



BIOLOGICAL CONTROL OF *STRIGA* USING *FUSARIUM OXYSPORUM*

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ABSTRACT

The study focuses on a low-cost biological control technology using *Fusarium oxysporum* to enhance smallholder farmers' livelihoods and explores developing fungicide-resistant variants of *Fusarium oxysporum* (FOXY 6A) for seed treatment, addressing the challenge of applying biological agents on fungicide-coated seeds. *Fusarium* strains were isolated from infected *Striga* plants, with three promising isolates identified through PCR. The experiments were conducted at IRAD Maroua, Cameroon. The pot experiment used a controlled study with a factorial design. It tested the effect of four *Fusarium oxysporum* strains on *Striga* control with two host (maize and sorghum). For each host, pots had a combination of *Fusarium* strain and conidial density, plus negative controls without *Fusarium*. Replication was done by using multiple pots per treatment group. For the fungicide resistant mutant tests, we had three treatments per fungicide. Pot trials demonstrated that applying four fungal strains at a concentration of 3×10^4 spores/mL significantly reduced *Striga* emergence in maize and sorghum compared to untreated controls. This reduction is attributed to toxins produced by *Fusarium oxysporum*, which inhibit parasite seed germination. The study found a positive correlation between *Striga* emergence and crop yield, indicating that higher *Striga* populations correspond to lower yields. Fungicide resistance tests on FOXY 6A indicated wider inhibition zones for Thiram and smaller zones for Captan, suggesting potential for developing fungicide-resistant biocontrol strains, although further validation is needed.

Keywords: *Fusarium*, *Striga*, Maize, Sorghum

INTRODUCTION

In Cameroon, farming is the backbone of many communities, with staple grains like maize, sorghum, rice, and millet feeding the population. But these key crops face a serious challenge from parasitic weeds, especially several *Striga* species like *Striga hermonthica*, *Striga asiatica*, and *Striga gesnerioides*. These three are the worst offenders economically among the 42 known *Striga* species (Tank *et al.*, 2006; Parker, 2012), each targeting different hosts. *Striga hermonthica* and *S. asiatica* mainly attack cereals (Parker, 2009; Pennisi, 2010), while *S. gesnerioides* goes after cowpea (*Vigna unguiculata*) (Ohlson and Timko, 2020). Among them, *Striga hermonthica* stands out as a huge threat to food security across Africa, driving hunger and poverty in many places (Pennisi, 2010; Khan *et al.*, 2014).

Though it's tricky to measure exactly, estimates suggest *Striga* impacts over 300 million Africans, causing crop losses worth billions every year of between 7 and 10 billion US dollars (Gressel *et al.*, 2004; Ejeta, 2007; Rodenburg *et al.*, 2010; Yang *et al.*, 2021; Badu-Apraku and Akinwale, 2015). Maize and sorghum suffer the most from these infestations (Ater a *et al.*, 2013). To keep *Striga* in check and improve soil health, farmers often rotate cereals with cotton or pearl millet. But high fertilizer prices mean many still don't use enough, which leaves soils weaker and crops more vulnerable to *Striga* attacks (Ejeta and Gressel, 2007). While cereals bear the brunt, *S. gesnerioides* specifically targets legumes like cowpea (*Vigna unguiculata*). Pressure on land and shorter fallow periods have made things worse by encouraging continuous cereal planting, which lets *Striga* build up steadily (Abunyewa and Padi, 2005; Ejeta and Gressel *et al.* 2004). Northern Cameroon has seen

heavy losses in maize and sorghum yields because of this (Tchouamo and Nguimbou, 2014). Traditional ways to fight *Striga* include pulling the weeds by hand, spraying herbicides, adding nitrogen fertilizer, rotating crops with non-host plants, and growing *Striga*-resistant or tolerant varieties (Joel *et al.*, 2013; Menkir and Meseke, 2019). But many farmers lack the resources or know-how to put these methods into practice effectively. Even hoe-weeding can backfire by disturbing the soil and helping *Striga* take hold (Marley *et al.*, 2004).

