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A microbial bioherbicide for *Striga hermonthica* control: production, development, and effectiveness of a seed coating agent

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Abstract

Witchweed (*Striga hermonthica*), also called striga, is a parasitic weed that causes high yield losses in maize on more than 200,000 ha in Kenya alone. A new commercial, biological herbicide developed in Kenya is able to control striga effectively. The product was approved for use by the Pest Control Products Board in Kenya in September, 2021. It is self-produced in villages using a secondary inoculum provided by a commercial company. The formulated product has some disadvantages, which are a complicated production process, a very short shelf life and high application rate. Additionally, the product has to be applied manually and therefore can only be used in manual production, leaving out the opportunity for farmers using mechanization. For this reason, efforts have been made to formulate the active ingredient *Fusarium oxysporum* f. sp. *strigae* strain DSM 33471, as a powder and to use it as a seed coating agent. This article deals with the production of the *Fusarium* spore powder, its properties, its application to the seed, and its herbicidal effect demonstrated in the first two field trials. The *F. oxysporum* strain was originally isolated from a wilting striga plant in Kenya. The strain was virulence enhanced to over produce the amino acids leucine, methionine and tyrosine. These amino acids are responsible for a second mode of action apart from the wilting causing effect of the fungus on striga. Whereas, leucine and tyrosine have a herbicidal effect, ethylene from methionine triggers the germination of striga seeds in the soil. Additionally, the strain has been improved to be resistant to the fungicide captan, which is commonly used to treat maize seed in Kenya. Seed coating tests conducted on 25 striga-infested small holder farms spread out in six counties of western Kenya reported yield increases of up to 88 %. A second trial carried out by the Kenyan Agricultural and Livestock Research Organization showed a 93% reduction of emerged striga plants.

Keywords: *Striga hermonthica*, *Fusarium oxysporum*, maize, biological herbicide, seed coating.

1. INTRODUCTION

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A microbial biological herbicide for pre-emergent and post-emergent control of the weed *Striga hermonthica* (striga) was approved and registered for use in maize in Kenya by the Kenyan regulatory body Pest Control Product Board in September, 2021. Striga is a parasitic weed that particularly affects monocot crops and negatively affects their yield. But the greatest damage is caused to maize.[1] The product contains a mixture of three strains of virulence-enhanced *Fusarium oxysporum* f. sp. *strigae*, a host-specific wilt pathogen of striga as the active ingredient.[2] Virulence enhancement of wild type *F. oxysporum* f. sp. *strigae* was necessary as wild type pathogens rarely kill their hosts, and there is always an equilibrium between a pathogen and host.[2,3,4,5] A virulence-enhanced pathogen from this overproduces three amino acids (methionine, leucine and tyrosine) that are harmful to the striga host at high concentrations, but are safe to the maize plant at these concentrations.[6] In the soil, methionine breaks down into ethylene gas, and ethylene stimulates the striga seeds in the soil to germinate prematurely.[7,8] Leucine and tyrosine interfere with biosynthesis of amino acids in striga, consequently killing the striga plant.[9] As a wilt pathogen, the mycelium and spores of *F. oxysporum* f. sp. *strigae* colonize and block striga plants vascular system blocking flow of water and nutrients, and this eventually leads to wilting and death of striga plants.[2,10]

The generation of virulence-enhanced *F. oxysporum* strains using mutation selection is described by Sands et al. (2001) and was used by Nzioki et al. (2016) for the development of the new biological herbicide.[6,9] The herbicidal effect of amino acids has already been found by Tiourebaev (1999).[11] Thus, the additional herbicidal effect of amino acids makes it possible to significantly increase the effectiveness of the *F. oxysporum* f. sp. *strigae* strain used in the product. The challenge now is to develop a production process and commercial system applicable to Kenyan conditions that will ensure farmers' access to effective striga control technology.

