Original Article: New evaluation of *Alternaria brassicicola* isolates against *Striga hermonthica* seeds emergence on maize in Kenya

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ABSTRACT

Striga hermonthica (Del.) Benth is also known as witchweed, is a highly noxious parasite of cereal in Sub- Saharan Africa leading to enormous economic losses of above 7 billion US$ yearly. Some of the suggested control methods have been ineffective, therefore, the weed has continued to increase its host range and area under infestation. The objective of this study was to evaluate *Alternaria brassicicola* isolates against *Striga hermonthica* seeds' emergence on maize. A greenhouse experiment was performed at KALRO/CYMMTRY Kibos station, Kisumu, Kenya during April to August, 2019. The experiment included local maize landrace known as "Rachar", *Striga hermonthica* seeds Kibos ecotype and four strains of *Alternaria brassicicola*. The treatments were; Soils (Treated) + two maize seed + *S. hermonthica* seeds + each isolate (LM017, LM013, LM019a and NY021) independently and soils (Treated) + three maize seeds + *S. hermonthica* seeds only (Control). *A. brassicicola* isolates LM019a, LM013 and LM017 inhibited *Striga* seeds emergence by 78.9%, 57.3% and 29.1% respectively. However, isolate NY021 enhanced *Striga* seeds emergence by 30.4% over the control. This study, established that some strains of *A. brassicicola* can inhibit the emergence of *Striga* weed. Therefore, the fungus could be used as a possible bio-agent in the production of mycoherbicide against *Striga* weed.

Introduction

Striga is a holoparasitic root weed that constrains cereals production worldwide particularly maize (*Zea mays* L.). The weed belongs to the Orobanchaceae family consisting of 30 species (Epee and Paul, 2017). Of all the species *Striga gesnerioides* (Willd.) Vatke, *Striga hermonthica* (Del.) Benth and *Striga asiatica* (L.) Kuntze is the most economically devastating weeds worldwide (Mbogo et al. 2016). *Striga* weed causes global yield losses translating to more than 40 billion US$ (Jamil et al. 2012), while in Sub-Saharan Africa, it results to yield losses of up to 100%, translating to approximately 7 billion US$ annually. In Western Kenya, *Striga* is the greatest bothersome weed, it distresses cereal such as millet, maize, sugarcane, rice, and sorghum. It results to cereal yield loss of up to 81 percent in Western Kenya (Mbogo et al. 2016). An assessment of 85 maize farms in Siaya County, shown that 73% of the field are infected by the weed, causing maize yield loss of 1.15 tons per hectare (MacOpiyo et al. 2010). However, in East Gem, Siaya County the damage is up to 2.8 tons per hectare of maize farm (Avedi et al. 2014).

Striga hermonthica weed has a highly synchronized lifecycle, it grows above and below the soil. In the existence of little phosphorous and nitrogen. The host plant exudes strigolactone, a plant hormone that stimulates germination. Its seeds germinate after 1-5 weeks and instantly develop haustorium in response to germination stimulants from the host plant. The haustorium penetrates the cortex, endodermis, phloem and xylem, consequently it attaches the host roots and siphons nutrients and water, leading to chlorosis and growth retardation of the host crop (Gacheru et al. 2002).

Despite the divesting challenges caused by the weed a wider range of control strategies has been exploited in the affected areas which include: manual weeding, crop rotation, intercropping maize with *Desmodium uncinatum jacq*, herbicides such as Pyrithiobac and Imazapyr, *Striga* resistant varieties of maize (Ejeta, 2007, Atera et al. 2013, Teka, 2014), use of *Smicronyx* species of insect (Vanlauwe, 2008). These methods are very expensive to small-scale farmers (Epee and Paul, 2017) and are ineffective, since the weed continues to cause more crop losses and expands its cover area. In Kenya soil, *Fusarium oxysporum f.* sp. *Strigae* (Foxy -2) strain as suggested by Avedi et al. (2014) was ineffective, need special storage facilities, their perspective has not been entirely utilized (Elzein et al. 2008). The strains also release Zearalenone, Trichothecenes and Fumonisin mycotoxins which are believed to be hazardous to animal and human (DMello et al. 1999).

The study responded to the need for bio-prospect of other fungi that are well adapted to different soil edaphic factors. Thus, calls for biological management of the weed using a cosmopolitan *Alternaria brassicicola* as a potential bio-agent against *Striga* weed. The fungus is a very aggressive necrotrophic plant pathogen that produces numerous toxic secondary metabolites and proteins (Kucharek, 2000). The fungus causes dark leaf disease on cabbage (Kirk et al. 2008), leading to defoliation of severely infected plants' leaves, decay, and death (Dillard and Joi, 2011). However, it is not pathogenic to maize since the defensive mechanism in maize inhibits it from colonization and attack.