Recent research points to improving soil fertility as a key part of beating *Striga* and boosting yields (Badu-Apraku and Akinwale, 2015). Biological control using *Fusarium oxysporum* has gotten attention because it can selectively suppress *Striga* without harming the crops (Elzein *et al.*, 2008; Ndambi *et al.*, 2011). Still, some *Fusarium* strains can harm plants like tomatoes and peppers, so farmers need to be careful when intercropping (Zarafi *et al.*, 2014). Another breakthrough comes from coating maize seeds that are resistant to imazapyr with imidazolinone herbicides. This treatment cuts down *Striga* emergence throughout the growing season and leads to much better yields (Menkir *et al.*, 2010; Habimana *et al.*, 2014; Makumbi *et al.*, 2015). Applying synthetic germination stimulants to fields also shows promise by forcing *Striga* seeds to germinate without a host, effectively shrinking the seed bank in the soil (Zwanenburg *et al.*, 2016; Kountche *et al.*, 2019). Combining host resistance with *Fusarium* treatment has proven effective in controlling *Striga* in maize (Mrema *et al.*, 2020; Shayanowako *et al.*, 2020). Similarly, seed coating with imazapyr cuts down infestation and boosts yields significantly (Kamara *et al.*, 2020). Using crop rotations that mix cereals with legumes along with synthetic stimulants helps reduce the *Striga* seed bank even more (Hailu *et al.*, 2018; Kountche *et al.*, 2019).

Despite these encouraging results in labs and trials, *Fusarium*-based biological control hasn't caught on widely yet. Problems like inconsistent performance, cost, and production hurdles hold farmers back. This study aims to push forward an affordable and effective biological control approach, including developing *Fusarium* strains resistant to fungicides so they can be safely used as seed treatments.

MATERIALS AND METHODS

Experimental Materials

Plant Material

Striga seeds were obtained from fields around Maroua. They were collected from mature *Striga* plants parasitizing maize plants in the IRAD research site. These seeds were stored dry in the dark in tightly closed bags under room temperature (25 °C).

These seeds were used for germination tests and for the growing in situ on the respective host plants for pathogenicity experiments. The crop plants used for the experiments were Sorghum and maize. Seeds of these plants were obtained from IRAD Maroua.

Fungal Strains

The *Fusarium oxysporum* strains used in the study were originally isolated from infected *Striga* plants collected from Maroua in 2021. These strains were coded based on the area of collection.



Figure 1. One week old *Fusarium oxysporum* grown on Potato Dextrose Agar (PDA) at 20 °C.

METHODS

Isolation of Fungal Pathogens from Infected *Striga* Plant Tissues

Sliced sections of the infected *Striga* plants were thoroughly rinsed in sodium hypochlorite solution and sterilized water before being placed on water agar medium containing 1% acetic acid in Petri dishes. After incubation for four to seven days at 20°C, the fungi grown out from the medium were isolated for further propagation.

Single *Fusarium* spores were isolated by standard dilution plate methods in which the germinating spore is removed from the plate under a stereoscope with a needle. Single spore from the *Fusarium* isolate was placed on Potato-Dextrose Agar medium in petri dishes and incubated at 20°C for 48 hours.

Table 1: Isolated *Fusarium* Strains

Name of strain	code	Remarks
Tchere field 2	Tch2f	Isolated from Tchere village
Mokio field 2	Mok2t	Isolated from Mokio village
Tchere field 3	Tch3t	Isolated from Tchere village
FOXY variant 6A	6A	Existing laboratory strain from Montana State University.

