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Screening for Resistance Mechanisms in Cowpea Genotypes on *Alectra vogelii*

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Abstract

Parasitic angiosperm *Alectra vogelii* Benth is a growing problem in Malawi, particularly with current emphasis on legumes. Therefore, two studies were set in order to understand the possible mechanisms of resistance in cowpea genotypes on their reaction to the parasitic weed. In the first experiment, *Mkanakaufiti*, IT99K-7-21-2-2XIT82E-16, Sudan 1 and IT82E-16 were grown in *Alectra* infested and non-infested pots. The experiment (2*4 factorial treatment combination) was arranged in an RCBD and replicated eight times. The second experiment, involved Petri-dish techniques where 4 genotype roots were assessed on their ability to stimulate the germination of *A. vogelii* as a proxy for germination stimulant production. The experiment was arranged in an RCBD and replicated five times. In the first experiment, data was collected on; the number of days to first *Alectra* emergence, *Alectra* shoot counts at 6, 8, 10, and 12 weeks after planting (WAP), *Alectra* attachment at 5 and 12 WAP, *Alectra* biomass at 12 WAP, cowpea biomass parameters at 5 and 12 WAP, yield and yield components per pot. While in the second experiment, number of germinated *Alectra* seeds per Petri dishes was recorded. The results indicated that IT82E-16 (33.25 days) and Sudan 1 (34.25 days) were earlier infested whilst late on IT99K-7-21-2-2XIT82E-16 (38 days) which correlated to the number of *Alectra* attachments. There were significant differences ($p = 0.05$) in weekly *Alectra* counts between cowpea varieties from 6 up to 10 WAP. *Mkanakaufiti* and IT99K-7-21-2-2XIT82E-16 were observed with no and few *Alectra* shoots infestation respectively which was an indicator of resistance mechanism in the study. Number of pods, grain weight (g) and harvest index per pot were significantly affected by inoculation protocol with lower yield on infested cowpea genotypes. The same trend was observed on cowpea varieties where *Mkanakaufiti* (21.9 g/pot) shown higher yield followed by IT82E-16 (12.5 g/pot) which is susceptible but with tolerance ability to the parasitic weed. The study has shown that resistance mechanisms can be categorized as no or few *Alectra* shoots, death of *Alectra* shoots and late infestation. In the Petri dishes, only 3 WAP grown *Mkanakaufiti* root media failed to induce the germination of *Alectra* seeds while the opposite occurred on IT82E-16, Sudan 1 and IT99K-7-21-2-2XIT82E-16. On the contrary, 4 WAP grown root media of the four genotypes stimulated *Alectra* germination which shed more light on the seed behaviour in the soil. This is worth exploring as more could be known to what causes termination of *Alectra* shoots on *Mkanakaufiti*. Still, intensifying resistant genotypes should be a goal in order to reduce *Alectra* seed banks in the soil, thereby, increasing cowpea yield.

Keywords

Alectra vogelii, Cowpea Genotypes, Mechanisms, Stimulant

1. Introduction

The widespread incidence of *Alectra vogelii* (Benth) on cowpea (*Vigna unguiculata* (L.) Walp) presents severe challenges on smallholder farmers with a record of yield reduction up to 100% [1]. However, resistance genotypes to *Alectra vogelii* are the most practical method as herbicide alternatives are costly [2]. Nevertheless, cultural control practices' benefits are long term solutions. Host plant resistance [3] controls seed bank levels in the soil as few or no growth is allowed. Reference [4] reported that cowpea resistance against *Alectra vogelii* is not easy to assess in a field due to a number of cofactors such as parasite variability, unpredictable environmental influences and imprecise selection criteria. However, resistance in cowpea on *Striga gesnerioides* has been assessed through the comparison of number and size of *S. gesnerioides* tubercles on accessions with notable reaction to the parasitic weed [5]. Two cowpea landraces, APL-1 and 87-2, showed absolute resistance to *Striga* strains sourced from Burkina Faso, Mali and Cameroon and partly Niger [5].

Reference [6] [7] reported that resistance mechanisms of cowpeas on *Alectra vogelii* can be easily studied on germination in relation to the levels of exudates produced by the crop. At germination, genotype of cowpeas has shown to support few or no *Alectra* shoots as there is low production of *Alectrol* or *Strigolatone* which is a prerequisite to germination of *Alectra vogelii* in a field [8] [9]. Reference [10] exploits that, low production of germination stimulants is an effective mechanism of resistance in sorghum material bred for *Striga* resistance with marginal consideration on *Orobanchaceae* in legumes [11]. However, [12] turns the coin, as it was discovered on a wide range of legumes.

Alectra vogelii cycle before ground emergence comprised of germination, haustorial induction, attachments to the host roots which allowed penetration of the host vascular cells [13] [14]. Each stage is critical for the successful development of the parasitic weed in different host plants [3] [15] [16]. However, *A. vogelii* seeds germinate only when they sense the presence of chemical compound produced by the host plant roots [6]. Furthermore, *A. vogelii* in a greenhouse study was observed to undergo all the stages of establishment and failure seed production later. This correlated to reduction of the seed banks (Bokosi, personal communication, 2016). The study of *Alectra vogelii* growth "*in-vitro*" using parasitized hosts, shed more light on the underlying mechanisms of resistance to *A. vogelii* at different root development stages.

