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MORPHOLOGICAL CHARACTERIZATION AND RESPONSE OF RED FLOWER RAG LEAF (*Crassocephalum crepidioides* Benth S. Moore) TO ORGANIC AND INORGANIC FERTILIZERS AND ARBUSCULAR MYCORRHIZAL FUNGUS

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ABSTRACT

Red flower rag leaf (Crassocephalum crepidioides) is one of the underutilized vegetables consumed globally. Pot trials were conducted to characterize 15 morphologically distinct accessions of C. crepidioides and assess the effects of treatment combinations of eggshell, NPK 15:15:15, poultry manure and arbuscular mycorrhizal fungus (Glomus mosseae) as soil amendments on growth and yield-related characters of C. crepidioides. Thereafter, 48 seedlings of the best performing accession were transplanted into perforated polythene bags filled with 7 kg of heat-sterilized soil. The experiment was laid out in a completely randomized design with three replicates. Treatment combinations were incorporated into in the polythene bags 1 week after transplanting, while control plants received no amendments. The results showed that accession NH/GKB-15 had the highest plant height (29.83 cm), stem length (27.67 cm), number of leaves (15) and length of internode at node 3 (1.13 cm) and node 4 (1.17 cm). The growth and yield characters of this best performing accession (NH/GKB-15) in response to soil amendments showed that poultry manure produced the tallest plants (55.17 cm), longest and widest stems (48.35 and 0.66 cm), longest and widest leaves (16.39 and 6.26 cm, respectively), and higher number of inflorescence (2.13). With NPK 15:15:15 the seedlings did not survive. Poultry manure should therefore be utilized for better plant nutrition and faster growth of C. crepidioides seedlings as well as for safer consumption of the leafy vegetable.

Key words: bio-inoculants, characterization, underutilized, ebolo, seedlings

INTRODUCTION

Crassocephalum crepidioides (Benth.) S. Moore is one of the underutilized African leafy vegetables belonging to family Asteraceae (Cronquist, 1981). Commonly referred to as red flower rag leaf, Okinawa spinach or thick head in English, it is called "Ebolo" by Yoruba speakers in South-western Nigeria (Burkill, 1995). The tender succulent leaves and stems of *C. crepidioides* are mucilaginous, and are used in preparation of soups and stews, especially in West and Central Africa (Sakpere *et al.,* 2013). The leaves of *C. crepidioides* are used to treat indigestion in southern Nigeria (Zollo *et al.,* 2000). The dried leaf powder is used by the Tanzanians as a snuff to stop nose bleeding and smoked to treat sleeping sickness (Zollo *et al.,* 2000).

Despite the numerous benefits of *C. crepidioides* to human health and nutrition, there are existing limiting factors that affect the effective production and yield of the crop. Ogbonna (2008) reported that soil fertility is one of the major problems limiting crop production. Soil fertility can be maintained by using mineral fertilizers to ensure sustainable crop production (Komolafe *et al.,* 2021). Aside poor soil fertility, other constraints responsible for low production of vegetable crops are high cost and unavailability of inorganic fertilizer, difficulty in obtaining adequate amount for large scale agriculture and delay in the release of the essential mineral nutrients for immediate use of the plant- as exhibited by organic manures (Olawuyi *et al.,* 2010).

Eggshells are waste materials from hatcheries, homes and fast-food industries (Amu *et al.,* 2005; Phil and Zhihong, 2009). Ground eggshells are a useful organic soil amendment due to the content of calcium carbonate (95%) and the potential as a source of lime to neutralize the pH of acidic soil (John and Paul, 2006). Poultry manure (PM), consisting of the bird's faeces, waste food and feathers, has been found to reduce parasitic nematode and increase soil nutrients (Babatola and Oyedunmade, 1992). It increases soil carbon, organic nitrogen and exchangeable calcium resulting in significant increase in pH, characterized with slow release of macronutrients most especially phosphorous (Sunassee, 2001; Nwangburuka *et al.,* 2012).

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Inorganic fertilizers like NPK 15:15:15 (NPK) are derived from mineral salts and are widely used by farmers due to fast and direct effect on plant growth. However, consequences from long term usage have degraded soil from its productive quality thereby reducing yield (Loveland and Webb, 2003; Phelan, 2004; Khan *et al.*, 2008). Due to those negative impacts, huge interest is recently geared towards promoting soil nutrients management techniques that could strengthen sustainable agricultural structure. Arbuscular mycorrhizal fungi (*Glomus mosseae*) have been explored as an alternative to inorganic fertilizer in soil amendment for crop growth and yield. Arbuscular mycorrhizal fungi (AMF) association enhances the uptake of the secondary and micro nutrients including calcium, zinc and copper (Ball, 2006; Phil and Zhihong, 2009). It plays key roles in nutrients cycling and protection of plants against environmental and biotic stresses (Odebode, 2005; Olawuyi *et al*., 2013; Olawuyi *et al*., 2014). The interactions of AMF and other bio-inoculants used in genetic improvement of crops have also enhanced yield, and reduced the challenges of soil pollution (Khan *et al*., 2008; Olawuyi *et al*., 2014).