Identification of *Fusarium oxysporum* Strains

After collecting many strains of *Fusarium* from various villages, 3 promising isolates were identified using the PCR method with specific primers of *F. oxysporum*. The genomic DNA of each strain was extracted using cetyltrimethylammonium bromide (CTAB) and stored at 4 °C for PCR use. PCR reactions were performed using a PTC-200 DNA engine cycler (MJ Research, MA, USA) in 50 µL volumes containing 50 ng of fungal DNA, 0.2 µmol L⁻¹ of single primers, and 25 µL 2X Reaction Mix (Tiangen, Beijing, China). The PCR products were separated using electrophoresis on 1.0% agarose gels in 0.5× Tris-borate-EDTA (TBE) buffer and photographed using a gel image system after staining with ethidium bromide (EB).

Preparation of Fungal Suspensions for Pathogenicity TestFor the preparation of *Fusarium* aqueous cell suspension, mycelia from 3 weeks old *Fusarium* cultures were scraped off from 10 Petri dishes and thoroughly smashed using a mortar. The smashed extract was filtered using a cheese cloth and the filtrate was made up to 35 mL using sterilized deionized water. The filtrate was made up to 50ml After shaking vigorously with an electric shaker; spore count was measured by haemocytometer. Concentrations of 3x10⁴ spores/mL were made for future pathogenicity tests. Preparation of Growth Media*Fusarium oxysporum* was cultured on potato dextrose agar medium or rice growth medium.

a. Potatoes Dextrose Agar (PDA) medium.

This was prepared by measuring 30g of PDA (Sigma Aldrich) and dissolving in 1litre of deionized water. The pH was adjusted to 5.8 before sterilizing. After sterilizing at 121 °C for 20 minutes, it was then poured into plastic petri dishes of diameter 9cm or test tubes and allowed to solidify for further use in inoculating the *Fusarium* strains.

b. Rice growth medium.

This was prepared by measuring 100g of commercial rice and soaking it in 30mL of deionized water in 500ml bottles. This was allowed to soak for 24 hours under room temperature before boiling for 15minutes. After boiling for 15 minutes, the content was then sterilized for 20 minutes at 121 °C. After sterilization, it was then allowed to cool for inoculation to be effected from *Fusarium oxysporum* earlier cultured on PDA.

Pot Experiment

Pot experiments were carried out to test the pathogenicity effect of the four strains of *Fusarium oxysporum* on *Striga* and to clarify two aspects of these fungi.

a) Which strain controls better the *Striga*.

b) which host plant is more tolerant to the *Striga/Fusarium*.



The experiments were carried out in the screen house with daytime temperature maintained at 25°C, whereas night temperature was kept at about 15°C.

The efficiency of various conidial density of *Fusarium oxysporum* to control *Striga hermonthica* after soil incorporation was evaluated in pot experiments. Soil was mixed with peat. The soil was then autoclaved for one hour at 121°C to kill pathogens. This was repeated after 24 hours to ensure complete disinfestation of the soil.

Two host plants were used: maize and Sorghum.

For each host plant species, 12 pots were used viz: 3 for the negative control (*Striga* spp. without *Fusarium* strains- 0t), 3 with *Fusarium* at a conidial density of 3×10^4 spores for each of the *Fusarium* strains (Tch2f, Mok2t, Tch3t and 6A). The pots measuring 12 x 12 x 18cm were filled with approximately 0.8kg soil/pot. Approximately 100 seeds of *Striga* sp. were sprinkled onto the surface in each pot. The seeds had been collected in Maroua in 2020 and showed a viability of approximately 80%. 5mL of each *Fusarium* aqueous cell suspension was sprayed on the soil surface of each pot. The pots were later filled with soil to the top. Soil irrigation was carried out immediately after inoculation and complete drying was avoided for the first 3 weeks in order to precondition the seeds. Subsequently, the soil was allowed to dry to a certain degree (i.e. irrigation was carried out every three days) not very harmful to the plants before irrigation. Host plants were sown alongside *Striga* species and later thinned to one plant per pot.

The experiment was evaluated when the host plants of the negative control as well as treatments with low inoculum doses of the fungus had stopped development due to *Striga* infestation. At this time, the number of emerged shoots was recorded. Shoots were considered emerged as from 0.5 cm above soil level. The shoots were also examined for symptoms of *Fusarium* infection.