In Malawi, *Mkanakaufiti* and IT82E-16 cowpea varieties have been released as resistant and tolerant to *Alectra vogelii* respectively [17] (Bokosi, personal communication, 2017). However, genotype B301 indicated that one dominant gene is responsible for resistance in *Striga*, whilst in *Alectra*, two genes are responsible for resistance in the genotypes [2] which is an indicator of variable resistance mechanism on the genotypes. In the present study, an *in vitro* system was used to evaluate a number of cowpea germplasm for their sources of resistance to *A. vogelii* and a further study was done on pot screening trial on the same cowpea genotype in order to confirm the results.

2. Materials and Methods

2.1. Cowpea Germplasm

Germplasm used in the study have a number of attributes and reactions to abiotic and biotic factors in the environment (**Table 1**). The genotypes were sourced from Crop and Soil Sciences Student Research Farm.

2.2. Pot Screening Study

Alectra-host interaction study was conducted in a well-ventilated plastic green house during the hot dry season (from October 2017 for 90 days) at Crop and Soil Sciences Student Research Farm. The site is located at 14˚35'S, 33˚50'E, with the elevation of 1200 metres above sea level, Lilongwe, Malawi. Dried and mature capsule *Alectra* plant were sampled from groundnut fields in Kalumba located

Table 1. Cowpea genotype germplasm and their characteristics.

Cowpea genotypes	Yield potential (kg·ha^{-1})	*Alectra* reaction	Common disease	Crop duration and growth habit	Drought reaction
Mkanakaufiti [17]	2500 and released	Resistant	Susceptible to Mosaic Virus	Dwarf and medium	Tolerant
IT99K-7-21-2-2XIT82E-16	**	Resistant	**	Climber and short	Tolerant
Sudan 1 [18]	2500 and released	Susceptible	Resistant to Mosaic virus	Dwarf and short	Tolerant
IT82E-16 [18]	2500 and released	Susceptible with tolerance	Resistant to Mosaic virus	Climber and short	Tolerant

Note: ** means not available.

at 14˚13.029'S, 033˚48.019'E with an elevation of 1187 metres above sea level during the 2016/17 growing seasons in June. Then after, the inoculum was sun dried for 30 days followed by cleaning.

2.2.1. Experimental Set-Up

There were sixty four (64) plastic pots with a uniform diameter of 18 and depth of 17 centimetres. The pots were filled with sandy loamy soil sourced from Bunda forest. Approximately, 0.015 g seeds of *Alectra* were inoculated on the four pots, after mixing with fine sandy and coarse sandy soil in the ratio of 1:2 respectively [19]. However, the other four pots were set as a control. Three seeds from the four cowpea genotype were planted per pot and thinned to two, 1 week after planting (WAP) representing an experimental unit. Irrigation was done on daily basis in regard to the water need of each crop and it was done up until the legumes reached their physiological maturity. All weeds, except *A. vogelii* were manually uprooted throughout the growing period. At 5 and 12 WAP, four reps were sampled. Their plant samples were uprooted gently using a hand trowel and then roots were thoroughly washed with tap water. Root and shoot fresh weights of samples per pots were taken and later on, the samples were oven dried for 48 hours at 70˚C.

There were two experimental factors: Cowpea variety; *Mkanakaufiti*, IT99K-7-21-2-2XIT82E-16, Sudan 1 and IT82E-16. Inoculation protocol; inoculated and non-inoculated. A 2*4 factorial treatment combination was arranged in an Randomised Complete Block Design (RCBD) and replicated eight times. The crops were planted on 10th October, 2017.

2.2.2. Data Collection

Data was collected on the number of days to first *Alectra* emergence, periodic number of *Alectra* shoots emerged per pot at 6, 8, 10, and 12 weeks after planting (WAP), *Alectra* biomass per pot at 12 WAP, number of *Alectra* attachment at 5 and 12 WAP.

Cowpea growth parameters were the number of days to first cowpea flowering, fresh and dry root, shoot weight (g), shoot to root ratio at 5 WAP and at harvest. Yield parameters included grain yield (g), pod weights (g), number of pods, seed weight (10 or 100 seed size) (g), shelling percent and harvest index. 10 seed size per pot was considered as the maximum number due to low yield on *Alectra* infested pots. However, a combined 100 seed size was recorded after combination of all replication per variety. Due to the nature of the experiment, soil analyses were not arranged.

2.3. *In Vitro* Study

A modified root cut assay technique adopted from [20] was utilized where four selected cowpea genotypes roots media were used.