The factors limiting the optimum growth and yield of crops can be checked by the application of fertilizers to the soil. Awodun (2007) reported that a combination of fertilizers gives beneficial effect on growth, leaf nutrient content and yield components. There is paucity of information on the effect of combined treatments of bio-inoculants and inorganic fertilizer. As a result, the study was aimed at investigating the effect of treatment combinations of eggshell, NPK, PM, and AMF on growth and yield related characters of *C. crepidioides*.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted in the screen house located at the Nursery Research Farm of the Department of Botany, University of Ibadan, Nigeria from Nov. 2019 to Dec. 2019. The area of the experimental screenhouse was 5,610 cm^2. The study area lies at 7° 26′ 30.12″ N and 3° 53′ 46.68″ E with an altitude of 185 m asl (Akin-Oriola, 2003), with a mean daily temperature of 24.6°C (Olawuyi *et al.,* 2017).

Source of Planting Materials, Bio-Inoculant, Organic and Inorganic Fertilizers

Seedlings of 15 accessions of *C. crepidioides* were collected from the Indigenous Vegetables Nursery of Genetic Resources Unit, National Horticultural Research Institute (NIHORT) Ibadan, Nigeria and placed in perforated trays. These accessions were derived through single seed descent (SSD) from a landrace population of *C. crepidioides* (NH/GKB) originally from Gbekuba in Ido Local Government Area of Oyo State, Nigeria. A pure culture of AMF (*Glomus mosseae*) was obtained from Genetics & Molecular Biology Unit in the Department of Botany, University of Ibadan, Ibadan, Nigeria. Poultry manure (PM, 1 to 3 weeks old) was collected from Oni-Aru Small Holder Poultry Farm at Alakia, Ibadan. Commercial grade NPK 15:15:15 fertilizer was obtained from the open market, while the eggshells were collected from a caterer in Sango area, Ibadan Nigeria, dried and ground into powder before use.

Design of the Experiment

This investigation was carried out in two stages:

i. Morphological evaluation: 15 accessions serially coded NH/GKB-01 to NH/GKB-15 were transplanted and left to acclimatize for one week before treatment application. The best performing accession was selected based on the following morphological growth characters: plant height, stem length, leaf length and width, number of leaves and length of internodes at node 3, 4 and 5.
ii. Soil amendment: The best performing accessions revealed in the morphological evaluation was subjected thereafter to different soil amendments. The experiment was laid out in a completely randomized design with three replicates. Forty-eight plants of the best performing accession were subjected to 15 treatment combinations and an untreated control as highlighted below:

T1, 2.67 g eggshell + 2.67 g AMF + 2.67g PM; T2, 2 g eggshell + 2 g AMF + 2 g NPK + 2 g PM; T3, 2.67 g eggshell + 2.67 g AMF + 2.67 g NPK; T4, 4 g NPK + 4 g PM;
T5, 2.67 g AMF + 2.67 g NPK + 2.67 g PM;
T6, 4 g eggshell + 4 g NPK;
T7, 2.67 g eggshell + 2.67 g NPK + 2.67 g PM; T8, 4 g eggshell + 4 g AMF;
T9, 4 g AMF + 4 g PM;
T10, 4 g eggshell + 4 g PM;
T11, 4 g AMF + 4 g NPK;
T12, 8 g PM;
T13, 8 g NPK;
T14, 8 g AMF;
T15, 8 g eggshell; and
T16, 0 g.

Soil Collection and Sterilization

Sandy loam soil for growing the experimental plants was obtained from the Research Nursery of the Department of Botany, University of Ibadan, Nigeria. The soil was sieved after which it was sterilized by heating for 2 h until the temperature rose to 100°C. The temperature was monitored using a thermometer. Sterilization was done to kill all indigenous microorganisms including AMF that may have been present. Then, 7 kg of sieved and sterilized soil sample was bagged in a polythene bag that was perforated to prevent waterlogging.

Planting and Cultural Practices

Seedlings were transplanted into the polythene bags, and placed on the slab in the screen house with an intra-row spacing of 10 cm and inter-row spacing of 12 cm. The AMF*,* PM, NPK fertilizer and ground eggshell were incorporated into the polythene bags based on each treatment combinations. After 1 week of transplant, the best accession was picked and treatments with bio-inoculants and inorganic fertilizer were applied 0.2 m away from the stem using the ring method. The experimental plants were watered daily with 25 cl of water. Manual removal of weeds around the potted plants was carried out weekly.

Data Collection

Data were taken according to Adjatin *et al.* (2013) with modifications. Plant height, stem length, stem diameter, number of leaves, leaf length, leaf width, length of internodes at node 3, 4, 5 from growing tip, time between bud emergence and achenes production, number of inflorescences, and number of branches were recorded weekly for six weeks after adding treatments. Plant height was measured from soil level to the tip of terminal flower head, while stem length was from the soil level to the last node before the inflorescence. Shoot biomass and root biomass were taken at six weeks after treatment. The fifth leaf from the tip was tagged and for the leaf measurements. Tagged leaves that wilted were replaced by the fifth leaf counting from the growing tip.

Statistical Analysis

Data were subjected to one way analysis of variance using Statistical Analysis Software (SAS) version 9.1. The means were separated using Duncan multiple range test at 5% probability level. Correlation matrix was used to show the various relationships that exist among the studied parameters.

RESULTS

Mean Square Effects of Accessions on Growth Characters of *C. Crepidioides*

Accession had highly significant ($p < 0.01$) effects on plant height, stem length, number of leaves, leaf length and leaf width, but not on length of internodes at nodes 3, 4 and 5 which were not significant (Table 1).

