0.15^{ns}	0.14^{ns}	0.18^{ns}	0.48**	-0.44**	**1.00*** **		
TCC	0.16^{ns}	0.30*	0.43**	0.23^{ns}	0.56**	0.26^{ns}	0.46**	-0.37*	0.88***	0.88***	

Significance levels: * P ≤ 0.05, ** P ≤ 0.01, *** P≤ 0.001, ns= not significant. PH=Plant Height, NoL=Number of leaves, NoB=Number of branches, SD=Stem diameter, Chl=Chlorophyll content, PV=Plant vigour, Soil Temp=Soil Temperature, LA=Leaf Area, LAI=Leaf Area Index, TCCC=Total Canopy Chlorophyll Content.

4. Discussion

The utilization of organic sources of mycorrhizae forms an integral part of a sustainable adaptation strategy to climate change in arid and semi-arid zones of South Africa. Results from soil analyses revealed that the soil pH levels of 7.02 at Ofcolaco and 5.97 at Syferkuil are generally suitable for the cultivation of most tropical and sub-tropical crops [30]. Arbuscular mycorrhizal inoculation has been reported to have a significant positive contribution to plant growth and yields [31,32]. On the other hand, its effects might equally suppress plants' growth under some growing conditions, especially where soil P is in abundance [33]. The percentage (8.14%) of AM contributions to the growth of the three Jew's mallow cultivars may be due to the differences in the clay content proportions of the two soils. The clay content is strongly related to the P mineralization in the two soils which varies with soil organic matter content, crop types and cultivars. The same reason could be attributed to variations in the percent root colonization on Ofcolaco and Syferkuil soils used in the study. Previous studies showed that AMF colonization decreased in soils with high phosphate concentrations [34]. Therefore, the available phosphate contents in soil could be a major factor influencing the colonization rates in the different soil types (Ofcolaco and Syferkuil) utilized in this study. The non-responsiveness of CGR in Ofcolaco and RGR in Syferkuil soils, to AM inoculation might be as a result of the colonization capabilities of the mixed inoculum (mycoGROW) fungi used in the study and their symbiotic capabilities to colonize specific plant roots in relation to both soil properties and host plant [35]. Different culture of AM behaves differently under the same ecological conditions, plant species and even in the same cultivar. Moreover, this may also be in connection with the short time frame or time lag used to conduct the study under greenhouse conditions, because some species of AMF need a long time to germinate, while others seem not to have the capability of germination [36].

The response of mycorrhizal growth on a plant depends on the plant species and cultivars within the same species. The present study revealed that arbuscular mycorrhizae isolates are present in the rhizosphere of Ofcolaco and Syferkuil semi-arid regions of South Africa and that colonization occurs in different habitats. The higher percentage of root colonization in the Syferkuil soil relative to that of Ofcolaco was the result of a higher number of AMF spores present in the soil. Specifically, the effects of the same AMF species on plants may differ depending on the soil type. AMF colonization is known to have a positive effect on plant growth, but the physio-chemical state of the soil could directly impact the symbiotic relationship between the plant and fungus [37]. AMF colonization in different semi-arid agro-ecological regions of South Africa in relation to soil properties, mostly pH and temperature, on Jew's mallow production had not been investigated by researchers, thus making this study a first of its kind in South Africa. The positive constant increase of "*Amugbadu*" to the applied AM compared to uninoculated on the two soils indicates the fungal contributions to the

 doi:10.20944/preprints202603.0949.v1

plants' physiological growth rate. This agreed with the study of [38], who reported a significant positive response of "*Amugbadu*" and "*Oniyaya*" cultivars to the applied AM under N and P fertilization on an alfisol. The positive association between plant height and chlorophyll concentration in the leaves demonstrated the synergistic effects of chlorophyll on plant growth. The chlorophyll content in chloroplasts facilitates photosynthesis, which influences plant development. The primary source of photosynthate materials in plants is the leaf, which is translocated to the roots (sink) and other parts of the plant [39]. Leaf Area (LA) positively correlated with leaf area index (LAI) at the vegetative stage of Jew's mallow plant, irrespective of the cultivar. These observations are supported by the findings of [40]. Varietal differences observed among the cultivars during the study are reflected in their genetically make-up characters and possibly the environmental factors. This is in corroboration with the study of [41].