2.3.1. Growing of Cowpea Cultivar Seedlings

There were twenty plastic pots with a uniform diameter of 22 cm and depth of

20 cm. These were filled with sandy loamy soil sourced from Bunda forest. Four seeds from the four selected cowpea varieties namely; *Mkanakaufiti*, IT99K-7-21-2-2XIT82E-16 and Sudan I, IT82E-16 were planted per pot and thinned to three, 1 WAP representing an experimental unit. Irrigation was done on daily basis in regard to the water need of each genotype for 3 and 4 WAP. After 3 and 4 WAP, the genotypes were removed gently from the pots with their roots washed free of soils with tap water and rinsed with distilled water.

2.3.2. Sterilization and Conditioning of *Alectra* Seeds

Alectra seeds were surface disinfected with an aqueous 1% NaOCL (Sodium hypochlorite) solution for about a minute. Then after, ten Petri dishes were lined with two Whatman No. 1 filter papers and moistened with 5 ml of distilled water. Then after, 0.05 g of *A. vogelii* seeds were placed on the filter papers. The Petri dishes were sealed with parasitic wrappers and covered with aluminum foil to prevent water losses and exclude light respectively. The 10 Petri dishes were incubated for 5 days at 33˚C which coincided with 21 and 28 days growth period of the test plants above.

2.3.3. Preparation of Root Cuttings and Inoculation Procedure

20 Petri dishes were lined with two layers of Whatman No. 1 filter paper moistened with 5.0 ml of distilled water. Roots from the four cowpea varieties were cut into small pieces and crashed using a knife. 1.0 g of root pieces from each cowpea variety was weighed and placed into the central aluminum foil ring where the precondition, *A. vogelii* seeds were placed. Then after, 2 to 3 drops of sterile distilled water were added on the roots media in order to facilitate diffusion of the root exudates across the filter paper. Thereafter, the Petri dishes were sealed with plastic wrappers and coated with aluminum foil followed by, incubation at 30˚C for five days.

The experimental factor was cowpea varieties (CV); *Mkanakaufiti*, IT99K-7-21-2-2XIT82E-16 Sudan I, and IT82E-16. Both grown cowpea genotypes and Petri dishes were arranged in a Randomized complete block design and replicated five times. The crops were planted on 27th December, 2017.

Germination on each Petric dish was checked on the 5th day of incubation. Germinated *Alectra vogelii* seeds from each Petri dish were counted with the aid of low power (×20) dissecting microscope.

2.4. Statistical Analysis

GenStat® 15 edition was used to perform analyses of variance (ANOVA). The difference between means of significant variables was separated using a least significant difference (LSD) at 5% and 10% level. Analysis on number of *Alectra* shoots, *Alectra* attachments; cowpea biomass parameters (g), and *Alectra* biomass (g) were performed after square root transformation of the data [$(x + 0.5)^{0.5}$] [21] [22] [23] as there was a high variability on the normal data. The maximum value of 40 and 84 days marks the end of the experiment in phases and indicated no *Alectra vogelii*. 100 seed size means were generated after combination of samples per treatment combination as grain yields were low due to *Alectra* infestation.

3. Results

3.1. Pot Screening Study Results

3.1.1. Number of Days to First *Alectra* Emergence and *Alectra* Attachments

Significant difference on the number of days to first *Alectra* emergence between cowpea varieties at 5 and 12 WAP was observed (Table 2). In both sampling times, *Mkanakaufiti* did not support emergences of *Alectra vogelii*. However, at 5 WAP, IT82E-16 was earlier in *Alectra* infestation while Sudan 1 at 12 WAP. Number of *Alectra vogelii* attachments at 5 and 12 WAP was significantly ($0.05 < p < 0.1$) affected by cowpea varieties. IT82E-16 cowpea variety registers a high number of attachments while *Mkanakaufiti* did not in all sampling times.

3.1.2. Cowpea Biomass Parameters

The result has revealed that inoculation protocol x cowpea varieties interaction effect did not significantly affect root biomass (g), shoot biomass (g), shoot to root ratio either fresh or dry at 5 WAP (Table 3). Fresh and dry root, biomass at 5WAP were significantly different on the inoculation protocol and cowpea varieties. However, high root biomass was observed on *Alectra* infested cowpea varieties. On the contrary, both fresh and dry shoot biomass were not significant at 5 WAP on the inoculation protocol. Shoot to root ratios showed a significant difference on the inoculation protocol. On the other hand, fresh and dry root biomass at 5 WAP were significantly ($p = 0.05$, $p = 0.01$) affected by variety effect. However, no significant difference was observed on fresh and dry shoot biomass, shoot to

Table 2. Effects of cowpea varieties on number of days to first *Alectra* emergence (NDFAE) and number of *Alectra* attachments at 5 and 12 weeks after planting (WAP) on the four cowpea varieties.