Morphological Characters of the Accessions

The results in Table 2 show that accession NH/GKB 15 was significantly ($p < 0.05$) different for stem length compared to other accessions with a mean value of 27.67 cm, whereas the effect of NH/GKB 15 on plant height was significantly ($p < 0.05$) different for all expect for accessions NH/GKB 04, 09 and 12 with values as 29.83, 23.27, 23.13 and 24.17 cm, respectively. Accessions NH/GKB 2 and NH/GKB 7 were not significantly ($p > 0.05$) different for plant height.

The effect of accession NH/GKB 12 on leaf length was significantly different ($p < 0.05$) for all except for accessions NH/GKB 04, 09, 10, 11 and 15. Also, accession NH/GKB 12 effect on leaf width was significantly different from all except accessions NH/GKB 04, 09, 10, 14 and 15. The result also showed that there was no variation in accessions for length of internodes at node 3 and node 4, resulting in all the accessions not significantly ($p > 0.05$) different.

Mean Square Variance of Treatments of *C. crepidioides* at Different Growth Stages

Treatment had very highly significant ($p < 0.001$) effects on all the characters evaluated. Weeks after planting had very highly significant ($p < 0.001$) effects on plant height, stem length, length of internodes at nodes 3, 4 and 5, leaf length and leaf width, but not on number of leaves, leaf width and stem diameter. Replication had no significant effect on any of the growth and yield characters evaluated (Table 3).

The Effects of Treatments on Growth and Yield Characters of *C. crepidioides*

The results in Table 4 show that the effects of PM on plant height and stem length with mean values of 55.17 and 48.35 cm, respectively were significantly ($p < 0.05$) different from all except AMF + PM and eggshell + PM (see Plate 1). Treatment NPK + PM and the control were similar ($p > 0.05$). Eggshell + AMF + NPK + PM was also similar to the control for plant height. Treatments eggshell + AMF + NPK and NPK were similar and had the lowest mean values. There was no significant difference between eggshell and control for stem length. Eggshell + PM had significant ($p < 0.05$) effect on number of leaves with a value 20.93. The PM, eggshell, eggshell + AMF + PM, eggshell + NPK + PM and eggshell + AMF were all showed similar number of leaves. The NPK + PM was not significantly different from control. The AMF had significant effect ($p < 0.05$) on length of internodes at node 3 (3.99 cm). The effect of AMF + PM (3.21 cm) and eggshell + PM (3.35 cm) were not significantly different on length of internodes at node 3. There was no variation in length of internode at node 4, showing no significant difference in treatment except treatment with NPK + PM (2.11 cm) and AMF + NPK + PM (1.90 cm) which were significantly different. Eggshell and PM produced significant ($p < 0.05$) effects on length of internode at node 5 (2.18 cm). The effect of AMF (2.18 cm) on length of internode at node 5 was not significantly different from that of control with mean value of 2.26 cm. Eggshell + AMF + PM, AMF + NPK + PM, eggshell + AMF and AMF + NPK treatments were not significantly ($p > 0.05$) different for length of internode at node 5 with mean values 1.37, 1.59, 1.52 and 1.42 cm, respectively.

Table 1: Mean square effects of accessions on growth characters of *Crassocephalum crepidioides*

Source of variation	Df (n-1)	PH (cm)	SL (cm)	Number of leaves	LIN 3 (cm)	LIN 4 (cm)	LIN 5 (cm)	LL (cm)	LW (cm)
Model	16	60.58	58.43	16.93	0.12	0.11	0.81	8.11	1.53
Accession	14	64.46^{***}	62.47^{**}	16.48^{***}	0.11^{ns}	0.11^{ns}	0.68^{ns}	8.97^{***}	1.75^{***}
Replicates	2	33.43^{ns}	30.15^{ns}	20.07^{ns}	0.16^{ns}	0.07^{ns}	1.78^{ns}	2.06^{ns}	0.01^{ns}
Error	28	15.49	17.19	3.40	0.21	0.13	0.69	1.80	0.41
Corrected total	44								

*denotes significant at $p < 0.05$, ** represents highly significant at $p < 0.01$, *** is highly significant at $p < 0.01$, ns - not significant.
Df - degree of freedom, PH - plant height, SL - stem length, LIN - length of internode at node, LL - leaf length, LW - leaf width

Table 2: Effect of accessions on morphological characters of *Crassocephalum crepidioides*