The fungus has been used in many studies that involve *Arabidopsis thaliana* (Puncelli and Faccini, 2005) control. This study was appropriate since *A. brassicicola* is a host non-specific plant pathogen that produces many non-host phytotoxins (Lou et al. 2013). The toxins are not limited, but includes, tentoxins, tenuazonic acid and AB toxins (Ayongwa, 2011). Tentoxin produced interferes with chlorophyll formation through over energization of thylakoids, thus inhibits chloroplast development (Avni et al. 1992), while tenuazonic acid was found to inhibit photophosphorylation (Liu et al. 2007).

Previous studies, revealed that some species of *Alternaria* have the ability to control several weeds that attack crops, for instance, *A. cassia* accessible as CASST mycoherbicide was used to control sickle weed, *A. macrospora* was also used to controls *Anoda cristate* weed in Soybeans (Lawrence et al. 2008). In USA, *A. macrospora* inoculum was used to control jimsonweed (Walker, 2010, Elzein et al. 2008). *Alternaria* sp. controlled *Orobanche crenata* and *Orobanche minor* in Morocco, Syria and France as reported by Linke et al. (1992). *Alternaria altanata* controlled *Chenopodium album*, a noxious weed of wheat (Iffat et al. 2010). *Alternaria brassicicola* was previously used as a model to control *Arabidopsis thaliana* in numerous experiments (Puncelli and Faccini. 2005). Therefore, the fungus *Alternaria brassicicola* can inhibit *Striga hermonthica* weed emergence in maize.

Materials and Methods

Isolation, purification and identification of *Alternaria brassicicola*.

The fungus was isolated from cabbage leaves in Limuru and Nyeri farms displaying characteristic symptoms of dark leaf spot diseases caused by *A. brassicicola*. The diseased parts of the leaves were cut into smaller portions together with healthy parts that encircled the spot and surface sterilized in 2% (v/v) Sodium hypochlorite solution for 3 minutes, washed five times with sterile distilled water, then placed on sterilized petri dishes which contains PDA under aseptic conditions and sealed with parafilm. The PDA plates were kept in the dark at room

temperature for 12 days with periodical observation for the fungal growth. The periphery of actively growing colony of the cultures were cut under aseptic conditions using 2 mm cork, sub-cultured on new PDA plates and stored at room temperature in the darkness for another 12 days. The isolates LM017, LMO13, LM019a and NY021 was morphological and molecularly identified as different strains of *Alternaria brassicicola*, their pol sequences were deposited at the Genbank under the following accession numbers: MN636299, MN636298, MN636303 and MN636302 respectively.

Preparation of Alternaria brassicicola spore's inoculum

The 12 days old conidia and mycelial in the sub-cultured PDA plate, five milliliters of sterilized distilled water were added to 12 days old culture, then agitated gently by shaking. The spores were then dislodged slowly using a sterilized bend glass rod. The content was sieved into a falcon tube through a Cheesecloth of three layers and centrifuged at 3600 rpm for 10 minutes. The spore's suspension obtained were splashed three times with sterilized water and stored at 20ºC.

Pot experiment protocol

One pot trial was undertaken during April to August 2019 at KALRO/CIMMYT Greenhouse in Kibos, Kisumu County (0º 02'S–34º 48'E), Kenya. The experiment consisted of one local white maize landrace known as 'Rachar', *Striga hermonthica* seeds of Kibos ecotype and 5 ml spore's suspension of *Alternaria brassicicola* isolates (LM017, LMO13, LM019a and NY021) inoculated separately in pots. Transparent five liters of pots containing sterilized soils from three different areas, in each block 12 pots were used. To 5 liters pots, a teaspoon of a mixture of sand and 0.25 mg of *Striga hermonthica* seeds (approximately 500-600 seeds) was thoroughly mixed with soil (heated at 100ºC for 30 minutes) at a depth of 5-10 cm and watered every day for pre-conditioning. Seven days later two maize seeds were sown at the same depth and thinned to a single seedling three weeks after emergence. The exact amount of viable and sterile *S. hermonthica* seeds were inoculated with 5ml of disinfected water for the control trial. The treatment was replicated three times in respective experimental block. *S. hermonthica* seeds emergences on 8^{th}, $10^{th,}$ 12^{th} and 14^{th} weeks after sowing and mean the number of *Striga* emergence was determined.