Incubation and Growth of *Fusarium* Strains

Single *Fusarium* spores were isolated and placed on Potato-Dextrose Agar (PDA) medium in petri dishes and incubated at 23 °C for 48 hours. For multiplication of biomass and production of enough metabolites, several Petri dishes of PDA (for screen house experiments) or boiled and sterilized rice (for laboratory bioassay) were later infected with plugs of PDA containing *Fusarium*. These cultures were incubated at 23 °C for one month before extraction.

Creating Fungicide Resistant Mutants

In creating fungicide resistant mutants, the following protocol was used: PDA and C-dox media were prepared and poured into 9 cm sterile plastic petri dishes. 0.5 ml of homogenized FOXY 6A was then added on the media and evenly spread with a sterilized glass spreader. A hole was made in the middle of the media using cork borer. Different concentrations of fungicide were prepared (1xT, 1.5xT, 2xT (T for thiram), 1xC, 1.5xC, 2xC (C for captan), 1xCT, 1.5xCT, 2xCT (thiram and captan combination) (Note 1x is the recommended rate of the fungicide). The prepared Fungicides were filled up in the holes of the culture media using a pipette, and the cultures were then incubated at room temperature. The culture media were observed after 48 and 72 hours. These mutants, which are resistance for the corresponding fungicides were identified within the zones of inhibition.

Data Analysis

Data were subjected to analysis of variance (ANOVA) using JMP software (version 4.0.2; SAS Institute Inc., USA) and Duncan's Multiple Range Test was adopted in separating means.

RESULTS

Identification of *Fusarium oxysporum*

Presence of *F. oxysporum* strains was confirmed by identification through PCR amplification using the specific primers of *F. oxysporum*. Foxy variant 6A was used as a positive control strain during PCR identification. The results confirmed that 3 strains belonged to *F. oxysporum*. These strains were then coded as Tch2f, Tch3t and Mok2t representing the three villages/fields where they were collected.

Pot Experiment

Fusarium cell suspensions from four strains: Tch2f (Tchere field 2); Mok2t (mokio field 2); Tch3t (Tchere field 3) and 6A (FOXY 6A variant) with inoculum density of 3×10^4 spores was used

based on a previous work by Paternotte 1987 whereby inoculum densities of 10^2 - 10^6 were used. The test conducted was to evaluate the effect of *Fusarium* on the germination and growth of *Striga* as expressed in the number of emerged *Striga* plants per pot.

Results obtained showed a significant difference between the treatments of application on the emergence of *Striga* in the two host plants i.e., maize (CMS 9015) and Sorghum (zoaye).

With regards to the effects of the various strains on the influence of the emergence of the parasite, there was significant difference (fig 2 and 3). From maize trial, the number of emerged *Striga* plants ranged from 1-2 for the different *Fusarium* treatments compared to 5 for the control where no *Fusarium* was applied (Table2). Similarly, for the *Sorghum* trial, treatments with 6A and Mok2t had a significantly lower number of emerged *Striga* plants compared to the control that had approximately 5 emerged *Striga* plants per crop plant (Table 2).

