

IMPACT OF ARBUSCULAR MYCORRHIZAL FUNGI ON ROOT CALCIUM CONTENT OF COWPEA VARIETIES (*Vigna unguiculata* L. WALP) UNDER *ALECTRA VOGELII* INOCULATED SOIL

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Abstract

Biological control methods can play an important role in managing plant parasites affecting cowpea production particularly in the face of climate change. This research evaluated the effect of *Glomus clarum* on the root calcium content of four cowpea varieties in an *Alectra vogelii* inoculated soil. The four cowpea varieties used were: SAMPEA 7, IFE 82-12, IT97K-499-35 and TVX 3236. The sterilized sandy-loamy soil used for this experiment consisted of a mixture of top soil and sand in ratio 1:1 (v/v). *Glomus clarum* treatments was applied at five rates: the zero and without *Alectra*, zero and with *Alectra*, 10, 20 and 30 g/pot each with *Alectra* (3.3 g). The treatments were arranged in complete randomized design. The root of cowpea plants was sampled for calcium content at 5 weeks after planting (WAP). Testing with ANOVA, the two years data based on *Glomus clarum* treatments, the *Glomus clarum* at 30 g/pot treatment resulted in highest root calcium content which was only comparable with that due to 20 g/pot *Glomus clarum* treatment. In addition, *Glomus clarum* treatments resulted in the highest root calcium content in IT97K – 499 – 35 which was only significantly higher than the lowest root calcium content observed in TVX 3236. In conclusion, *Glomus clarum* treatments significantly increased root calcium content for the cowpea varieties on *Alectra* inoculated soil and thus recommended as a biological control agent in *Alectra vogelii* infested fields.

Keywords: *Alectra vogelii*, Cowpea, *Glomus clarum*, Root, Calcium

INTRODUCTION

The risk to global food security due to climate change has been identified as one of the biggest challenges facing humankind (FAO, 2010). The challenge is producing sufficient food for the escalating population growth with limited water supplies and breeding for drought tolerance and water use efficiency (Lydia *et al.*, 2022). To ensure adequate food for an expanding global population in the face of climate change there is the need to ensure the control of parasitic plant parasites to enhance bumper harvest, ensure environmental sustainability and economic growth. Cowpea

(*Vigna unguiculata* L. Walp), is a diploid annual herbaceous legume crop known for its high protein content (18% - 25%) which is equal to certain meat types. This crop is resilient to heat and drought stresses and therefore finds place in several cropping systems across the world (Narayana and Angamuthu, 2021). Cowpea is an important grain legume for human and livestock nutrition worldwide. Its grains represent a valuable source of protein for rural families in Sub-Saharan Africa while its haulms offer nutritious fodder for livestock, especially, in the Sahel regions (Togola *et al.*, 2023). Apart from its nutritional component, the crop has multiple advantages to farmers,

including its ability to grow and produce high yields on poor, sandy soils unsuitable for the production of other crops, high rates of symbiotic nitrogen fixation, and lower fertilizer requirements. Biological control method is a well-established strategy for controlling pest populations by releasing the natural enemies of the pest into the field to keep pest populations below the threshold level (Heimpel and Mills, 2017). Arbuscular mycorrhizal fungi (AMF) represent a group of soil fungus which resides in symbiotic association with plant roots (Uwamungu *et al.*, 2022). Establishment of an active mycorrhizal symbiosis is an important beneficial factor in terms of improved plant nutrient uptake, such as calcium, phosphorus *et.c* (Wahab *et al.*, 2023). Experimental trials on tomato plants inoculated with AMF have shown increased leaf area, and nitrogen, potassium, calcium, and phosphorus contents, reflecting enhanced plant growth (Balliu *et al.*, 2015). *Alectra vogelii* is a troublesome parasitic weed that reduces the yield of various leguminous crops, including cowpeas, by 50-100%, depending on the severity of infestation in most parts of Africa (Musango *et al.*, 2022). The current pest control measures being used by some farmers to control parasitic weeds (such as cultural, mechanical, physical, chemical *et.c*) have disadvantages. For instance, the chemical control method could lead to land pollution and death of aquatic organisms. Therefore, due to the limitations of each control method there is a need to search for an effective control measure that can be suitable for the host plant, safe for the environment, control the parasite and can be easily adopted by poor resource farmers.

This research assessed the tripartite interactions between cowpea varieties, Arbuscular Mycorrhizal Fungi and *Alectra vogelii* with emphasis on the role of the fungi on root calcium content of cowpea varieties. This is valuable to cowpea breeders in planning future cowpea selection and to contribute to development programmes aimed at improving cowpea yield. It is of great importance because some farmers grow cowpea plant particularly to use it as a fodder crop and hence a need for a biological control method that sustains the environment.