5. Conclusions

The study revealed that mycorrhizae isolates are present in the rhizosphere of Ofcolaco and Syferkuil, a semi-arid region of South Africa and that colonization occurs in different habitats. Application of AMF resulted in a greater Relative Growth Ratio and Crop Growth Rate in the Ofcolaco soil, while inoculation of AMF led to a greater Crop Growth Rate and Average Growth Rate in the Syferkuil soil. "*Amugbadu*" cultivar consistently responded positively to AMF amendment on South African Plinthic Oxisol soils due to their synergetic effects compared to the other cultivars. The effect of AMF inoculation was not reflected in the physiological growth rate of the other cultivars ("*Oniyaya*" and Landrace), indicating that the benefit will not always be realized early in the growing cycle of these species. However, the direct benefits of AMF can be accessed via nutrient uptake of the elements within the plants. Thus, further studies might reveal this under a long period of cultivation.

Author Contributions: Conceptualization, Oni S. and Ayisi K..; methodology, Oni S and Ayisi K..; software, Oni S..; validation, Oni S. and Ayisi K..; formal analysis, Oni S.; investigation, Oni S.; resources, Ayisi K.; data curation, Oni S. and Ayisi K.; writing—original draft preparation, Oni S.; writing—review and editing, Oni S., Ayisi K., Ayodele V. and Mogale Tlou; visualization, Oni S., Ayisi K. and Mogale Tlou; supervision, Ayisi K. and Ayodele V.; project administration, Oni S. and Ayisi K.; funding acquisition, Ayisi K. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Acknowledgments: This study acknowledges the funding provided by the Department of Science, Technology (DSTI) and the National Research Foundation (NRF) through the Centre for Global Change (CGC), University of Limpopo. The contribution of resources provided by the Department of Plant Production, Soil Science and Agricultural Engineering, University of Limpopo, is also acknowledged.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Loumerem, M. and Alercia, A. Descriptors of jute (*Corchorus olitorius* **L**.). *Genet Resource Crop Evolution,* **2016**, 63, 1103-1111.
2. Denton, O.A. and Nwangburuka, C.C. Morphological Diversity among *Corchorus olitorius* Accessions Based on Single Linkage Cluster Analysis and Principal Component Analysis. *Jordan Journal of Biological Sciences,* **2012**, 5, 191-196.
3. Buso, P.H., Oliveira, R.A., Daros, E., Zambon, J.C., Venancio, W.S., Souchie, E.L., Buso, E.K.R.P. and Díaz-Zorita. Plant growth analysis describing the soybean plants response on dryland field to seed co-inoculation. *Ciência Rural,* **2021**, 51, 1-13.
4. Akinrinlola, R.J. Evaluation of bacillus strains for plant growth-promotion and predictability of efficacy by in vitro physiological traits. Available online:

 doi:10.20944/preprints202603.0949.v1

https://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1588&context=plantpathpapers (accessed on 15 February 2021).