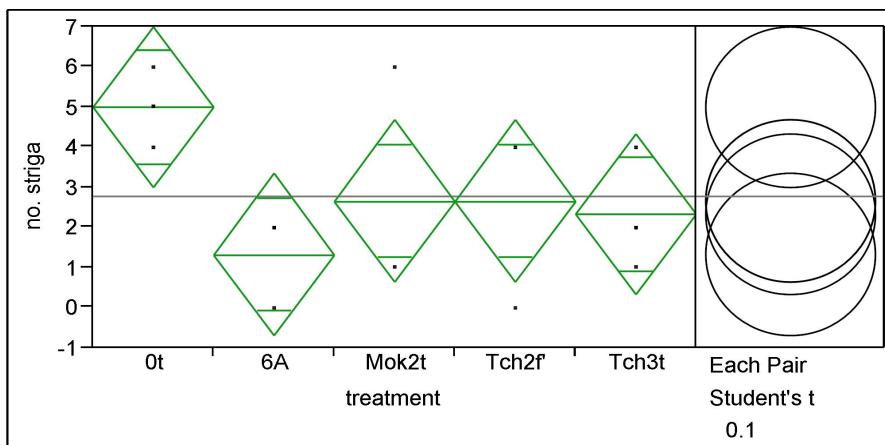


Figure 2: Effects of the Treatments on Number of Striga on Maize

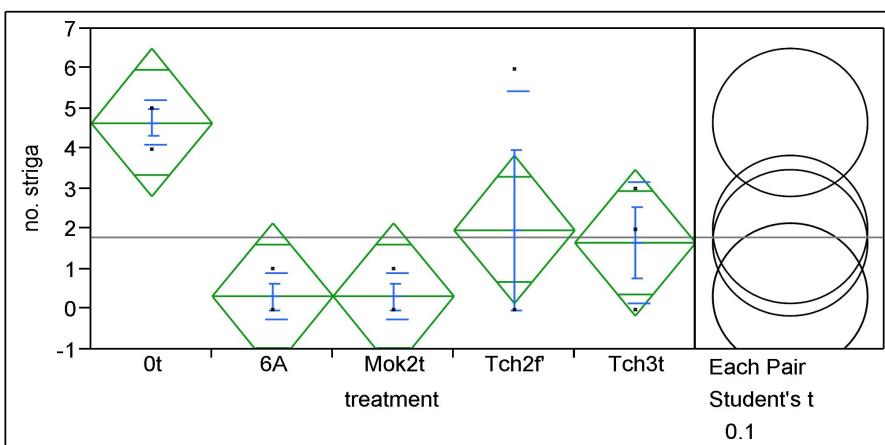


Figure 3: Effects of the Treatments on Number of Striga on Sorghum

Table 2: Emergence of *Striga* Plants in Relation to *Fusarium* Strains

Inoculum Strain	No. of <i>Striga</i> shoots/pot when inoculum was applied pre-emergence	
	Maize	Sorghum (zoaye)
0t	5.0000000 ^a	4.6666667 ^a
Tch2f	2.6666667 ^{ab}	2.0000000 ^{ab}
Mok2t	2.6666667 ^{ab}	0.3333333 ^b
Tch3t	2.3333333 ^{ab}	1.6666667 ^{ab}
6A	1.3333333 ^b	0.3333333 ^b

Means on same column not connected by same letter are significantly different.

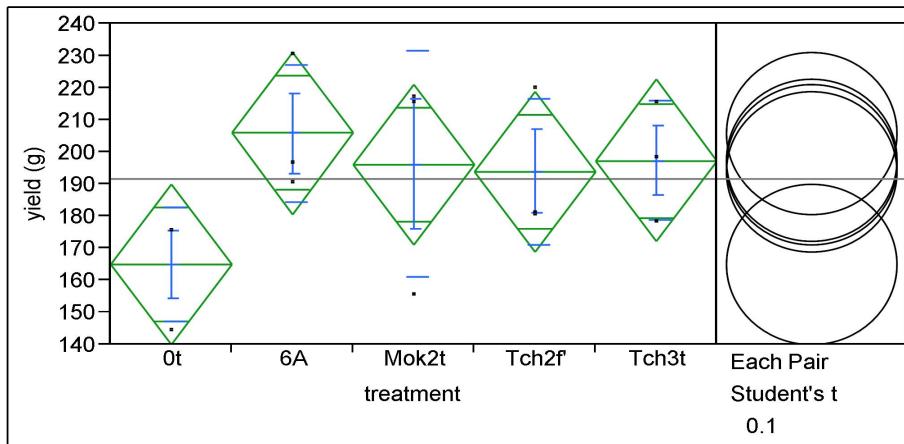


Figure 4: Effects of the Treatments on Yield of Maize

Results from maize yields (fig 4) obtained showed treatments with 6A strains gave higher significant yields compared to the control. While 6A gave yields of 205.9 g, the control gave a yield of 164.93g per plant. However, there was no significant difference between the different *Fusarium* strains.