Data collection and analysis

Data were tested for homogeneity of variance using Bartlett test. The number and mean *S. hermonthica* seeds emergence were analyzed using one-way ANOVA at 0.5% confidence, while Tukey's Honest Significant Differences (HSD) test was used for post-hoc analysis at 0.95 confidence intervals to separate the feasible means in R software (Version 3.5.0).

Results and Discussion

Effect of A. brassicicola spore's suspension on S. hermonthica incidence on soil

Maize emerged one week after planting (WAP) and by the 14^{th} WAP, 98% of the maize had germinated, eight weeks later *S. hermonthica* weed started to emerge. *A. brassicicola* isolates were found to inhibit *S. hermonthica* incidence in the greenhouse as compared to the control experiment (Fig. 2). Fungi are known to biodegrade *S. hermonthica* seeds in the soil, this might be due to their ability to produce phytotoxins and enzymes as reported by Meena et al. (2017). The compounds are able to kills plant cells and tissues (Ayongwa, 2011).

According to the results, there was significant ($p = 0.029$) variation in the number of *S. hermonthica* seeds emergence in relation to weeks after planting maize seeds (WAP). The weed emergence increased progressively with time then fall drastically (Fig. 1a). The number of weed emergence was higher on 12^{th} week (1.53±1.59) and 10^{th} week (1.0±1.25) and lowered on the 8^{th} week (0.33±0.72) and 0.53±0.64 on 14^{th} week (Fig. 1b). This could either be due to exudation of more strigolactone by the maize roots, which declines as the plant grows to maturity or due to exhaustion of resources such as organic carbon which acted as the source of energy for the fungi. However, when the seeds were inoculated with spores of isolate LM017. *Striga* seeds emergence were reduced on the 8^{th} and 10^{th} week and increased on 12^{th} week by isolate LM017 spores (Fig. 1a).