Accessions	Plant height (cm)	Stem length (cm)	Number of leaves	LIN 3 (cm)	LIN 4 (cm)	LIN 5 (cm)	Leaf length (cm)	Leaf width (cm)
NH/GKB-01	19.33^{bcde}	15.67^{bcd}	10.33^{cdefg}	1.07^{a}	0.90^{a}	1.63^{ab}	7.33^{def}	3.30^{cde}
NH/GKB-02	12.50^{e}	9.83^{d}	7.33^{g}	0.83^{a}	0.93^{a}	1.33^{ab}	6.47^{f}	2.47^{e}
NH/GKB-03	15.73^{cde}	13.50^{bcd}	8.67^{egf}	0.60^{a}	0.53^{a}	1.23^{ab}	7.20^{ef}	2.73^{de}
NH/GKB-04	23.27^{abc}	20.00^{b}	13.00^{abcd}	0.77^{a}	0.77^{a}	1.03^{ab}	9.80^{abcd}	4.33^{abc}
NH/GKB-05	19.83^{bcde}	17.00^{bcd}	11.00^{bcdef}	0.33^{a}	0.70^{a}	1.00^{b}	8.83^{bcde}	3.47^{cde}
NH/GKB-06	18.50^{bcde}	15.33^{bcd}	8.33^{gf}	0.70^{a}	0.87^{a}	1.43^{ab}	6.63^{f}	2.83^{de}
NH/GKB-07	14.00^{e}	11.33^{cd}	9.00^{efg}	0.63^{a}	1.13^{a}	1.47^{ab}	8.10^{cdef}	2.83^{de}
NH/GKB-08	19.83^{bcde}	18.50^{bc}	10.00^{defg}	0.70^{a}	0.97^{a}	1.20^{b}	6.33^{f}	2.73^{de}
NH/GKB-09	23.13^{abc}	18.50^{bc}	12.67^{abcd}	0.87^{a}	1.03^{a}	1.07^{b}	10.97^{ab}	4.70^{ab}
NH/GKB-10	21.80^{bcd}	19.27^{bc}	13.67^{abc}	0.70^{a}	0.57^{a}	1.00^{b}	9.83^{abcd}	4.30^{abc}
NH/GKB-11	16.73^{bcde}	13.83^{bcd}	11.33^{bcdef}	0.90^{a}	0.97^{a}	2.90^{a}	9.63^{abcde}	3.53^{bcde}
NH/GKB-12	24.17^{ab}	20.00^{b}	14.00^{ab}	0.70^{a}	1.10^{a}	1.83^{ab}	12.07^{a}	4.90^{a}
NH/GKB-13	15.67^{cde}	12.00^{bcd}	12.00^{abcde}	0.73^{a}	1.03^{a}	1.53^{ab}	8.37^{cdef}	3.60^{bcde}
NH/GKB-14	14.67^{de}	12.33^{bcd}	13.67^{abc}	0.80^{a}	1.03^{a}	1.53^{ab}	9.37^{bcde}	3.93^{abcd}
NH/GKB-15	29.83^{a}	27.67^{a}	15.00^{a}	1.13^{a}	1.17^{a}	1.33^{ab}	10.17^{abc}	3.83^{abcd}

Means with the same superscript in the same column are not significantly different at $p > 0.05$. LIN - length of internode at node

The highest mean value (16.39 cm) from PM was not significantly ($p > 0.05$) different from treatment eggshell + PM with mean value 14.87 cm for leaf length. Treatments eggshell + AMF + PM, NPK + PM and eggshell were not significantly ($p > 0.05$) different for leaf length with mean values of 9.23, 9.93 and 10.06 cm, respectively. Treatments AMF + NPK + PM and eggshell + NPK + PM were also not significantly different for leaf length with mean values 8.87 and 8.95 cm, respectively. There was variation in leaf width, showing significant difference in treatment except control (3.16 cm) and eggshell + NPK + PM (3.41 cm) which were not significantly different. The highest mean value (0.66 cm) from PM was similar to that (0.65 cm) from eggshell + PM for stem diameter. The NPK + PM, AMF + PM and AMF were not significantly different from one another for stem diameter with mean value 0.54, 0.60 and 0.56 cm, respectively. AMF + NPK + PM and eggshell + NPK + PM were not significantly ($p > 0.05$) different from control for stem diameter with mean value 0.42, 0.45 and 0.44 cm, respectively. Eggshell + AMF+ PM, eggshell + AMF + NPK and eggshell + AMF were not significantly different from each other for stem diameter with mean values 0.33, 0.30 and 0.35 cm, respectively.

The PM had highly significant ($p < 0.05$) effect on number of inflorescence (s) with mean value of 2.13 cm compared to other treatments. Eggshell + PM was not significantly different from the use of just eggshell as treatment, although eggshell + PM had higher mean value of 1.80 cm than eggshell (1.73 cm) for number of inflorescence (s). Eggshell + AMF + PM, AMF + NPK + PM, eggshell + AMF, AMF + NPK and AMF were not significantly different from one another for number of inflorescence (s) with mean values 0.73, 0.93, 0.67, 0.80 and 1.00 cm, respectively. Eggshell had significant effect on number of branches with mean value 1.13 cm. Eggshell + AMF + PM, NPK 15:5:15 + PM, AMF + NPK + PM, eggshell + NPK, eggshell + AMF, AMF + PM, PM and AMF were not significantly different with mean values 0.53, 0.13, 0.07, 0.07, 0.27, 0.33, 0.53 and 0.40 cm, respectively.

Treatments with eggshell + AMF + NPK and NPK recorded lowest values which were not significantly ($p > 0.05$) different for all growth characters, while treatments with eggshell + AMF + NPK + PM, eggshell + AMF + NPK and NPK recorded lowest values which were not significantly ($p > 0.05$) different for all yield parameters.