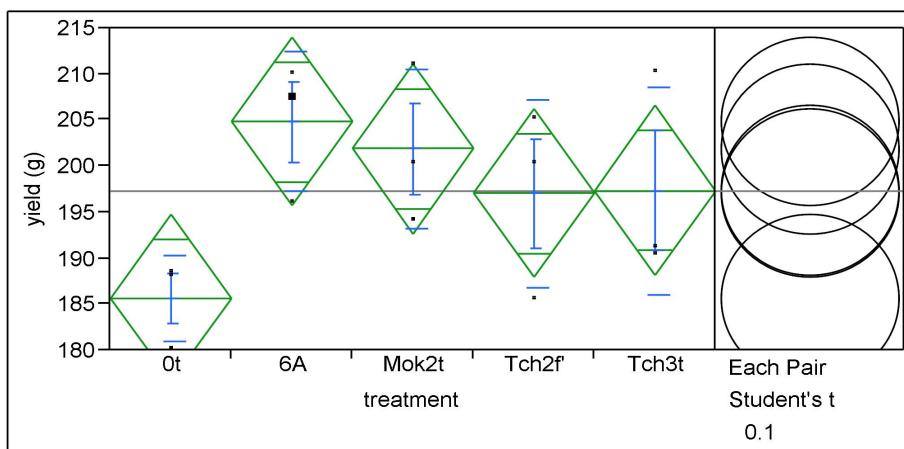


Figure 5: Effects of the Treatments on Yield of Sorghum (zoaye)

Results from *Sorghum* yields (fig 5) obtained showed treatments with 6A and Mok2t strains gave higher significant yields compared to the control. While 6A and Mok2t gave yields of 204.86g and 201.9 g respectively compared to the control of 185.63g per plant. However, there was no significant difference between the different *Fusarium* strains.

It was also noticed that there is a positive correlation between the emergence of *Striga* and the yield obtained. Where the number of emerged *Striga* is high, the yield consequently is lower. In a similar observation, where we had higher yields from treatments with *Fusarium*, the number of emerged *Striga* was significantly lower compared to the control where no *Fusarium* was applied.

Creating Fungicide Resistant Mutants

Results from Fungicide (Thiram and Captan) resistance FOXY 6A variant shows a wider inhibition zone in the treatment of Thiram (T) and a wider inhibition zone was observed at increased concentration of T (2xT). The inhibition zone created by Captan (C) was smaller and uniform at different concentrations (Fig 6). In each inhibition zone some growth of FOXY strain was observed. The Foxy strains growing within the zone of inhibition are resistant to the respective fungicide concentrations and their resistance needs to be confirmed after more trials. However, these results give an indication of the resistances FOXY to fungicides.



Figure 6: Culture of FOXY 6A with treatment of common fungicides (Captan (C) and Thiram (T)), notice the inhibition zone (clear zone) created by fungicide treatment.

DISCUSSIONS

The study focuses on *Fusarium oxysporum* as a source of effective agrochemicals for controlling parasitic weeds, specifically *Striga* species. Pathogenicity tests demonstrated that *Fusarium* strains 6A, Mok2t, Tch2f, and Tch3t can infect *Striga* at pre-emergence stages, causing necrosis, chlorosis, and inhibiting seed germination—effects consistent with toxin production. Factors influencing *Fusarium* pathogenicity include temperature (Lee and Kim 2020), pH, and nutrition (Lopez *et al* 2023), which align with the observed pathogenic effects on *Striga*.