Cowpea varieties	NDFAE at 5 WAP	NDFAE at 12 WAP	Number of *Alectra* attachments at 5 WAP *	Number of *Alectra* attachments at 12 WAP*
Mkanakaufiti	40.000^{b}	84.000^{c}	0.707^{a}	0.707^{a}
IT99K-7-21-2-2XIT82E-16	38.000^{ab}	35.000^{ab}	3.168^{ab}	2.29^{ab}
IT82E-16	33.250^{a}	36.000^{b}	5.243^{b}	1.625^{ab}
Sudan 1	34.250^{ab}	31.750^{a}	4.722^{ab}	3.240^{b}
LSD 5%	3.891	3.268	2.965	1.932
F. prob	0.011	<0.001	0.029	0.080
Grand mean	36.380	46.690	3.460	1.966
CV %	6.700	4.400	53.600	61.500

Note: *means analysis was performed after square root transformation of data $[(x + 0.5)^{0.5}]$. +means the maximum value of 40 and 84 days marks the end of the experiment in phases and indicates that no emergence of *A. vogelii* occurred.

Table 3. Effects on *Alectra* inoculation protocol, cowpea varieties, and inoculation protocol x cowpea varieties on cowpea biomass (g) parameters at 5 weeks after planting (WAP).

Factors	Fresh root biomass (g)	Fresh shoot biomass (g)	Shoot to root ratio (fresh) *	Dry root biomass (g)	Dry shoot biomass (g)	Shoot to root ratio (dry)*
Inoculation protocol						
Inoculated	46.100^{a}	130.400^{a}	1.937^{a}	9.510^{b}	19.600^{a}	1.794^{a}
Non-inoculated	16.800^{b}	142.900^{a}	3.114^{b}	3.700^{a}	24.300^{a}	2.883^{b}
LSD 5%	10.910	17.250	0.345	2.701	5.130	0.5231
F. prob	<0.001	0.147	<0.001	<0.001	0.070	<0.001
Cowpea varieties						
Mkanakaufiti	23.200^{a}	130.100^{a}	2.692^{a}	3.580^{a}	18.700^{a}	2.719^{a}
IT99K-7-21-2-2XIT82E-16	29.000^{ab}	144.300^{a}	2.605^{a}	6.310^{ab}	24.800^{a}	2.275^{a}
IT82E-16	29.300^{ab}	134.800^{a}	2.594^{a}	6.790^{ab}	21.900^{a}	2.431^{a}
Sudan 1	44.100^{b}	137.300^{a}	2.210^{a}	9.730^{b}	22.400^{a}	1.930^{a}
LSD 5%	15.440	24.400	0.4881	3.820	7.260	0.7398
F. prob	0.060	0.682	0.203	0.026	0.392	0.195
F. prob for interaction						
Inoculation protocol * cowpea varieties	0.289	0.387	0.938	0.258	0.181	0.944
Grand mean	31.400	136.700	2.525	6.600	21.900	2.339
CV %	47.300	17.200	18.600	55.600	31.800	30.400

Note: *means analysis was performed after square root transformation of data $[(x + 0.5)^{0.5}]$.

root ratio between the cowpea varieties. Interestingly, low shoot to root ratio was observed on inoculated pots as compared to non-inoculated.

3.1.3. Cowpea Biomass Parameters at 12 WAP

Inoculation protocol by x cowpea varieties interaction was significant on fresh shoot biomass (**Table 4**). However, the inoculation protocol x cowpea varieties did not significantly affect fresh root weight, dry root weight, dry shoot weight, fresh and dry shoot to root ratio (**Table 5**). Similarly, all the cowpea biomass parameters were not significantly affected by inoculation protocols. Fresh root weight, dry root weight, dry shoot weight and dry shoot to root ratio were not significantly affected by variety effect. Nevertheless, fresh shoot, dry shoot biomass and fresh shoot to root ratio were significantly affected by cowpea varieties. Interestingly, *Mkanakaufiti* was observed with higher biomass.

3.1.4. Weekly *Alectra* Shoot Counts on the Four Cowpea Varieties

Alectra counts at all sampling times was significantly affected by the cowpea varieties. Sudan 1 and IT82E-16 were observed in supporting a higher number of *Alectra* shoots from 6 WAP (**Table 6**). On the contrary, *Mkanakaufiti* was

Table 4. Effects inoculation protocol x cowpea varieties interaction on fresh shoot biomass* (g) at 12 weeks after planting (WAP).

	Inoculation protocol	
Cowpea varieties	Non-inoculated	Inoculated
IT82E-16	7.426	4.756
IT99K-7-21-2-2XIT82E-16	6.658	5.772
Mkanakaufiti	11.062	8.506
Sudan 1	6.242	9.524
LSD 5%	3.298	

Table 5. Effects of inoculation protocol, cowpea varieties and inoculation protocol x cowpea varieties on cowpea biomass (g) parameters at 12 weeks after planting (WAP) 12.