Manufacturing a packaged product in a central facility would be associated with high costs and a high level of logistical effort, including substantial funding for the establishment. The costs would primarily be caused by the required drying, additional storage, packaging and distribution of the product. Additionally, a live inoculum was thought to be a faster approach because striga attaches to the crop roots within 72 h of planting.[6] Therefore, a strategy was developed that enables the primary inoculum to be embedded into toothpicks and delivered to the village level where a live active inoculum product is manufactured. Proof-of-concept experiments showed strong maize yield improvement can be achieved by creating a field-ready inoculum with rice as a culture medium for the selected fungus strain.[6]

Toothpick Company Limited (www.toothpickcompany.co.ke), named for the primary inoculum storage method on toothpicks, was formed to be responsible for the production and commercialization of the product. The company was founded in March 2018 in Kenya according to the Kenyan Companies Act, 2015. The company is based in western Kenya, in a region where *S. hermonthica* causes very high yield losses, especially among small-holder farmers. Based in the city of Kakamega, it is particularly active in the counties of Vihiga, Siaya, Kakamega, Bungoma, Kisumu, Busia, Homa Bay, and Migori. The company recruits and trains the village-based producers or VIPs (Village Inoculum Producers), provides them with the necessary inoculum production materials and oversees the production process. The VIPs, in turn, are responsible for selling the product to the farmers and instructing the farmers on how to use the product correctly when planting the maize. In the long and short rains season of 2022, 135 VIPs worked for the Toothpick Company, supplying a total of about 4,500 farmers with product and supporting them in its application. The product is sold to the farmers immediately after it is made, and they apply it by hand at 1.5 g per planting hole. The price of the product is 120 KES or about 1.2 US$ per kg. Farmers use an average of 75 kg of product per ha (i.e., 90 US$ per ha).

With rice prices currently rising as a result of the war in Ukraine, it is questionable whether this price can be maintained. Additionally, with food shortages due to drought and climate change, using a food as the culture medium is less than ideal. Other disadvantages are a relatively high risk of contamination during the manufacturing process, a limited storage stability and a labour-intensive application method.

While the advantages outweigh the disadvantages based on yield improvement for farmers, identifying alternatives to the existing system could improve the outcomes and the ability to reach more farmers more easily. The disadvantages could be eliminated if it were possible to develop a product based on the active strain that could be used as a seed treatment.

Scientists have previously attempted to coat *F. oxysporum* f. sp. *strigae* to the seed of susceptible plant species (especially maize and sorghum) to facilitate application of the fungus. For example, Bastiani tested the applicability of strain M12-4A in millet cultivation in Mali.[12] Although some striga plants were infected with *F. oxysporum*, no effect of the treatment on yield could be determined. Later, in other studies, this isolate was found to be less effective than other strains.[13]

Venne et al. tested both the application of a *Fusarium* pesta granule, made by encapsulating fresh chlamydospores of *F. oxysporum* in a matrix composed of durum wheat-flour, kaolin, and sucrose, in the planting holes of maize and millet and the application of a pesta spore powder (ground pesta granules) to the seed.[13] The trials were carried out in Burkina Faso and Benin. Although, the application in the planting holes had a slightly better effect, the seed coating caused a reduction of the number of striga plants by about half compared to the untreated control, especially when using the isolates PSM197 and Foxy 2.

Elzein and Beed found a 53% reduction in the number of emergent striga plants as a result of the seed treatment.[14] This resulted in a 42% reduction in the number of flowering striga plants and a 21% increase in maize yield. The trials took place in Nigeria. The strains PSM197 and Foxy 2 were used again. In conclusion, it was pointed out that seed treatment of maize with effective *F. oxysporum* strains, particularly in combination with the chemical herbicide imazapyr use on imazapyr-resistant maize cultivars, had good prospects for commercialization. Avide et al., on the other hand, could not confirm the effect of seed treatment in the tested Kenyan soils.[15]

Oula et al. isolated five strains of *F. oxysporum* f. sp. *strigae* from striga plants from maize fields in Siaya County, Kenya. Strains FK1 to FK5 were attached to maize seed and tested in field trials.[16] In a total of six field trials (three locations, two seasons), clear differences between the strains in terms of their effect on the number of striga plants that had emerged could be determined. In particular, the strains FK3 and FK5 were superior to the other strains. In some cases, the number of striga plants that had emerged was reduced to 10% compared to the control as a result of seed treatment with the strains. However, a statistically significant difference in yield ($p < 0.05$) between the treatments and the untreated control could only be found at one site during the short rainy season.