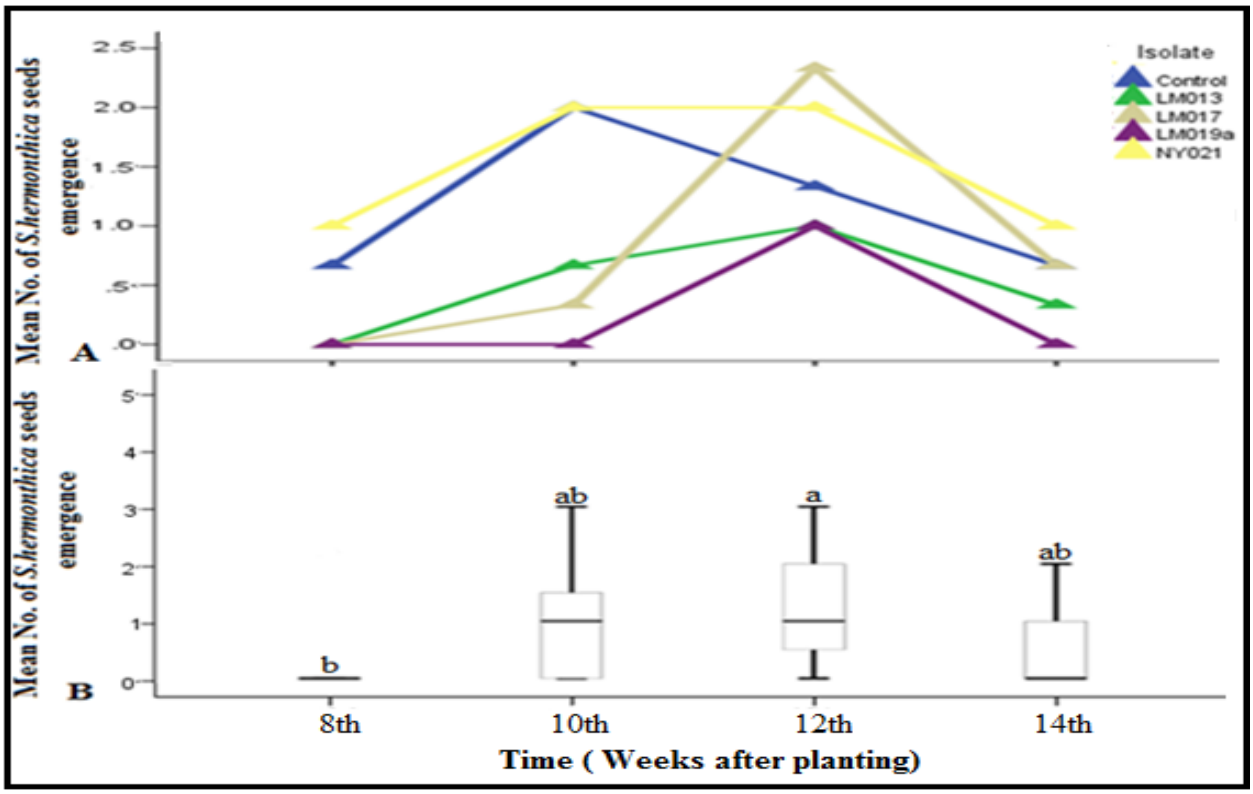

Figure 1. Inoculated *S. hermonthica* weed emergence on 8th, 10th, 12th and 14th WAP (Weeks after planting). (A) The line plot showing the trend of mean number of *S. hermonthica* emergence when its seeds were inoculated with different *A. brassicicola* strains. (B) The boxplot of the mean number of *S. hermonthica* emergence in each time. The data are presented as the mean of three replicates. The vertical bars represent the mean ± SE and the letters above each bar indicate significant difference according to Tukey's HSD at level 0.05. Time with the same letters are not significantly different. LM; Limuru isolates, NY; Nyeri isolates.

There was significant (p = 0.0285) variation in the number of *Striga* emergence, when the seeds were inoculated and when not. The number of inoculated *Striga* seeds emergence varied from 0.25±0.45 (LM019a) to 1.50±1.83 (NY021) as in figure 3. Though, fewer *Striga* seeds emerged in the control experiment contrary to our anticipation. This could be attributed to the fact that; the heated soil might have changed soil abiotic and biotic factors contributing to *Striga* emergence. *Striga* seeds viability was also not determined prior to their use. *Alternaria brassicicola* strains inhibited *S. hermonthica* emergence by between 29.1% (LM017) to 78.9% (LM019a) over the control (Fig. 4). This could be attributed to their immense production of various degrading enzymes, acids and phytotoxins which are bioactive (Lou et al. 2013) and possible because, *A. brassicicola* spores inoculated on the *Striga* seeds germinated and grows upon acquiring energy from organic carbon available in soil. The symbiosis interaction between the maize and strains of the fungus might have significantly reduced strigolactone production by the maize plant (Lopez-RÃ¡ez et al. 2011). However, when the seeds were inoculated with spore's suspension of isolate NY021, *Striga* emergence was enhanced by 30.4% over the control (Fig. 4). This is in agreement with El-Kassas et al. (2005), that some strains of fungi when applied on soil are able to produce growth hormones such as ethylene which stimulate the germination of *S. hermonthica* weed.

Figure 2. Selected pots showing *S. hermonthica* emergence on 14th WAP maize seeds. (A) Pots with soils sampled from Bondo and *Striga* seeds inoculated with different strains of *A. brassicicola* isolates. (B) Pots with soils sampled from Ugenya and *Striga* seeds inoculated with different strains of *A. brassicicola* isolates. (C) Pots with soils from Gem and *Striga* seeds inoculated with isolate LM017. (D) Pots of Gem soils inoculated with sterilized distilled water (control). The red arrows indicate some *S. hermonthica* seedlings.