Table 3: Mean square variance of treatments of *Crassocephalum crepidioides* at different growth stages

Source of variance	Df (*n*-1)	PH	SL	NL	LIN 3	LIN 4	LIN 5	LL	LW	SD	NI	NB
Model	21	3256.69	2504.18	394.38	20.09	20.64	13.16	238.17	38.88	0.38	10.96	2.23
Treatments	15	4007.97***	3151.83***	545.80***	21.39***	22.40***	15.53***	329.00***	53.55***	0.52***	7.16***	1.63***
Weeks	4	1931.57***	1258.75***	13.95^{ns}	23.89***	24.31***	9.41***	11.43***	1.49^{ns}	0.03^{ns}	30.11***	5.50***
Replicates	2	272.33^{ns}	137.65^{ns}	21.07^{ns}	2.76^{ns}	0.14^{ns}	2.89^{ns}	10.52^{ns}	3.33^{ns}	0.02^{ns}	1.22^{ns}	0.18^{ns}
Error	218	306.23	227.27	40.37	2.81	2.91	1.72	19.00	2.83	0.04	1.87	0.56
Corrected total	239											

*significant at $p < 0.05$, **highly significant at $p < 0.01$, ***very highly significant at $p < 0.001$, ns - not significant at $p < 0.05$, Df - degree of freedom
PH - plant height, SL - stem length, NL - number of leaves, LIN 3, 4 and 5 - length of internode at node at 3, 4 and 5, respectively
LL - leaf length, LW - leaf width, SD - stem diameter, NI - number of inflorescence, NB - number of branches

Table 4: Effect of treatments on growth and yield characters of *Crassocephalum crepidioides*

Treatments	Growth characters									Yield characters	
	PH (cm)	SL (cm)	NL	LIN 3 (cm)	LIN 4 (cm)	LIN 5 (cm)	LL (cm)	LW (cm)	SD (cm)	NI	NB
Eggshell+AMF+PM	29.60^{defg}	20.05^{efg}	11.60^{cdef}	1.74^{cdefg}	1.17^{cde}	1.37^{def}	9.23^{cd}	3.63^{defg}	0.33^{cd}	0.73^{bcde}	0.53^{bc}
Eggshell+AMF+NPK+PM	15.04^{h}	13.13^{g}	8.13^{ef}	0.81^{fgh}	0.57^{de}	0.81^{efg}	5.67^{efg}	2.30^{ghi}	0.30^{cd}	0.00^{e}	0.00^{c}
Eggshell+AMF+NPK	1.43^{i}	1.27^{h}	0.60^{h}	0.04^{h}	0.04^{e}	0.04^{g}	0.35^{h}	0.14^{j}	0.00^{e}	0.00^{e}	0.00^{c}
NPK+PM	35.69^{cdef}	31.37^{cde}	12.80^{bcde}	2.21^{bcde}	2.11^{bc}	1.85^{cde}	9.93^{cd}	4.41^{cde}	0.54^{ab}	1.53^{abc}	0.13^{bc}
AMF+NPK+PM	28.22^{defgh}	25.77^{cdef}	8.93^{def}	2.07^{bcdef}	1.90^{bcd}	1.59^{def}	8.87^{cdef}	2.95^{fgh}	0.42^{bc}	0.93^{bcde}	0.07^{bc}
Eggshell+NPK	14.51^{h}	11.40^{gh}	3.27^{gh}	0.62^{gh}	0.59^{de}	0.56^{fg}	2.87^{gh}	1.11^{ij}	0.17^{de}	0.27^{de}	0.07^{bc}
Eggshell+NPK+PM	23.54^{fgh}	20.50^{efg}	11.27^{cdef}	1.21^{defgh}	1.05^{cde}	1.09^{ef}	8.95^{cdef}	3.41^{efg}	0.45^{bc}	0.47^{cde}	0.00^{c}
Eggshell+AMF	26.09^{efgh}	22.63^{defg}	10.87^{cdef}	1.13^{efgh}	1.29^{cde}	1.52^{def}	6.83^{def}	2.39^{ghi}	0.35^{cd}	$_{0.67}$bcde	0.27^{bc}
AMF+PM	46.05^{abc}	38.00^{abc}	14.14^{bcd}	3.21^{ab}	3.78^{a}	2.79^{abc}	11.12^{bc}	4.84^{bcd}	0.60^{ab}	1.27^{abcd}	0.33^{bc}
Eggshell+PM	51.91^{ab}	46.13^{ab}	20.93^{a}	3.35^{ab}	3.71^{a}	3.41^{a}	14.87^{a}	5.91^{ab}	0.65^{a}	1.80^{ab}	0.67^{ab}
AMF+NPK	19.14^{gh}	16.81^{fg}	6.73^{fg}	1.11^{efgh}	1.27^{cde}	1.42^{def}	5.52^{fg}	1.64^{hi}	0.23^{d}	0.80^{bcde}	0.00^{c}
PM (Poultry manure)	55.17^{a}	48.35^{a}	17.73^{ab}	2.73^{abc}	2.99^{ab}	3.14^{ab}	16.39^{a}	6.26^{a}	0.66^{a}	2.13^{a}	0.53^{bc}
NPK (NPK 15:15:15)	0.00^{i}	0.00^{h}	0.00^{h}	0.00^{h}	0.00^{e}	0.00^{g}	0.00^{h}	0.00^{j}	0.00^{e}	0.00^{e}	0.00^{c}
AMF (Arbuscular mycorrhizal fungi)	39.43^{bcde}	34.33^{bcd}	15.40^{bc}	3.99^{a}	2.67^{ab}	2.18^{bcd}	13.42^{ab}	5.24^{abc}	0.56^{ab}	1.00^{bcde}	0.40^{bc}
Eggshell	41.19^{bcd}	36.12^{bc}	17.80^{ab}	2.59^{bc}	2.75^{ab}	2.38^{abcd}	10.06^{cd}	3.86^{def}	0.49^{abc}	1.73^{ab}	1.13^{a}
Control	36.96^{cdef}	35.38^{bc}	13.00^{bcde}	2.52^{bcd}	2.74^{ab}	2.26^{bcd}	9.08^{cde}	3.16^{efg}	0.44^{bc}	1.60^{abc}	0.60^{abc}