Despite numerous, well-planned approaches, it has not yet been possible to commercialize the seed coating process. None of the strains used in the above-mentioned studies have been commercialized in any other application form. Apparently, based on the results available so far, potential industrial partners were not willing to take the risk of commercializing the process. The virulence-enhanced strains, paired with the seed coating appear to be an opportunity to finally successfully commercialize a biological herbicide for striga.

2. MATERIAL AND METHODS

2.1. Selection of a captan-resistant strain

The fungal strain used was *F. oxysporum* f. sp. *strigae* 6A, one of the three strains used in the commercial product, originally isolated from a striga plant in Kenya. The strain subsequently was virulence enhanced at the Department of Plant Science and Plant Pathology, Montana State University, Bozeman, MT, USA.

This strain was subjected to further mutation selection for increased resistance to the fungicide captan, which is commonly used on seeds. To this end, five captan-containing Potato Dextrose Agar (PDA) media were prepared by the addition of 0.02; 0.05; 0.125; 0.3 and 0.75 g/L of the active ingredient captan. The medium with the lowest concentration of captan was used first. Each 25 ml of this was poured into ten 90-mm Petri dishes. After cooling, 100 µl of a conidia suspension containing about 1×10^7 colony forming units (cfu) per millilitre of the *Fusarium* strain were smeared per plate using a Drigalski spatula.

After 7 days, isolated fungal colonies were visible on the surface of the agar medium, which had to be assumed to be capable of development at the concentration of captan used, i. e. having already reached a certain degree of resistance to the fungicide. These were removed using a sterile scalpel and further cultivated on PDA in other Petri dishes. The selected isolates were then transferred to the next higher concentration of captan using the same method. Again, colonies could be found growing on the medium containing the fungicide. The fungal isolates obtained from this were further cultivated and again subjected to the selection process. This was continued until a captan concentration of 0.75 mg/L agar medium was reached.

2.2. Production of a *Fusarium oxysporum* spore powder

Two hundred ml each of potato dextrose broth were placed in four, 1-L Erlenmeyer flasks. These were closed with a cotton plugs and aluminium foil and then autoclaved. The cooled broth was inoculated per Erlenmeyer flask with eight pieces of agar (approximately 6×6 mm) covered with the mycelium of strain DSM 33471 from the edge area of a fungal colony growing on a PDA culture medium. The cultures were then shaken at 90 rpm for 7 days at about 26 °C. As a result of the shaking culture, about 1×10^8 microconidia per millilitre and some mycelium fragments were counted.

The spore suspensions were then placed in a sterile glass beaker and refrigerated for 24 h. During this time, the microconidia and mycelium fragments settled to the bottom of the beaker. The supernatant water was poured off, leaving 300 ml of a highly concentrated spore suspension in the beaker.

To manufacture the spore powder, 600 ml of tap water were mixed well with 400 g of a beech wood powder (*Fagus* sp.) from J. Rettenmaier & Söhne GmbH & Co.KG (Holzmühle 1, D-73494 Rosenberg, Germany) with the brand name “Lignocel® HB 120”, placed in a beaker and autoclaved. The wood powder consists of particles with an edge length of 40 - 120 µm and has already been heat-treated so that it contains few potential contaminants. After cooling, the wood powder was transferred to a sterile tub under a biosafety cabinet and the 300 ml of spore suspension obtained previously were added. The wood powder was mixed well with the spore suspension using a large spoon so that a homogeneous mixture of wood particles and spores (including some mycelium fragments) was formed. This was then transferred to a sterile drying vessel (layer height 4 cm) and dried at 25 °C under the laminar flow of a biosafety cabinet for 6 days. During the drying process, the powder was shifted twice a day. The concentration of the spore powder after drying was about 2×10^8 cfu/g. The 400 g of highly concentrated spore powder were then mixed well with a further 1200 g of wood powder, resulting in a final product with a concentration of approximately 5×10^7 cfu/ml. This was used for all further experiments.