5. Mishra, S., Joshi, B., Dey, P., Nayak, P. and Guru, S.K. Effect of shading on growth, development and reproductive biology of Phalaris minor Retz. *Journal of Pharmacognosy and Phytochemistry*, **2020,** 9, 803-807.
6. Horel, Á. and Zsigmond, T. Plant Growth and Soil Water Content Changes under Different Inter-Row Soil Management Methods in a Sloping Vineyard. *Plants*, **2023**, 12, 1549-1554.
7. Makinde M. Sensitivity of Okra Growth Indices to Various Moisture Conditions. *American Journal of Agricultural and Biological Sciences*, **2022**, 17, 51-57.
8. Rakesh, P., Vijay, P., Madurima, D., Mahesh, M. and Ramesh, C.M. Plant Growth Analysis. *Manual of ICAR Sponsored Training Programme on "Physiological Techniques to Analyze the Impact of Climate Change on Crop Plants" 16-25 January, 2017, Division of Plant Physiology, IARI, New Delhi.* 102-107 pp.
9. Harman, G. Multifunctional fungal plant symbionts: New tools to enhance plant growth and productivity. *New Phytologist*, **2011**, 189, 647-649.
10. Wareing, P.F. The physiologist's approach to tree growth. *Forestry Supplements*, **1966**, 7, 18.
11. Queiroz Rego, C.H. Co-inoculation with *Bradyrhizobium* and *Azospirillum* increases yield and quality of soybean seeds. *Agronomy Journal*, **2018**, 110, 2302-2309.
12. Hungria, M. Soybean Seed Co-inoculation with *Bradyrhizobium* spp. and *Azospirillum brasilense*: A New Biotechnological Tool to Improve Yield and Sustainability. *American Journal of Plant Sciences*, **2015**, 6, 811-817.
13. Gong, M. An effective method for screening and testing the true phosphate-solubilizing fungus that enhances corn growth. *Journal of Agricultural Science*, **2014**, 6, 60-70.
14. Elbon, A. and Whalen, J.K. Phosphorus supply to vegetable crops from arbuscular mycorrhizal fungi: a review, Biological Agriculture and Horticulture: *An International Journal for Sustainable Production Systems*, **2015**, 31, 73-90.
15. Janos, D.P. Plant responsiveness to mycorrhizas differs from dependence upon mycorrhizas. *Mycorrhiza*, **2007**, 17, 75-91.
16. Bray, R.H. and Kurtz, L.T. Determination of total N, organic C and available forms of phosphorus in soils. *Soil Sci*ence, **1945**, 59, 39-45.
17. van Reeuwijk, L.P. Procedure for Soil Analysis, 6th ed. (van Reeuwijk, L.P. Ed.); International Soil Reference and Information Centre (ISRIC)/Food and Agricultural Organization: Wageningen. The Netherlands, 2002, (pp. 120).
18. Bremner, J.M. and Mulvaney, C.S. Nitrogen-Total. In: Methods of soil analysis, Part 2. Chemical and microbiological properties, Page, A.L., Miller, R.H. and Keeney, D.R. Eds., American Society of Agronomy, Soil Science Society of America, Madison, Wisconsin, 1982, (pp. 595-624).
19. Rayment, G.E. and Higginson, F.R. Australian Laboratory Handbook of Soil and Water Chemical Methods; Inkata Press: Port Melbourne, Australia, 1992, (pp. 102).
20. Walkley, A.J. and Black, I.A. Estimation of soil organic carbon by the chromic acid titration method. *Soil Science*, **1934**, 37, 29-38.
21. Nelson, D.W. and Sommers, L.E. *Methods of Soil Analysis*: Part 3 Chemical Methods. Soil Science of America Book Series no. 5, 1996, (pp. 961-1010).
22. Bouyoucos, G.J. Hydrometer method improved for making particle size analyses of soils. *Soil Science Society Proceedings*, **1962**, *26*, 446-465.
23. Özalkan, Ç., Sepetoğlu, H. T., Daur, I. and Şen,O. Relationship between some plant growth parameters and grain yield of Chickpea (*Cicer arietinum* **L**.) during different growth stages. *Turkish Journal of Field Crops*, **2010**, 15, 79-83.
24. Sepetoğlu, H., Budak, N. Bitki Büyüme Analizi: Ege Üni. Ziraat Fak. *Dergisi*, **1994**, 31, 297-301.
25. van der Heijden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel,R., Boller, T., Wiemken, A., Sanders, I.R. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, **2002**, 396, 69-72.
26. Rosilaine, C., Sandra, F.B.T., Vera, L.R.B. and Eraldo, S.S. The effect of different soil properties on arbuscular mycorrhizal colonization of peanuts, sorghum and maize. *Acta bot. bras*., **2007**, 21, 723-730.