Extracts from 3-4 week-old *Fusarium oxysporum* cultures caused mortality in germinating seeds of *Striga crenata*. Application of inoculum pre-emergence significantly reduced the number of emerged *Striga* plants compared to controls (Table 2). These results support earlier findings by Muller-Stover (2001), who reported a 78-89% reduction in broomrape emergence following pre-emergence *Fusarium oxysporum* application at 0.5 g/kg soil. Similarly, Saremi and Okhovvat (2008) observed a decrease in broomrape species in field conditions with pre-emergence *Fusarium* mycelia treatment.

No adverse effects on host plants were recorded in this or related studies (Abouzeid and Tarabily, 2010), suggesting host specificity and safety. The reduced number of parasite shoots in pre-emergence treatments likely results from the combined attack by *Fusarium oxysporum* on seeds, germ tubes, and tubercles in the soil.

The mechanisms behind *Striga* suppression may involve toxin production that inhibits parasitic seed germination or the induction of suicidal germination through ethylene production by certain fungal strains (El-Kassas *et al.*, 2005). Furthermore, Lakshmipriya (2019) proposed that fungal toxins act as germination inhibitors by reducing protein synthesis via peptidyltransferase inhibition.



Application of spore suspensions from the four *Fusarium oxysporum* strains at a density of 3 $\times 10^4$ spores/ml consistently reduced *Striga* emergence (Table 2).

Field deployment of actively growing *Fusarium oxysporum* strains, particularly those selected for enhanced excretion of amino acids inhibitory to *Striga hermonthica*, has demonstrated significant yield improvements for smallholder farmers under real production conditions. This promising approach justifies further molecular studies to clarify the dynamics of amino acid excretion in situ and its precise impact on *Striga* development. Although the complexity and cost of such studies may delay wider adoption, our work confirms the effectiveness of Fusarium as a biocontrol agent, even as the detailed mechanisms remain to be fully elucidated.

Despite the difficulty of controlling parasitic weeds without harming host crops and the prolific seed production and long soil viability of *Striga* seeds, the Fusarium on-farm inoculum method presents an ideal, sustainable strategy for *Striga* biocontrol (Adhikary *et al.*, 2024) because this biological approach offers a viable alternative. Overcoming these obstacles is crucial not only for weed suppression but also for improving crop yields, which depend on seed quality, soil fertility, and water availability.

Beyond direct economic benefits, successful *Striga* biocontrol can empower rural communities by promoting women's empowerment, improving nutrition, and supporting education. Effective management reduces crop failure risk and encourages adoption of better agronomic practices, thereby contributing to sustainable food security for smallholder farmers.

Moreover, this biocontrol method aligns well with agroecological farming systems. Unlike approaches that amplify plant hormones or phytotoxins, selecting Fusarium strains for enhanced amino acid excretion is considered safer. Amino acid overproduction is common in industrial contexts for animal feed and human nutrition, making this method less risky than selecting toxin-producing strains. With many plants sensitive to amino acids and the prevalence of host-specific pathogens, this strategy could extend to controlling other weeds.

From the experiments conducted, no adverse effect of the inoculums was recorded on the host plants confirming the work of Lee and Kim (2020), that *Fusarium* exclusively attacks *Striga*. Mantzoukas *et al.* 2022 reported that conidial dose and time variation as the main factors in pathogenesis of *Fusarium*. Similarly, Saremi and Okhovvat (2008) reported that pre-emergence application of *Fusarium* mycelia caused broomrape (*O. ramosa* and *O. aegyptiaca*) reduction in the field.

However, it is likely that the combined attack on seeds, germ tubes and tubercles in soil by *Fusarium oxysporum* was responsible for the reduced number of parasite shoots in pot experiments of the current study as well as those conducted by the above-mentioned researchers.