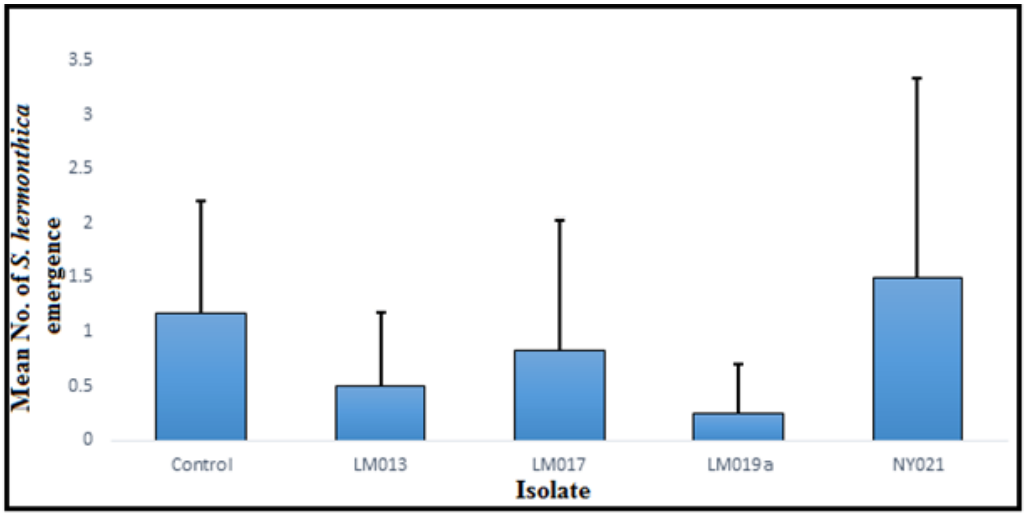

Figure 3. *Striga* emergence on 14th week after planting. The vertical bars represent the mean ± SE number of *Striga* emergence, according to Tukey's HSD at ($P \leq 0.05$). LM; Limuru isolates, NY; Nyeri isolates.

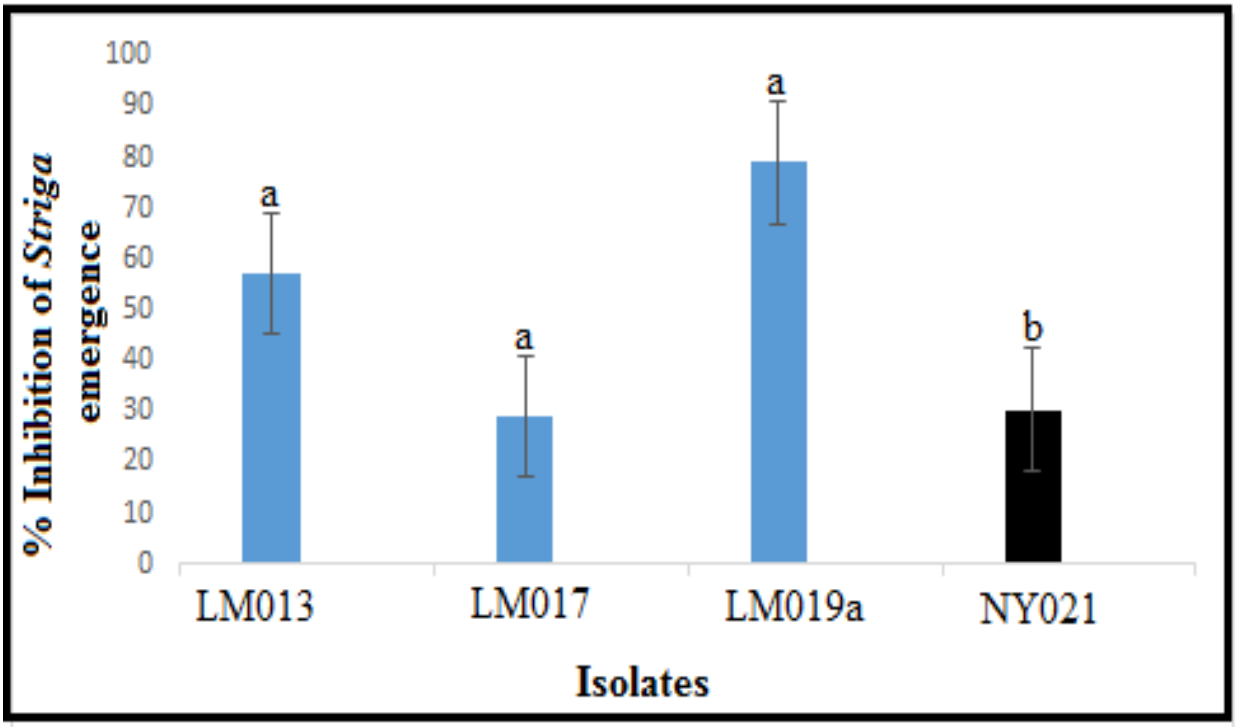

Figure 4. Percentage inhibition of inoculated *S. hermonthica* seeds emergence on 14th WAP (Weeks after planting). The data are presented as the mean of three replicates. The vertical bars represent the mean ± SE and the letters above each bar indicates a significant difference according to Tukey's HSD at ($P \leq 0.05$). Isolates with the same letters are not significantly different.

Conclusion

The number of *S. hermonthica* emergence was higher on 10th and 12th week and lowered on the 8th and 14th week after planting maize. Strains of *A. brassicicola* inhibited *S. hermonthica* by between 29.1% to 78.9% over the control. Therefore, we recommend further extraction, identification, analysis of various bioactive metabolites and enzymes from isolate LM013 as a potential bioagent against *Striga* weeds.

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding this paper.

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