Factors	Fresh root biomass* (g)	Fresh shoot biomass* (g)	Dry shoot biomass* (g)	Dry root biomass* (g)	Fresh shoot: root ratio*	Dry shoot to root ratio*
Inoculation protocol						
Inoculated	3.692[a]	7.847[a]	6.221[a]	2.354[a]	2.431[a]	3.441[a]
Non-inoculated	3.096[a]	7.139[a]	5.606[a]	1.925[a]	2.444[a]	3.487[a]
LSD 5%	0.701	1.649	1.494	0.618	0.465	1.094
F. prob	0.092	0.382	0.402	0.164	0.954	0.932
Cowpea varieties						
Mkanakaufiti	3.506[a]	9.784[b]	7.433[b]	2.106[a]	2.992[b]	4.033[a]
IT99K-7-21-2-2XIT82E-16	3.480[a]	6.215[a]	5.055[a]	2.339[a]	2.046[a]	2.866[a]
IT82E-16	2.819[a]	6.091[a]	4.825[a]	1.865[a]	2.388[ab]	3.223[a]
Sudan 1	3.770[a]	7.883[ab]	6.340[a]	2.250[a]	2.326[a]	3.736[a]
LSD 5%	0.991	2.332	2.113	0.875	0.657	1.547
F. prob	0.259	0.011	0.062	0.697	0.045	0.421
F. prob for interaction						
Inoculation protocol * cowpea varieties	0.262	0.050	0.128	0.325	0.241	0.678
Grand mean	3.394	7.493	5.913	2.140	2.438	3.464
CV %	28.100	29.900	34.400	39.300	25.900	43.000

Note: *means analysis was performed after square root transformation of data $[(x + 0.5)^0]$.

Table 6. Weakly *Alectra* shoot counts (AC) on the four cowpea varieties.

Cowpea varieties	AC at 6 WAP*	AC at 7 WAP*	AC at 8 WAP*	AC at 9 WAP*	AC at 10 WAP*	AC at 11 WAP*	AC at 12 WAP*
Mkanakaufiti	0.707[a]	0.707[a]	0.707[a]	0.707[a]	0.707[a]	0.707[a]	0.707[a]
IT99K-7-21-2-2XIT82E-16	2.184[ab]	3.502[b]	4.053[ab]	4.280[ab]	3.623[ab]	2.924[a]	2.215[a]
IT82E-16	2.964[ab]	5.839[b]	7.887[c]	4.966[ab]	3.060[ab]	3.112[a]	2.491[a]
Sudan 1	4.573[b]	5.696[b]	6.411[bc]	7.944[b]	7.345[b]	6.568[a]	3.375[a]
LSD 5%	2.432	2.451	3.368	4.512	4.459	5.053	3.838
F. prob	0.035	0.003	0.005	0.035	0.049	0.141	0.498
Grand mean	2.607	3.936	4.765	4.474	3.684	3.328	2.197
CV %	58.300	38.900	44.200	63.000	75.700	94.900	109.200

Note: *means analysis was performed after square root transformation of data $[(x + 0.5)^{0.5}]$. WAP means weeks after planting.

observed with no *Alectra* shoot during the entire growing period. There was an increase in the number of *Alectra* shoots on the different genotypes used with time. However, from 10 WAP up to 12 WAP *Alectra* shoot counts were dropping.

3.1.5. Cowpea Flowering Parameters

There was no significant interaction between inoculation protocol by cowpea varieties on the number of days to first flowering (NDFF), 50% and 100% flowering (Table 7). NDFF was significantly affected by cowpea varieties with IT99K-7-21-2-2XIT82E-16 as earlier in flowering while *Mkanakaufiti* flowered late. However, NDFF on inoculation protocol was not significant. Number of days to 50% and 100% flowering was insignificant on the inoculation protocol. Nevertheless, number of days to 50% and 100% flowering was different between the cowpea varieties.

3.1.6. Cowpea Yield and Yield Components

The results revealed that inoculation protocol x cowpea varieties interaction effect did not significantly affect number of pods, pod weight (g), grain weight (g), seed weight (10 seed size) (g), shelling percent and harvest index per pot (Table 8). Number of pods, yield (g) and harvest index per pot were significantly

Table 7. Effects of inoculation protocol, cowpea varieties and inoculation protocol x cowpea varieties interaction on cowpea flowering parameters.

Factors	NDFF	Number of days to 50% flowering	Number of days to 100% flowering
Inoculation protocol			
Inoculated	49.440^{a}	56.940^{a}	63.810^{a}
Non-inoculated	51.060^{a}	56.440^{a}	63.810^{a}
LSD 5%	2.470	1.921	1.931
F. prob	0.186	0.594	1.000
Cowpea varieties			
Mkanakaufiti	58.380^{c}	63.120^{b}	71.000^{c}
IT99K-7-21-2-2XIT82E-16	44.750^{a}	53.620^{a}	60.250^{a}
IT82E-16	48.380^{b}	54.120^{a}	60.750^{ab}
Sudan 1	49.500^{b}	55.880^{a}	63.250^{b}
LSD 5%	3.493	2.717	2.731
F. prob	<0.001	<0.001	<0.001
F. prob for interaction			
Inoculation protocol * cowpea varieties	0.334	0.303	0.141
Grand mean	50.250	56.690	63.810
CV %	6.700	4.600	4.100

Note: NDFF means number of days to first flowering.

Table 8. Effects of inoculation protocol, cowpea varieties and inoculation protocol x cow pea varieties interaction on cowpea yield and yield components.