 doi:10.20944/preprints202603.0949.v1

27. Vierheilig, H., Coughlan, A.P., Wyss, U., and Piché, Y. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Applied Environmental and Microbiology*, **1998**, 64, 5004-5007.
28. Giovannetti, M. and Mosse, B. An evaluation of techniques for measuring Vesicular-arbuscular mycorrhizal infection in roots. *New Phytologist*, **1980**, 84, 489-500.
29. Fertiliser Society of South Africa (FSSA). 2010. Fertiliser Handbook. Seventh Edition. Available online: http://www.fssa.org.za/ (access on 30 May 2015)
30. Mogale, T.E., Kingsley, K.K., Munjonji, L. and Kifle, Y.G. Yield Responses of Grain Sorghum and Cowpea in Binary and Sole Cultures under No-Tillage Conditions in Limpopo Province. *Agriculture*, **2022**, 12, 733-741.
31. Sadhana B. Arbuscular Mycorrhizal Fungi (AMF) as a Bio-fertilizer- A Review. *International Journal of Current microbiology and Applied Sciences*, **2014**, 3, 384-400.
32. Smith S E, Read D J. Mycorrhizal Symbiosis. New York, USA, Academic Press, 2008, (pp. 5-110).
33. Khosro M, Shiva K, Yousef S. and Gholamreza H. Beneficial Effects of the Mycorrhizal Fungi for Plant Growth. A Review: *Journal of Applied Environmental and Biological Sciences*, 2011, 1, 310-319.
34. Kaeppler S M, Parke J L, Mueller S M, Senior L, Stuber C, Tracy W F. Variation among maize inbred lines and detection of quantitative trait loci for growth at low phosphorus and responsiveness to arbuscular mycorrhizal fungi. *Crop Science*, 2000, 40, 358-364.
35. Hayman D S. Influence of soils and fertility on activity and survival of VAM fungi. *Phytopathology*, **1982a**, 72**,** 1119-1125.
36. Olfat K, Jalil K. Spore density and root colonization by arbuscular mycorrhizal fungi in some species in the northwest of Iran. *International Research Journal of Applied and Basic Sciences*, **2012**, 3, 977-982.
37. Daniels B A, Skipper H D. Methods for the recovery and quantitative estimation of propagules from soil. In: Schenck N C, editor, Methods and principles of mycorrhizal research. St. Paul (MN): American Phytophathological Society, 1982, (pp. 29-35).
38. Olaniyi J O, Ajibola A T. Growth and Yield Performance of *Corchorus olitorius* Varieties as Affected by Nitrogen and Phosphorus Fertilizers Application. *American-Eurasian Journal of Sustainable Agriculture*, **2008**, 2, 234-241.
39. Medrano H, Flexas J, Ribas-Carbó M, Gulías J. Measuring water use efficiency in grapevines. In: Delrot S, Medrano H, Or E, Bavaresco L, Grando S, eds., Methodologies and Results Grapevine Research. Springer, Germany, 2010, (pp. 57-60).
40. Lopez-Bellido F C, Lopez-Bellido R J, Khalil S K, Lopez-Bellido L. Effect of planting date on winter kabuli Chickpea growth and yield under rain-fed Mediterranean conditions. *Agronomy Journal*, **2008**, 100, 957-964.
41. Nwangburuka C C, Olawuyi O J, Oyekale K, Ogunwenmo K O, Denton O A, Nwankwo. A.A. Growth and yield response of *Corchorus olitorius* in the treatment of *Arbuscular mycorrhizae* (AM), *Poultry manure* (PM), Combination of AM-PM and Inorganic Fertilizer (NPK). *Advances in Applied Science Research*, **2012**, 3, 1466-1477.