2.3. Shelf-life test

The powder described under 2.2. was portioned and stored at +25, +7 and -10 °C. The number of cfu in the product was determined after 3, 6, and 9 months. For this purpose, the product was suspended in different concentrations in sterile water and homogenized with an Ultra-Turrax T25 from the company IKA (Janke & Kunkel-Str. 10, 79219 Staufen, Germany). While the homogenization process was still in progress, 100 µl of the suspension were taken and placed in the centre of a PDA Petri dish and smeared using a Drigalski spatula. Since it was not clear how many cfu would still be in the powder, this process was carried out with different concentrations of the powder in the suspension and repeated twice each time. The number of colonies which developed on the PDA medium was converted to the number of cfu per gram of spore powder.

2.4. Field trials

Two trials were carried out to determine the influence of a *F. oxysporum* seed coating. The first one to determine the yield effect was carried out under farmer practice conditions at different farms in different counties (see Section 2.4.3.). The second one to determine the influence of the treatment on the number of emerging and developing striga plants was carried out at the KALRO trial station in Kibos, Kenya.

2.4.1. Seed coating with spore powder

Ten g of sucrose were stirred in 100 ml of water until the sucrose had completely dissolved. Then, 50 ml of the sucrose solution was mixed with 2.0 g of the *F. oyxsporum* spore powder producing a suspension and resulting in a concentration of about 2 x 10^6 cfu per ml. 1000 g of maize seed was homogeneously mixed with this spore powder suspension resulting in a concentration of a bit more than 20,000 cfu per seed grain. The sugar in the suspension served as a surfactant and the powder completely stuck to the seed. To test if the cfu were attached and vital on the maize seed, seed grains were placed on a PDA Petri dish until the fungus was growing out and the maize was germinating (Figure 3). It could be observed that *F. oxysporum* grew out very vigorously and that neither the seedling root nor the shoot was damaged by the fungus.

For the trial in Kibos, 3 g of spore powder was used to treat 1 kg seed. The commercial seed used for this treatment had previously been coated with the fungicide captan.

2.4.2. Seed coating with mycelium suspension

Wooden dowels (5 mm x 30 mm) coated with the non-captan resistant *F. oxysporum* strain (6A) were removed from storage container (50 ml sterile falcon tube) placed in a refrigerator at 4 °C. Using sterile forceps, three dowels were separately placed on labelled 9-cm diameter Petri dishes containing SNA (soil nutrient agar) in a biosafety cabinet. The dowels were then incubated at 25 °C - 28 °C. After 10 days of incubation, pure culture of the growing fungus was scraped off the plates and placed in labelled 0.5-L sterile media bottles, each containing 250 ml of sterile distilled water. The mixture was then homogenized using an Ultra-Turrax for about 5 min. The number of cfu from each respective homogenized mycelium suspension was determined by serial dilutions on PDA (three replications). Only inoculum showing at least more than 1 x 10^5 cfu per ml was used to coat maize seeds.

One hundred g of maize seed (not previously treated with the fungicide captan) were coated with mycelium of the *F. oxysporum* strain 6A. The seeds were placed in a sterile

0.5-L media bottle and mixed with 6 ml of sugar solution (see above) and 2 ml of the homogenized mixture of the *Fusarium* strain. The media bottle was capped and thoroughly shaken by hand to uniformly coat the seed. The coated seed were subsequently dried on sterile, paper napkins in a running biosafety cabinet. The dried seed were subsequently placed in sterile paper envelopes and stored at room temperature until planting.