Factors	Number of pods per pot	Pods weight/pot (g)	Yield/pot (g)	10 seed size/pot (g)	100 seed size (g)	Shelling %	Harvest index
Inoculation protocol							
Inoculated	9.880[a]	16.400[a]	11.600[a]	1.241[a]	12.54 ± 1.405	72.400[a]	16.13[a]
Non-inoculated	14.000[b]	23.800[a]	16.500[b]	1.130[a]	11.78 ± 1.652	71.670[a]	27.77[b]
LSD 5%	4.08.000	7.750	4.810	0.1194	**	7.473	7.823
F. prob	0.048	0.061	0.048	0.066	**	0.841	0.006
Cowpea varieties							
Mkanakaufiti	18.250[b]	30.400[b]	21.900[b]	1.176[b]	12.590 ± 0.566	72.160[a]	20.07[ab]
IT99K-7-21-2-2XIT82E-16	8.750[a]	14.000[a]	10.700[a]	1.365[c]	13.460 ± 0.622	75.490[a]	24.92[b]
IT82E-16	11.250[a]	16.600[a]	12.500[a]	1.354[c]	11.430 ± 2.708	75.010[a]	29.11[b]
Sudan 1	9.500[a]	19.300[a]	11.100[a]	0.848[a]	11.160 ± 0.629	65.470[a]	13.70[a]
LSD 5%	5.770	10.970	6.800	0.1688	**	10.568	11.063
F. prob	0.010	0.027	0.007	<.001	**	0.2090	0.049
F. prob for interaction							
Inoculation protocol * cowpea varieties	0.751	0.342	0.255	0.138	**	0.596	0.216
Grand mean	11.940	20.100	14.000	1.186	**	72.030	21.950
CV %	46.500	52.500	46.600	13.700	**	14.100	48.500

Note: **means not available.

affected by inoculation protocols. However, pod weight (g), 10 seed size (g) and shelling index was not significant on the inoculation protocol. On the other hand, number of pods, pod weight (g), grain weight (g) and harvest index per pot registered higher output on non-inoculated cowpea genotypes. On variety difference a similar significant trend was observed on number of pods, pod weight (g), yield (g) per pot on their reaction to *Alectra* infestation or not. Seed weight (g) was significant on between the cowpea varieties with IT99K-7-21-2-2XIT82E-16 and IT82E-16 registering higher seed size whilst Sudan 1 was the least. However, shelling index was not significant on the cowpea varieties. *Mkanakaufiti*, IT99K-7-21-2-2XIT82E-16 and IT82E-16 were significantly different to Sudan 1 on the harvest index parameter. Even though, harvest index was drastically reduced by *Alectra* infestation on Sudan 1 as compared to other genotypes used.

3.1.7. *Alectra* Biomass at Harvest per Pot

No significant difference was observed on both fresh and dry *Alectra* biomass at

12 WAP between the cowpea varieties. However, no *Alectra* weight was observed on *Mkanakaufiti* whilst Sudan 1 registered higher amount (Table 9).

3.2. *In Vitro* Study Results

Germinated *Alectra* Seed per Petri Dishes

Alectra shoot counts per Petri dish were significantly different between the cowpea varieties root media prepared at 3 and 4 WAP (Table 10). Only, 3 WAP *Mkanakaufiti* root media failed to support *Alectra* seed germination. However, at 4 WAP root media shown germinated *Alectra* seeds, developing mycelia and attachment tubes on all the cowpea varieties.

4. Discussion

Number of days to first *Alectra* emergence between the cowpea varieties was significant at 5 and 12 WAP. Earlier infestation of *Alectra vogelii* on IT82E-16 at 5WAP and Sudan 1 at 12 WAP could be probably due to the level of exudates

Table 9. Effects of cowpea varieties on *Alectra* biomass (g) per pot at 12 weeks after planting (WAP).

Cowpea varieties	*Alectra* fresh biomass* (g) per pot	*Alectra* dry biomass* (g) per pot
Mkanakaufiti	0.707[a]	0.707[a]
IT99K-7-21-2-2XIT82E-16	3.133[a]	1.986[a]
IT82E-16	3.646[a]	2.675[a]
Sudan 1	4.909[a]	3.692[a]
LSD 5%	5.333	3.903
F. prob	0.393	0.415
Grand mean	3.099	2.265
CV %	107.600	107.700

Note: *means analysis was performed after square root transformation of data $[(x + 0.5)^{0.5}]$.

Table 10. Effects of cowpea varieties on number of *Alectra* seeds germinated per Petri dish.