Means with the same superscript in the same column are not significantly ($p > 0.05$) different using Duncan's Multiple Range Test (DMRT).
PH - plant height, SL - stem length, NL - number of leaves, LIN 3, 4 and 5 - length of internode at node at 3, 4 and 5, respectively
LL - leaf length, LW - leaf width, SD - stem diameter, NI - number of inflorescence, NB - number of branches

Plate 1: *Crassocephalum crepidioides* treated with 8 g of poultry manure four weeks after treatment (WAT)

Effects of Treatment on Growth and Yield Characters of *Crassocephalum crepidioides*

The shoot biomass and root biomass had no significant ($p > 0.05$) effect across the replicates. Treatment had significant ($p < 0.05$) effect on root biomass, while the treatment had no significant ($p > 0.05$) effect on shoot biomass (Table 5).

Effect of Treatments on Shoot and Root Biomass

The results in Table 6 show that the effect of PM on root biomass was significantly different ($p < 0.05$) for all except AMF + PM, eggshell + PM, AMF and control (Plate 2b). All soil amendments except Eggshell+ AMF + PM, eggshell + AMF + NPK, eggshell + NPK and eggshell + AMF on shoot biomass were not significantly ($p > 0.05$) different (Plate 2a).

Table 5: Mean square effects of treatments and replicates on shoot biomass and root biomass

Source of variation	Df (n-1)	Shoot biomass	Root biomass
Model	17	149.78	5.38
Treatments	15	163.80^{ns}	6.03*
Replicates	2	44.63^{ns}	0.57^{ns}
Error	30	103.00	3.02
Corrected total	47		

*Significant at $p < 0.05$, ns - not significant at $p < 0.05$,
Df - degree of freedom

Table 6: Effects of treatment on shoot biomass and root biomass

Treatments	Shoot biomass (g)	Root biomass (g)
Eggshell+AMF+PM	4.28^{bc}	0.91^{b}
Eggshell+AMF+NPK+PM	7.51^{abc}	1.20^{b}
Eggshell+AMF+NPK	0.00^{c}	0.00^{b}
NPK+PM	6.11^{abc}	0.91^{b}
AMF+NPK+PM	10.25^{abc}	1.44^{b}
Eggshell+NPK	1.43^{c}	0.20^{b}
Eggshell+NPK+PM	12.67^{abc}	1.30^{b}
Eggshell+AMF	4.23^{bc}	0.90^{b}
AMF+PM	8.39^{abc}	2.34^{ab}
Eggshell+PM	21.68^{ab}	3.37^{ab}
AMF+NPK	8.74^{abc}	1.96^{b}
PM (Poultry manure)	25.49^{a}	5.46^{a}
NPK (NPK 15:15:15)	0.00^{c}	0.00^{b}
AMF (Arbuscular mycorrhizal fungi)	12.60^{abc}	2.75^{ab}
Eggshell	17.65^{abc}	2.22^{ab}
Control	6.03^{abc}	2.70^{ab}

Means with same superscript in the same column are not significantly ($p > 0.05$) different using Duncan's Multiple Range Test (DMRT).

Plate 2a: Highest shoot biomass of *C. crepidioides* treated with 8 g of poultry manure

Plate 2b: Highest root biomass of *C. crepidioides* treated with 8 g of poultry manure

Correlation of Growth and Yield Characters of *Crassocephalum crepidioides*

The results of correlation coefficient of growth and yield characters in Table 7 show that plant height was positive and strongly related ($p < 0.01$) with stem length, number of leaves, length of internode at node 3, length of internode at node 4, length of internode at node 5, leaf length, leaf width, stem diameter and number of inflorescence, with r = 0.98, 0.81, 0.83, 0.81, 0.91, 0.84, 0.82, 0.84 and 0.73, respectively.

Stem height was highly significant and strongly associated with number of leaves, length of internode at node 3, length of internode at node 4, length of internode at node 5, leaf length, leaf width, stem diameter and number of inflorescence, with r = 0.82, 0.85, 0.83, 0.92, 0.85, 0.82, 0.85 and 0.71, respectively. Number of leaves had a strong positive correlation with length of internode at node 3, length of internode at node 4, length of internode at node 5, leaf length, leaf width, stem diameter, with r = 0.85, 0.63, 0.71, 0.88, 0.84, 0.71, respectively. Length of internode at node 3 was highly significant and positively correlated with length of internode at node 4, length of internode at node 5, leaf length, leaf width, stem diameter and number of inflorescence with r = 0.79, 0.82, 0.66, 0.66, 0.73 and 0.64, respectively. Length of internode at node 4 had a strong positive correlation with length of internode at node 5, leaf length, leaf width, stem diameter and number of inflorescence with r = 0.81, 0.61, 0.63, 0.71 and 0.67, respectively. Length of internode at node 4 had no correlation with number of branches, bud emergence to fruiting, shoot biomass, root biomass and treatment. Length of internode at node 5 had a strong positive correlation with leaf length, leaf width, stem diameter and number of inflorescence with r = 0.73, 0.73, 0.78 and 0.71, respectively. Leaf length had a strong positive correlation with leaf width and stem diameter with r = 0.96 and 0.72, respectively. Leaf width only had a strong positive correlation with stem diameter (r = 0.70). Stem diameter had strong positive correlation with number of inflorescence having r value as 0.61.