2.4.3. Planting and trial design

To test the effect of the spore powder on the plant yield plots with a size of 10 x 10 m were planted with coated maize at a spacing of 0.75 cm between rows and 0.25 cm within rows during the long rainy season in March 2021. The trials were conducted on striga-infested farms in Bungoma (6 farms), Kakamega (5 farms), Siaya (4 farms) and Vihiga (2 farms) counties in western Kenya. Immediately adjacent to the plot on which the coated seed was planted, a control plot of the same size was sown with non-coated seed of the same maize variety. The seed used were not treated with any fungicide. All plots were treated with the recommended agronomic practices (thinning, weeding, and fertilization). The trials were repeated during the long rainy season of 2022 in counties Homa Bay (4 farms) and Migori (4 farms). The maize varieties used are shown in Table 2 (see point 3.2.). All varieties were hybrids.

The plant yield at the time of harvest was used as a criterion for the effect of the seed treatment. The average yields for the individual counties were determined. The individual farms can be handled as a random sample from a population in a linear model.[17] It is not possible to compare average treatment values per farm, but comparisons of average treatment levels (treated, not treated) per county are possible. The statistical analysis was carried out with the model:

y = treat + count + treat x count + farm

where ***y*** is the yield, ***treat*** is the treatment (spore powder or untreated control), ***count*** is county, and ***farm*** is the farm. For the sake of simplicity, the model notation from Piepho et al. was applied.[18]

In order to test the effect of the spore powder and the mycelium suspension on the number of emerging and developing striga plants, a trial with the hybrid maize variety DK 8033 was set in October 2021 (short rainy season). The variety was planted because it is susceptible to striga weed but less susceptible to maize lethal necrosis (MLN) disease compared to the other varieties. The design was a randomized block replicated three times. Each treatment consisted of paired 2.5-m long plots. Two maize seeds were planted per hole at a depth of 3-4 cm at the recommended spacing of 25 cm within rows and 75 cm between rows. Two weeks after planting, the maize plants were thinned to one plant per hole.

Data on the number of emerged and number of flowered striga plants were collected at intervals of 2 weeks up to 12 weeks after planting (WAP). The data were collected from striga plants within a radius of 15 cm from the maize plants. Data on crop reaction symptoms (phytotoxicity) caused by the Foxy pathovar containing treatments were similarly collected 6 to 8 WAP using a crop reaction symptoms scale of 0-9. Analysis of the data was conducted using statistical analysis systems software (SAS Institute, Cary, NC, USA). Significant treatment means were separated by Fishers LSD test at $p < 0.05$.

3. RESULTS

3.1. Captan resistance and shelf-life

The selection of a captan-resistant strain resulted in a strain which is able to grow on an agar-medium containing 0.75 mg/L of captan (active ingredient in the fungicide Captan 50WP). The strain was designated as DSSN 6. It was deposited at the German Collection of Microorganisms and Cell Cultures in Braunschweig under the strain designation DSM 33471.

Investigating the shelf-life of the spore powder, well-developed colonies of the fungus *F. oxysporum* could be seen under the microscope after just 24 h (Figure 1). After 64 h, the colonies could even be counted with the naked eye (Figure 2). The results of the shelf-life studies are shown in Table 1. The storage stability of the cfu in the spore powder clearly depends on the storage temperature and the storage duration.

3.2. Yield results of the on-farm trials

In the long rainy season of 2021 as well as in the long rainy season of 2022, clear differences in yield were found between the treated variant and the untreated control. Seed treatment with *F. oxysporum* spore powder resulted in yield increases of between 27.26 and 88.45% respectively. Mean yields measured in the counties are shown in Table 2. According to the F-test of analysis of variance, there are statistically significant differences in the main treatment effects (Num DF = 1, Den DF = 19, F = 113.18, $P < 0.001$) whereas no statistically significant differences were found between counties or between treatment-county interactions.