In this study, it is assumed that the reduction of emerged *Striga* when *Fusarium oxysporum* strains were applied in the soil at time of planting is because they produced some toxins that inhibited the germination of the parasitic seeds. Other potentially effective method of controlling parasitic weeds such as *Striga* is to stimulate germination of their seeds in the absence of the host crop. When the germinated seedling fails to find the roots of a susceptible host, they die and thereby reducing the quantity of the parasite in the seed bank. An alternative method has been reported by other researchers such as El-Kassas *et al.*, 2005 that some strains of fungi when applied to the soil produce ethylene which stimulate the germination of the parasite. The fungi are said to produce some toxins that act as germination inhibitor by reducing protein synthesis through the inhibition of peptidyltransferase.

In summary, agriculture grapples with resistant pathogens and pests, alongside environmental and health issues linked to chemical pesticide use (Waller *et al.*, 2005; Ashrafi *et al.*, 2007). Natural products from fungi like Fusarium offer promising bioactive alternatives for pest management (Newman *et al.*, 2003; Demain *et al.*, 2005). Our pathogenicity tests and field trials confirm *Fusarium* strains' capacity to control parasitic weeds such as *Striga* and broomrape, through toxin-mediated seed germination inhibition and ethylene-induced suicidal germination (Muller-Stover, 2001; El-Kassas *et al.*, 2005; Lakshmipriya, 2019).

Deploying *Fusarium oxysporum* strains selected for enhanced amino acid excretion can improve yields for smallholder farmers, reduce reliance on chemical herbicides, and support



sustainable food security. While challenges in consistent field efficacy and technology adoption remain, this biological control strategy offers a practical, environmentally friendly alternative in integrated weed management programs (Ejeta and Gressel *et al.*, 2004).

CONCLUSION AND RECOMMENDATION

Natural products have long been crucial in drug discovery and agricultural development, with plants and fungi standing out as major contributors. *Fusarium oxysporum*, in particular, is known to produce a variety of metabolites with phytotoxic effects, some of which target specific plant species. This study focused on *Fusarium oxysporum* isolated from infected *Striga* plants in Cameroon, demonstrating its selective phytotoxicity against *Striga* spp. This is the first report of such selective activity in this region.

Field application of *F. oxysporum* strains, naturally pathogenic to *Striga hermonthica* and selected for their ability to excrete certain amino acids, showed significant potential to boost maize yields for smallholder farmers in Cameroon. Compared to untreated controls, Fusarium treatments led to notable yield improvements. The technology is cost-effective and simple to produce, making it accessible for small-scale farmers. By helping control *Striga* infestations and stabilize crop yields, this biocontrol method could empower farmers to invest in high-quality seeds and fertilizers without fearing losses caused by the parasite.

Using endemic *Fusarium* strains adapted to local environments also lowers the risk of side effects, provided toxicogenic strains are avoided. The approach enhances strain virulence through targeted mutations affecting amino acid biosynthesis, relying on conventional selection rather than genetic engineering. This makes it a sustainable, low-input technology suitable for integration into pest management systems. It promotes reduced reliance on herbicides and toxins, aligns with environmental safety goals, and supports the preservation and optimization of crop germplasm diversity.

Overall, this study confirms that *Fusarium oxysporum* isolates from *Striga* in Cameroon offer a promising, environmentally friendly biocontrol strategy. The development of fungicide-resistant *Fusarium* strains compatible with seed treatments further facilitates adoption by smallholder farmers. This approach provides a sustainable, affordable option for managing *Striga* in the Sudano-Sahelian zone and similar agroecosystems, improving yields and supporting farmer livelihoods while reducing chemical pesticide use.

ACKNOWLEDGEMENT

We heartily acknowledge the following institutions and persons for their support towards this work:

1. International center for genetic engineering and biotechnology (ICGEB) for financial support through the ICGEB research grants.
2. Institute of Agricultural research for Development (IRAD), Cameroon for providing research laboratories and space.
3. Kenya Agricultural livestock research organization (KALRO), Katumani for providing research laboratory and space.
4. Prof Davids Sands for providing FOXY 6A variant
5. Dr Peter Lueth for technical assistance through the toothpick company
6. Sands Claire for technical assistance through the toothpick company

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