Principal Component Analysis of Growth and Yield Characters of *Crassocephalum crepidioides*

The results in Table 8 delineate the *C. crepidioides* into seven principal component axes; Prin. 1, 2, 3, 4, 5, 6 and 7. Prin. 1 which constituted the highest accounted for 0.70 of total proportion with eigen value of 8.35, while Prin. 7 was the least with proportion of 0.02 and eigen value of 0.24. In Prin. 1, plant height (0.34) and stem length (0.34) were closely related as well as length of internode at node 3 and node (0.29). Also, length of internode at node 5, leaf length and leaf width were closely related with eigen value 0.31. No eigen values were closely related in Prin. 2 and Prin. 3. Leaf length and leaf width (–0.16) were closely related in Prin. 4. In Prin. 5, number of leaves and leaf length (–0.16) were closely related, while in Prin. 6, stem height and length of internode at node 5 (–0.07) were closely related.

DISCUSSION

The findings from the first stage of the experiment showed variations in morphological characters among the 15 accessions of *C. crepidioides.* The best performing accession based on morphological evaluation was NH/GKB 15, while the accession with the worst performance was NH/GKB 02. The variations shown by the characters may be as a result of high genetic diversity, differences of growing type and differences on the type of adaptation. This is in accordance with the reports that variations in characters were due to high genetic diversity and differences of growing type and type of adaptation (Mujica and Jacobsen, 2003; Oraegbunam *et al.,* 2016; Umezinwa *et al*., 2020).

Table 7: Correlation of growth and yield characters of *Crassocephalum crepidioides*

	PH	SL	NL	LIN 3	LIN 4	LIN 5	LL	LW	SD	NI	NB	BEF	SB	RB	T
PH															
SL	0.98**														
NL	0.81**	0.82**													
LIN 3	0.83**	0.85**	0.85**												
LIN 4	0.81**	0.83**	0.63**	0.79**											
LIN 5	0.91**	0.92**	0.71**	0.82**	0.81**										
LL	0.84**	0.85**	0.88**	0.66**	0.61**	0.73**									
LW	0.82**	0.82**	0.84**	0.66**	0.63**	0.73**	0.96**								
SD	0.84**	0.85**	0.71**	0.73**	0.71**	0.78**	0.72**	0.70**							
NI	0.73**	0.71**	0.46	0.64**	0.67**	0.71**	0.46	0.45	0.61**						
NB	0.47	0.44	0.41	0.41	0.41	0.39	0.28	0.25	0.40	0.44					
BEF	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
SB	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00			
RB	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
T	0.25	0.30	0.25	0.24	0.26	0.26	0.22	0.18	0.15	0.20	0.19	0.00	0.00	0.00	0.00

*significant at $p < 0.05$, **highly significant at $p < 0.01$, ***very highly significant at $p < 0.001$; PH - plant height, SL - stem length, NL - number of leaves, LIN 3, 4 and 5 - length of internode at node at 3, 4 and 5, respectively; LL - leaf length, LW - leaf width, SD - stem diameter, NI - number of inflorescence, NB - number of branches, BEF - bud emergence to fruiting, SB - shoot biomass, RB - root biomass, T - treatments

Table 8: Principal component analysis (PCA) of growth and yield characters of *Crassocephalum crepidioides*

Characters	Prin. 1	Prin. 2	Prin. 3	Prin. 4	Prin. 5	Prin. 6	Prin. 7
Plant height	0.34	0.02	–0.02	0.02	–0.06	–0.05	0.05
Stem length	0.34	–0.02	–0.06	–0.02	0.00	–0.07	0.01
Number of leaves	0.30	–0.06	0.35	–0.17	–0.16	0.23	–0.23
Length of internode at node 3	0.29	0.04	–0.31	–0.23	0.40	0.21	0.62
Length of internode at node 4	0.29	0.07	–0.33	0.01	0.48	0.48	–0.50
Length of internode at node 5	0.31	–0.00	–0.26	–0.09	0.03	–0.07	–0.04
Leaf length	0.31	–0.22	–0.22	–0.16	–0.16	0.15	0.20
Leaf width	0.31	–0.25	0.23	–0.16	–0.15	0.28	0.19
Stem diameter	0.30	–0.15	0.10	–0.04	0.00	–0.75	–0.46
Number of inflorescence	0.24	0.29	–0.48	0.32	–0.66	0.05	0.02
Number of branches	0.14	0.88	0.39	–0.14	0.08	–0.04	0.03
Bud emergence to fruiting	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Shoot biomass	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Root biomass	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Eigen value	8.35	0.91	0.79	0.63	0.40	0.26	0.24
Proportion (%)	0.70	0.08	0.07	0.05	0.03	0.02	0.02

The variations also suggest that the characters are under genetic control and as such be liable to genetic improvement. This provides opportunity to improve desirable morphological traits of *C. crepidioides.* Adjatin *et al.* (2013) also reported genetic variation and diversity among different populations of *C. crepidioides.* The diversity noted in crop performance or the growth characters in this study could be explained by differences in agro-climatic conditions and the individual's selection practices prevailing in the area where the seedlings were collected.