Cowpea varieties	Number of germinated *Alectra* seeds on the 3 WAP root media	Number of germinated *Alectra* seeds on the 4 WAP root media
Mkanakaufiti	0.707[a]	10.610[a]
IT99K-7-21-2-2XIT82E-16	2.151[bc]	11.500[ab]
IT82E-16	1.651[ab]	11.730[ab]
Sudan 1	3.360[c]	13.310[b]
LSD 5%	1.420	1.842
F-prob.	0.011	0.048
Grand mean	1.970	11.790
CV %	52.400	11.300

Note: *means analysis was performed after square root transformation of data $[(x + 0.5)^{0.5}]$.

produced by the different host crops, suggesting that the cultivars are highly susceptible [24] [25]. Reference [25] reported that early emergence of *Alectra vogelii* could probably result in 100% yield reduction on the host crops. The absence of *Alectra vogelii* in *Mkanakaufiti* in all sampling times indicated host incompatibility of the variety on the parasitic weed. Number of *Alectra* attachments at 5 and 12 WAP sampling time was significantly different between the cowpea varieties which corresponded to the number of day to first *Alectra* emergence. This could be due to host specificity and susceptibility levels which corresponded to the level of exudates and the level of photo-assimilates produced by the host crops. Reference [26] reported that few or no *Alectra* shoots on cowpea varieties could be attributed to very limited food reserves on *Alectra* seeds such that they only survive for a few days after their germination, unless, they reach host root and xylem [15]. This is why, few or no attachments on IT99K-7-21-2-2XIT82E-16 and *Mkanakaufiti* was not a surprise. The behavior mirrored resistance mechanisms on the two cultivars while the other two happened to support higher number of *Alectra* attachment which could be attributed to the level of root exudates produced by the host crops and corresponded to the high demands of photo assimilates in supporting the shoots [27]. Reference [28] reported that non-emergence of *Alectra* in resistant genotype confirms the characterization of variety as resistant to the parasitic weed which was revealed on *Mkanakaufiti*.

Alectra vogelii counts at all sampling times indicated a significant difference between the cowpea varieties. This indicated the level of susceptibility and host specificity. However, *Mkanakaufiti* was observed with no *Alectra* shoots which agrees with its resistance ability in hotspot areas [17]. Even though, all susceptible cowpea varieties were found with *Alectra* shoots at 6 WAP. Higher *Alectra* shoot counts were observed on Sudan 1 which corresponded to the suitability of the host [29]. Probably, this could be attributed to the level of root exudates produced by the host plant [26]. There was an increase in the number of *A. vogelii* shoot counts overtime on the susceptible cowpea varieties which indicated resource availability from the host crops. However, a drop in *Alectra* shoot counts in the late stages on the host crops was noted which revealed that susceptible cultivars tend to support few *Alectra* shoots as photo-assimilates decreases [28]. This is one of the means of reducing *Alectra* seed banks in the soil. IT99K-7-21-2-2XIT82E-16 was observed to support few *Alectra* shoot counts which reflected resistance ability, as it is considered resistant to the parasitic weed. This agreed to one of the McKnight trial results at Mitundu (Bokosi, personal communication, 2017). The mechanisms of resistance in the study could probably be reflected by no and few *Alectra* infestation on *Mkanakaufiti* and IT99K-7-21-2-2XIT82E-16 cowpea variety respectively.

Surprisingly, NDFF, number of days to 50% and 100% flowering were not significantly affected by inoculation protocol. On the other hand, it was assumed that infested pots could probably be observed in delayed flowering parameters. The results corresponded to [24] where no effect on flowering was observed on infested cowpea genotypes. NDFF was significantly affected by variety effect which could be attributed to the genotypes characteristics as *Mkanakaufiti* was a late maturing variety while IT82E-16, Sudan 1 and IT99K-7-21-2-2XIT82E-16 were early maturing cultivars [18]. IT99K-7-21-2-2XIT82E-16 was earlier in flowering as it supported fewer *Alectra* shoots which probably reflected no delay in flowering in the study.

Inoculation protocol by x cowpea varieties interaction was not significant on the number of pods, pod weight (g), grain yield (g), 10 seed size (g), shelling percent and harvest index per pot which indicated no correlation on the two factors together. However, number of pods, grain yield (g) and harvest index per pot were significantly affected by inoculation protocol. This indicated that *Alectra* shoots probably reduced yield as infested pots registered low yield which agreed with 100% reduction of yield in Botswana, observed on the susceptible variety [30] [31] [32]. However, shelling percent, 10 (100) seed size (g) was higher on inoculated pots which indicated that attributes were associated with variety genetics not *Alectra* infestation. The higher shelling percentage on inoculated corresponded to [22] findings. The number of pods, pod weights (g), grain yield (g) per pot was significantly different between the cowpea varieties. This correlated to [24] results where infested cowpea genotype registered low output. Furthermore, any attachment of the parasitic weed to the host plant increases sink demands in the roots thereby, diminishing carbon transfer to the shoot biomass demands which led to yield reduction [27]. This was why, higher yield was observed on a resistant released cowpea variety called *Mkanakaufiti*. However, significant higher yield was noted on IT82E-16 as it had tolerance ability to *Alectra vogelii* infestations [33] [34] [35]. Durable and sustainable tolerance of *Alectra vogelii* by host crops probably could be attributed to multiple genes which control various components of resistance [25] which was reflected by a released IT82E-16 cowpea variety in the study. Interestingly, IT99K-7-21-2-2XIT82E-16 was observed with low grain yield though supporting few *Alectra* shoots during the entire growing period. This indicated that resistance attribute had a trade off on grain yield though led to low *Alectra* seed banks in the soils. Reference [28] reported that genotypes which supported higher emerged *Alectra* shoots and heavy infestations probably recorded low yield and yield parameters of the crop. 10 seed size (g) was significantly differently affected by cowpea varieties which could be attributed to varietal genetics. Harvest index parameter was significant between the cowpea varieties. The results are in agreement with [22] where harvest index reduction was observed in *Vuli* I, *Tumaini* and IT 03K-378-4 due to *Alectra* infestation but not in the other three genotypes.