3.3. Effect of different seed treatments on the number of emerged striga plants

Figure 4 shows the results in terms of the number of striga plants that had emerged and the number of flowering striga plants 12 weeks after the planting date at the Kibos site. The use of seed treated with a mycelium suspension showed a decrease of the emerged and flowering striga plants in comparison with the untreated control. This reduction was statistically significant at $p < 0.10$. The treatment of the seed with the spore powder worked best ($p < 0.05$).

The results also make it clear that the herbicidal effect of the captan-resistant fungal isolate is not prevented by treating the seed with captan. Despite the treatment of the seeds with captan, an average reduction in the number of emerged striga plants compared to the untreated control of 93% and an average reduction in number of flowering striga plants of 96% could be determined by an additional treatment with the spore powder.

The formation of the striga fruit capsules was completely prevented in the variants "Seed treatment with mycelium" and "Seed coating with spore powder" (data not shown). None of the treatments resulted in any damage to the maize plants. Unfortunately, it was not possible to determine the yield due to significant vertebrate pest damage (birds, mongoose).

4. DISCUSSION AND CONCLUSIONS

It was possible to select a *F. oxysporum* strain with high captan resistance. Since captan is the most widely used fungicide for seed treatment in Kenya next to thiram, this property allows for easier commercialization of the product. However, it may be possible that the fungicide treatment of the seed will not play such an important role in the future. Even treating the seed with just the fungus seems to have a fungicidal effect on certain soil-

borne fungi.[19] A treatment of the seed with a fungicide can therefore possibly be omitted in the future if the seed is treated with the new spore powder.

In 2001, Tiourebaev et al. produced a fungus powder with the microconidia of *F. oxysporum*. At that time sawdust from birch (*Betula* sp.) wood was used.[20] However, the sawdust mixed with certain nutrients served only as a carrier material for the further culture of the fungus. The powder was dried after a certain incubation time when the fungal mycelium became visible. The spore powder used in this work, on the other hand, is produced by mixing a spore suspension with a fine wood powder. After drying, a product is created that can already be used. It is therefore a novel formulation of a biological active ingredient that has been registered for a patent.

The shelf-life meets the requirements for conducting initial efficacy tests, but still needed to be improved for the commercialization of a spore preparation for seed treatment. A good shelf-life is necessary so that the product can be sold to the final consumer via intermediaries. The product is temporarily stored both by the producer and by the middleman, i. e. also by agricultural trade in the villages. During these storage times, the vitality of the fungus in the product must not fall below a specified value (cfu/g). The effect would be lost. Ideally, the shelf life should guarantee use of the product over two growing seasons. Unsold product in the rural trade could still be sold in the next rainy season. According to Rhodes, a biological pesticide should have a shelf life of at least 12 - 18 months at room temperature.[21] Therefore, already commercialized biological pesticides based on fungal spores have a significantly higher storage stability. This can be achieved through the addition of protectants and stabilizers during formulation process, through the use of protective gases during storage and through the use of certain materials to manufacture the storage vessels.[22,23,24] One of us has had good experiences using High Density Polyethylene bottles overlaying the product with pure nitrogen (P.L., unpublished). However, even during the cultivation of the fungus to produce the conidia, the subsequent storage stability can be influenced.[25] Garcia Riaño et al. achieved a storage stability of the conidia of *Beauveria bassiana* of more than 3 years at approx. 6 °C, and more than 2 years at 18 °C.[26] Further investigations are required to increase the shelf life of the cfu in the spore powder as well as on the seed.