The second stage of the experiment showed that the growth and yield characters of accession NH/GKB 15 of *C. crepidioides* were significantly increased in response to the application of PM. This might be as a result of the highest release of nitrogen and phosphorus from the organic fertilizer. This is in conformity with earlier reports that organic amended soils possess essential nitrogen twice the level of nitrogen in conventional soils (Dauda *et al.,* 2005; Delate *et al.,* 2008). Poultry manure had also been reported to enhance the growth parameters of crops (Dauda *et al.,* 2008; Nwite *et al*., 2013; Nnadi *et al*., 2020) due to high content of essential plant nutrients resulting to high photosynthetic activities (John *et al.*, 2004). Organic fertilizer apart from releasing nutrient elements to the soil has also been shown to improve other soil chemical and physical properties which enhance crop growth and development (Dauda *et al.*, 2008; Ogbonna, 2008; Uko *et al*., 2009).

The PM produced the highest plant height, stem length, stem diameter, leaf length, leaf width, number of inflorescence, shoot biomass and root biomass. This observation is in line with Dossou *et al.* (2019) who reported that adding either cow dung or poultry manure as organic fertilizer improved the growth of *C. crepidioides* plants. Ojeniyi *et al*. (2008) also reported higher growth parameter values with the addition of poultry manure, whereas Abdulmaliq *et al.* (2019) found that sole application of poultry manure resulted in better growth and yield of tomato than NPK fertilizer alone. Bawa (2010) also observed taller plants, more leaf number and height, as well as enhanced vegetative growth and yield of amaranth with poultry manure application. Poultry manure was also reported by Ayeni *et al.* (2016) to produce the highest fresh root weight, fresh shoot weight dry root weight and dry shoot weight in *Solanum nigrum.*

Eggshell + AMF had lowest biomass compared to the single application of eggshell and AMF. This is not in agreement with Valentine *et al*. (2001) who reported that colonization of AMF to produce highest biomass is depended on the low concentration of phosphorus and high concentration of other nutrients; as eggshell has low concentration of phosphorus but in combination with AMF, it did not perform better. This might be as a result of high phosphorus and high nutrient concentration which inhibited an optimum performance of AMF to produce highest biomass.

There was no significant effect on leaf length and stem diameter when eggshell + PM and PM were used; although treatment with eggshell + PM performed better than that of PM only. This could be as a result of the additional amount of essential secondary nutrients (Ca and Mg) and micronutrients (B, Cu, Fe, Mn, Mo) obtained from ground eggshell which supports plant growth. This agrees with Nguyen *et al*. (2016) that application of the bio-mixture containing spent coffee and ground eggshells to the soil helps to increase soil nutrients, soil health and okra yield. It is also in agreement with Ledbetter *et al*. (2011) study which revealed that red cover plants fertilized with eggshells grew at an average of more than 10 mm larger than plants without eggshells.

The same quantity of PM and ground eggshell did not show similar effect on *C. crepidioides*. This might be due to more quantity of eggshell needed to display similar significant effect. This is in accordance with Radha and Karthikeyan (2019) report whose study noted a better performance of cowpea planted on 10 g of eggshell compared to 8 g of eggshell. The treatment NPK + PM did not perform best in the experiment. This was not in accordance with reports by Awodun (2007) and Fonge *et al.* (2016).

There were negative responses on survival from seedlings treated with 8 g of NPK. Seedlings treated with NPK did not survive. This might be due to the quantity of the fertilizer applied. Dachung *et al.* (2019) reported that an increase in dose of fertilizer (at dose 6 and 9 g, whose mean is approximately 8 g) caused decrease in survival percentage of *Tamarindus indica* seedlings. Grzyb *et al.* (2013) also observed high mortality of plants following a high application rate of NPK mineral fertilizer on apples.

The comparison of AMF with AMF + NPK effect on growth and yield parameters showed that AMF performed better than AMF + NPK. This could be as a result of the nitrogen released from NPK which affected the colonization of AMF. This agrees with Liu *et al*. (2000) who showed that root colonization of maize by AMF and production of extra-radical hyphae were suppressed by high application rates of nitrogen and phosphorus fertilizers. Similar observation of addition of nitrogen and phosphorus fertilizers reducing the colonization of AMF was also reported by Bakhshandeh *et al.* (2017).

CONCLUSION AND RECOMMENDATION

In the first experiment, there were morphological variations among the accessions of *Crassocephalum crepidioides* of which the best accession was NH/GKB-15. The addition of poultry manure produced better growth at the early and maturity stages of *C. crepidioides*. The NH/GKB-15 accession of *C. crepidioides* could be recommended and utilized for future experiments involving poultry manure and other bio-inoculants for better plant nutrition and faster growth of *C. crepidioides* seedlings.

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