MATERIALS AND METHODS

This pot experiment was conducted on a fenced farmland at Trikania, Kaduna in 2016 and 2017 wet

seasons (two years data collected). Four cowpea varieties comprising of two susceptible varieties (SAMPEA 7 and TVX 3236) and two moderately resistant varieties to *Alectra* (IFE 82-12 and IT97K-499-35) were obtained from the Seed Production Unit, Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria. Also, the *Alectra* seeds and AM inoculum were gotten from IAR farms, Zaria and University of Ibadan, Ibadan respectively. The method of Heckman and Angle (1987) was used to prepare *Glomus clarum* inoculum. Soil composed of a mixture of topsoil and sharp sand in ratio 1:1 was sieved, sterilized and placed in polythene bags (used as pots) and used for planting. Four seeds each of the different cowpea varieties were planted in each polythene bag. These pots were arranged at an intra-row spacing of 0.30 m. The cowpea plants were inoculated with propagules of *Glomus clarum* depending on the treatments (control without *Alectra*, control with *Alectra*, 10, 20 and 30 g per pot) with a constant quantity of *Alectra* (3.3 g). The AM fungal inoculum was mixed with the top 3 cm of the soil for the relevant treatments. Each of the treatment was assigned eight pots in three replicates. The treatments were arranged in Complete Randomized Design (CRD).

The plants were thinned to two plants per pot at two weeks after planting. Weeds with the exception of *Alectra* were controlled by hand pulling as at, when necessary, from 2 WAP. The cowpea seedlings were sprayed with Benlate (Benomyl) and Dithane M45 (Carbendazim) at the product rate of 0.6 kg/ha and 2.5 kg/ha respectively (to control fungal diseases) and Rogor (dimethoate) at 0.75 L/ha at 4 WAP, to prevent viral diseases. Sherpa plus (cypermethrin + perfekthion) was applied fortnightly at the rate of 1.0 L/ha, beginning from 7 WAP until harvest, to control insect pests during flowering and pod development. Weeds except *Alectra* were controlled by hand pulling as at, when necessary, from 2 WAP. At each sampling, cowpea plants were carefully uprooted from three pots. The sampled plants were brought to the laboratory in labeled polythene bags, washed carefully with tap water and the surface water was allowed to drain. The selected cowpea plants were separated into root and shoot using a knife and each part was then put in labeled envelopes; oven dried at 70 °C (to ensure preservation for analysis). The dried roots were ground separately and stored in air tight labeled

specimen tubes for mineral element analysis at Department of Soil Science, Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria. The root calcium was determined by the flame photometer (Jackson, 1962; Black, 1965).

The data obtained on root calcium content were subjected to analysis of variance (ANOVA) as described by Lawes Agricultural Trust (1980), to compare the varietal reaction of cowpea varieties to the presence of Arbuscular mycorrhizal fungi. Significant differences between treatments means were compared using Duncan Multiple range test (DMRT). The two years data on each parameter were pooled and subjected to ANOVA.

RESULTS AND DISCUSSION

Table 1: Effects in *G. clarum* on root calcium content in cowpea varieties at 5 WAP in 2016 and 2017

Cowpea variety	VAM Conc.(g)	PLANT'S AGE (WAP) Calcium (%)	
		2016	2017
SAMPEA 7	O-	2.18b	0.09d
	O+	2.75ab	0.10c
	10	2.90a	0.11b
	20	2.95a	0.06e
	30	3.00a	0.13a
	Mean	2.76	0.10
	SE ±	0.20	0.003
IFE 82-12	O-	2.46b	0.15a
	O+	2.50b	0.11c
	10	2.16b	0.13b
	20	3.37a	0.11c
	30	3.30a	0.09d
	Mean	2.76	0.12
	SE ±	0.16	0.0003
IT97K-499-35	O-	2.53b	0.12a
	O+	2.85b	0.11a
	10	2.49b	0.11a
	20	3.23a	0.07b
	30	3.45a	0.11a
	Mean	2.91	0.10
	SE ±	0.11	0.003
TVX 3236	O-	2.01d	0.11a
	O+	2.39c	0.10ab
	10	2.75b	0.09b
	20	2.80b	0.10ab
	30	3.38a	0.11a
	Mean	2.67	0.10
	SE ±	0.08	0.005

Note: Means followed by the same letter(s) in each column, under each variety, in each year are not significantly different ($P \leq 0.05$), using DMRT. WAP- Weeks after Planting

Source; Author's Field Work

Root calcium content of cowpea varieties and *Glomus clarum* treatments

At 5 WAP, the control treatments mostly resulted in lower root calcium than the *Glomus clarum* treatments in the cowpea varieties in 2016 (Table 1). The control without *Alectra* treatment resulted in the highest root calcium content in IFE 82-12, IT97K-499-35 and TVX 3236 in 2017. Also, 20 g/pot *Glomus clarum* treatment resulted in the lowest root calcium content in SAMPEA 7 and IT97K-499-35 (Table 1). The ANOVA of the two years data based on *Glomus clarum* treatments showed that, *Glomus clarum* at 30 g/pot treatment resulted in highest root calcium content which was only comparable with that due to 20 g/pot *Glomus clarum* treatment. This was followed by that due to the control plus *Alectra* treatment.