Significant interaction between inoculation protocols by x cowpea varieties on fresh shoot biomass was observed at 12 WAP. This could be attributed to the difference in reaction of the four cowpea varieties on the parasitic weed. However, fresh and dry root biomass at 5 WAP was significantly different on the inoculation protocol. This could be due to the presence of *Alectra* shoots on the susceptible cowpea varieties. This is in agreement as it was assumed that more growth resources were shunted to the root than the shoots [36]. Both fresh and dry root biomass of cowpea were significantly affected by cowpea varieties which reflected *Alectra* infestation level as in both cases as *Mkanakaufiti* was observed with low root biomass. On the contrary, both fresh and dry shoot biomass at 5 and 12 WAP were not significantly affected by variety effect. This suggested that *Alectra* reaction on the four varieties did not affect the shoot biomass. *A. vogelii* infected cowpeas had significantly ($p < 0.001$) higher fresh shoot to root ratio compared to uninfected plants at 5 WAP which correlated to [24]. However, upon oven drying the opposite was observed. A higher shoot to root ratio was observed on non-infested pots which indicated that infestation of *Alectra* on the four cowpea varieties increased the roots biomass thereby, decreasing the ratio.

On the contrary, fresh shoot, dry shoot biomass and fresh shoot to root ratio were significant on cowpea varieties at 12 WAP due to varietal reactions on *A. vogelii*. In general, cowpea biomass at 12 WAP were low as compared to 5 WAP probably due to aging of the varieties as they had lost most of their leaves. Reference [25] reported that lower biomass in infested cowpea genotypes was apparently due to intensive *Alectra* infestation which agreed to the study findings. A higher significant cowpea biomass on *Mkanakaufiti* variety revealed photo-assimilates full utilization on the crop for growth and development. Even though, *Alectra* biomass was not significant on the inoculation protocol and variety difference, higher biomass was observed on susceptible varieties with *Mkanakaufiti* registering no *Alectra* biomass. This reflected the yield decrease on the susceptible varieties as photo-assimilate sinks changed to the roots in favour of mobilizing to the parasitic weed [28].

In vitro, *Alectra* induction to germination was significantly affected by variety factor. This could be due to the biochemistry and specificity of the exudates produced by the varieties. Only *Mkanakaufiti* failed to support *Alectra* seed germination on root media harvested from 21 days grown cowpea plants. This could be probably due to *Mkanakaufiti* age where it was observed to be infested in the late stages of the crop under pot study. It is interesting to note that IT82E-16 supported few number compared to IT99K-7-21-2-2XIT82E-16 resistant genotype. This correlated to pot screening results especially on NDFAE at 12 WAP.

Interestingly, *Alectra* seed germinated on all the four cowpea root media grown for 28 days which agrees with [26]. This revealed that root exudates of all the four cowpea cultivars induced probably the germination of *Alectra* seeds in the soil as shown in the Petri dishes. However, susceptible cultivar allows further growth above the ground while resistant cultivar utilized apoptosis, thereby, supporting very few or no *Alectra vogelii* shoots which was reflected on IT99K-7-21-2-2XIT82E-16 and *Mkanakaufiti* in a pot screening trial (Kabambe, personal communication, 2017). The mechanisms of resistance could not be related to the exudates produced but what stops further growth of *Alectra* shoots on the *Mkanakaufiti* cowpea variety is worthy exploring. Even though, both

IT99K-7-21-2-2XIT82E-16 and *Mkanakaufiti* induced the germination of a few *Alectra* seeds *in vitro*.

5. Conclusion

In the study, resistance mechanism was observed on *Mkanakaufiti* with no *Alectra* shoots during the entire growing period of the crop. However, IT99K-7-21-2-2XIT82E-16 supported few *Alectra* shoots which dropped in the late stages of growth. Furthermore, death of *Alectra* shoot on susceptible genotypes and late infestation were the signs of resistance. Yield output was higher on the two cultivars as compared to the two susceptible cultivars but IT82E-16 supported high number of *Alectra* shoots but produced reasonable yield than Sudan 1. Age of *Mkanakaufiti* root media affects *Alectra* seed germination which corresponded to juvenile resistance. However, other genotypes used induced *Alectra* germination at all times of sampling. This revealed that in the field, the host crops have mechanisms that stop a further growth of the parasitic weed and it's worth exploring. Still, *Mkanakaufiti* and IT99K-7-21-2-2XIT82E-16 are suitable varieties for production in Malawi as the inoculum in the soil banks will decrease, thereby, decreasing the level of infestations on other suitable hosts.

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