The control without *Alectra* treatment resulted in the lowest root calcium content than that due to all the other treatments (Table 2). The 30 g/pot *Glomus clarum* treatment resulted in the highest calcium in cowpea roots. This suggests that, the AMF species reduced the impact of the parasite (*Alectra*) on the cowpea plant. Also, it suggests that a high concentration of AMF might have led to an efficient AMF colonization which in turn stimulated nutrient uptake in the plants (Chen *et al.*, 2017; Mitra *et al.*, 2019). The inoculation with AMF must have increased the percentage of colonized roots. Host root systems extended by widespread extra radical mycelia, enabling colonized roots to reach more water and nutrient pools unavailable to uncolonized roots (Katalin and Nguyen, 2019). Also, *Glomus clarum* treatments resulted in the highest root calcium content in IT97K – 499 – 35 which was only significantly higher than the lowest root calcium content observed in TVX 3236 (Table 2). *Glomus clarum* having its highest values for root calcium in cowpea variety IT97K-499-35 might be due to differences in nutrition efficiency of the various cowpea varieties. From physiological perspective, the nutrition efficiency refers to the

ability of the genotype to absorb the nutrient from the soil, distribute it and use it internally (Mengel and Kirkby, 2006). Also, it can be attributed to genotypic differences in the cowpea varieties response to AMF colonization. Genotypic variation to AMF responses may arise from differences in the degree of the fine root development (Leb ron *et al.*, 2012). Rolden- Fajardo (1994) posited that each plant has a specific reaction to certain associated mycorrhizal fungal strain.

The root calcium content of the cowpea varieties resulting in 2016 having significantly higher values than in 2017 (Table 2) might be due to environmental factors such as rainfall, temperature e.t.c. It was reported that heavy rainfall favored excessive vegetative growth and caused lower seed quality in cowpea (Karungi *et al.*, 2000). Cowpea grows best at day temperature of 25 to 35°C; night temperature should not be less than 15°C (Brink and Belay, 2006). Also, it might be due to effect of climatic changes on the development of arbuscular mycorrhizal fungi. Gemma and Koske (1988) reported that, seasonal and climate changes, including temperature fluctuations, can affect the temporal structuring of AMF communities.

Table 2: Effect of *G. clarum* on root calcium content of Cowpea Varieties (combined 2016 and 2017)

Calcium			
Treatment		SE ±	0.04
0-	1.21c		
0+	1.36b	Year	
10	1.34b	2016	2.77a
20	1.59a	2017	0.11b
30	1.70a	Mean	1.44
Mean	1.44	SE ±	0.003
SE ±	0.04		
Variety		Interactions	
SAMPEA 7	1.43ab	Var*Conc	*
IFE 82-12	1.44ab	Var*Year	NS
IT97K-499-35	1.51a	Conc*Year	*
TVX 3236	1.38b	Var*Conc*Year	*
Mean	1.44		

Note: Means followed by the same letter(s) in each column, under each variety, in each year are not significantly different ($P \leq 0.05$), using DMRT. WAP- Weeks after Planting

Source; Author's Field Work

Generally, in 2016 and 2017 there was no emerged *Alectra* plants recorded under the 7 and 9 WAP (Table 3; Table 4). The recorded cases of unemerged *Alectra* plants on cowpea varieties were mostly under varieties SAMPEA 7 and TVX 3236

(Table 3; Table 4). No unemerged *Alectra* shoot was found on IT97K-499-35 at 7 and 9 WAP in 2016 (Table 3). In 2017, no *Alectra* shoot (emerged or unemerged) was recorded at 9 WAP (Table 4). Also, the highest percentage of mycorrhizal

colonization at 5 WAP was recorded in cowpea variety SAMPEA 7 followed by TVX 3236 while the lowest was in cowpea variety IFE 82-12 (Table 5). The highest record of unemerged *Alectra* plants in cowpea variety SAMPEA 7 and TVX 3236 might be due to its genetic make-up which makes it more susceptible to *Alectra* infestation. The genotypes promote the ability of the two cowpea varieties to support high levels of infection and exhibit reduced growth and yield compared to resistant varieties. It has been reported that resistant genotypes improve yields by improving an effect on one or

combination of the yield components such as: minimizing or preventing reduction of number of pods per plant, reduction in pods weight, reduction in number of seeds per pods among other parameters (Kutama *et al.*, 2013; Karanja *et al.*, 2013). The highest record of unemerged *Alectra* plants in year 2016 than that in year 2017 might also be due to environmental changes. This is in agreement with work done by Mbwaga *et al.* (2010) who deduced that environmental factors such as drought reduces the virulent effect of *Alectra vogelii*.

Table 3: Effect of *Glomus clarum* on *Alectra vogelii* growth parameters at 7 and 9 WAP in 2016

Cowpea Variety	VAM CONC (g)	<i>Glomus clarum</i>					
		7 WAP		9 WAP			
		No. of emerged plants	No. of unemerge d plants	Fresh weight (g)	No. of emerged plants	No. of unemerge d plants	Fresh weight (g)
SAMPEA 7	0 – parasite	-	-	-	-	-	-
	0+ parasite	-	-	-	-	-	-
	10	-	1	0.2	-	-	-
	20	-	2	0.5	-	-	-
	30	-	9	14.2	-	1	0.6
IFE 82 -12	0 – parasite	-	-	-	-	-	-
	0+ parasite	-	1	0.4	-	-	-
	10	-	-	-	-	-	-
	20	-	-	-	-	-	-
	30	-	-	-	-	-	-
IT97K – 499 – 35	0 – parasite	-	-	-	-	-	-
	0+ parasite	-	-	-	-	-	-
	10	-	-	-	-	-	-
	20	-	-	-	-	-	-
	30	-	-	-	-	-	-
TVX 3236	0	-	-	-	-	-	-
	0+ parasite	-	-	-	-	-	-
	10	-	1	1.4	-	-	-
	20	-	-	-	-	-	-
	30	-	2	1.5	-	-	-

Note: The symbol “-” indicates absence of emerged or unemerged *Alectra vogelii* plants

Source; Author's Field Work

Table 4: Effect of *Glomus clarum* on *Alectra vogelii* growth parameters at 7 and 9 WAP in 2017

Cowpea Variety	VAM CONC (g)	<i>Glomus deserticola</i>					
		7 WAP		9 WAP			
		No. of emerged plants	No. of unemerge d plants	Fresh weight (g)	No. of emerged plants	No. of unemerge d plants	Fresh weight (g)
SAMPEA 7	0 – parasite	-	-	-	-	-	-
	0+ parasite	-	-	-	-	-	-
	10	-	-	-	-	-	-
	20	-	2	1.3	-	-	-
	30	-	-	-	-	-	-
IFE 82 -12	0 – parasite	-	-	-	-	-	-
	0+ parasite	-	-	-	-	-	-
	10	-	-	-	-	-	-
	20	-	-	-	-	-	-
	30	-	-	-	-	-	-
IT97K – 499 – 35	0 – parasite	-	-	-	-	-	-
	0+ parasite	-	-	-	-	-	-
	10	-	-	-	-	-	-
	20	-	-	-	-	-	-
	30	-	-	-	-	-	-
TVX 3236	0	-	-	-	-	-	-
	0+ parasite	-	-	-	-	-	-
	10	-	-	-	-	-	-
	20	-	-	-	-	-	-
	30	-	3	1.5	-	-	-

Note: The symbol “-” indicates absence of emerged or unemerged *Alectra vogelii* plants

Source; Author’s Field Work

Table 5: Percentage Colonization for Cowpea Variety Sampled at 5 WAP

Cowpea variety	Percentage Colonization (%) for <i>Glomus clarum</i>
SAMPEA 7	84.62
IFE 82-12	50.00
IT97K-499-35	62.50
TVX 3236	64.29

Source; Author’s Field Work

CONCLUSION AND RECOMMENDATIONS

In conclusion, the result of this work shows that, *Glomus clarum* treatments had significant increase on the root calcium content of the four cowpea varieties studied. In the tripartite interaction between cowpea varieties, AMF and *Alectra*,

significant impact on the host plant was observed. Therefore, the null hypotheses that AM fungi have no impact on the root calcium content of *Alectra* infected cowpea varieties are rejected.

Based on the research findings, the following are recommendations:

1. The use of each *Glomus clarum* at 30 g/pot treatment in soils, with *Alectra* is recommended for farmers to obtain higher values for calcium content of cowpea root.
2. Farmers can cultivate cowpea varieties IT97K-499-35 on soils infected with *Alectra* for higher values for calcium content of cowpea root.
3. Further research work is also needed to determine the interaction between the cowpea varieties, fertilizer application, AMF with *Alectra* under both sterilized and unsterilized conditions. This will provide relevant information necessary agricultural and horticultural advancement.

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