The effect of a seed treatment was impressively demonstrated in both trials. The use of the spore powder produced according to the method described above (see Section 2.2.) showed a significantly better effect, both in the control of *S. hermonthica* and in relation to the resulting yield increase than the seed treatments in previous tests (see point 1.). According to the authors, this can be attributed to various reasons. In contrast to the strains used previously, strain DSM 33471 has two mechanisms of action. In addition to acting as a wilt pathogen on *S. hermonthica*, the amino acids excreted by the fungus in the soil have a clear enhanced herbicidal effect.[9]

In addition, the powder formulation consists mainly of the conidia of *F. oxysporum*, whereas the formulations previously used contained chlamydospores in particular. According to Elzein et al. chlamydospores of *F. oxysporum* (Foxy 2) have a better storage stability than conidia.[27] On the other hand, chlamydospores may need a trigger (e.g., root exudates) or a certain nutrient concentration in the soil in order to germinate .[28]

Thirdly, the way in which the spore powder was bound to the maize seed could have played a role. While earlier experiments used almost exclusively arabic gum[12,14,16] as the glue, the new spore powder was attached to the seed using a 10% sugar solution. This sugar solution offers the very rapidly developing *Fusarium* germs an ideal starter supply of nutrients. The importance of the sugar solution is also confirmed when using a mycelium suspension in the field trial carried out in Kibos. None of the other authors cited mentioned supplemental nutrition[12,14,15,16], but Venne et al.[13]. They used sucrose in their pesta formulation.

The results were achieved even though the application rates used were significantly lower than in previous seed treatment trials. For example, Woomer et al.

reported 40 g of a *F. oxysporum* product per 600 grains of maize seed.[29] For this reason, much lower costs for the treatment of the maize seed can be expected. With an application rate of just 2 g spore powder per kg seed, according to initial calculations costs of less than KES 1200 (about 12 US$) per ha can be assumed. This would make the new technology affordable even for poor subsistence farmers.

The rice formulation was specifically developed for maize cultivation by smallholder farmers due to its low storage stability, freshness and manual application. Due to the higher sowing quantity per unit area, the application was not suitable for other crops such as sorghum, millet or dryland rice, nor for larger farms where sowing is done mechanically. The introduction of a seed treatment technology would expand the market through additional distribution channels. With a striga infestation of 217,000 ha in western Kenya and a seed rate of 25 kg per hectare, around 11,000 kg of spore powder would be required annually.[30] This amount could be produced with a relatively small production facility. The seed treatment could potentially continue to take place at the village level, driving the economy locally and giving farmers the opportunity to coat maize seed suited for their area.

The good results of the first field trials and the expected commercial feasibility prompted us to press ahead with the registration of the spore powder in Kenya. A registration dossier is currently being prepared and Good Experimental Practice field trials are being carried out by an independent certified company.

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Table 1. Shelf life of cfu in the spore powder at +25, +7, and -10 °C after storage for 3, 6 and 9 months

Date	Spore viability in cfu/g dependant on different storage temperatures		
	+25 °C	+7 °C	-10 °C
18/02/2021 (start)	4.81×10^7	4.81×10^7	4.81×10^7
21/05/2021 (3 months)	0.83×10^7	4.35×10^7	4.83×10^7
17/08/2021 (6 months)	2.33×10^4	2.97×10^7	4.45×10^7
23/11/2021 (9 months)	0	1.91×10^7	3.21×10^7

Table 2. Effect of treatment of maize seed with *Fusarium oxysporum* spore powder on maize yield in six Kenyan counties after infestation of the cultivated area with the parasitic weed *Striga hermonthica*

County	Maize varieties	Number of farms	Yield in kg per plot (10 x 10 m)		Yield increase in %
			Untreated control	2 g of spore powder per kg of seed	
Bungoma	Duma 43	6	11.76 a*	16.90 b	+43.73
Kakamega	Duma 43	5	11.02 a	18.16 b	+64.79
Siaya	Tempo 73	4	10.05 a	18.94 b	+88.48
Vihiga	Tempo 73	2	11.61 a	14.78 a	+27.26
Homa Bay	Pannar 3M05	4	10.48 a	17.01 b	+62.89
Migori	Pannar 3M05	4	10.66 a	17.85 b	+68.33

*Means in a row followed by the same letter are not significantly different at $p < 0.05$ by t-test.

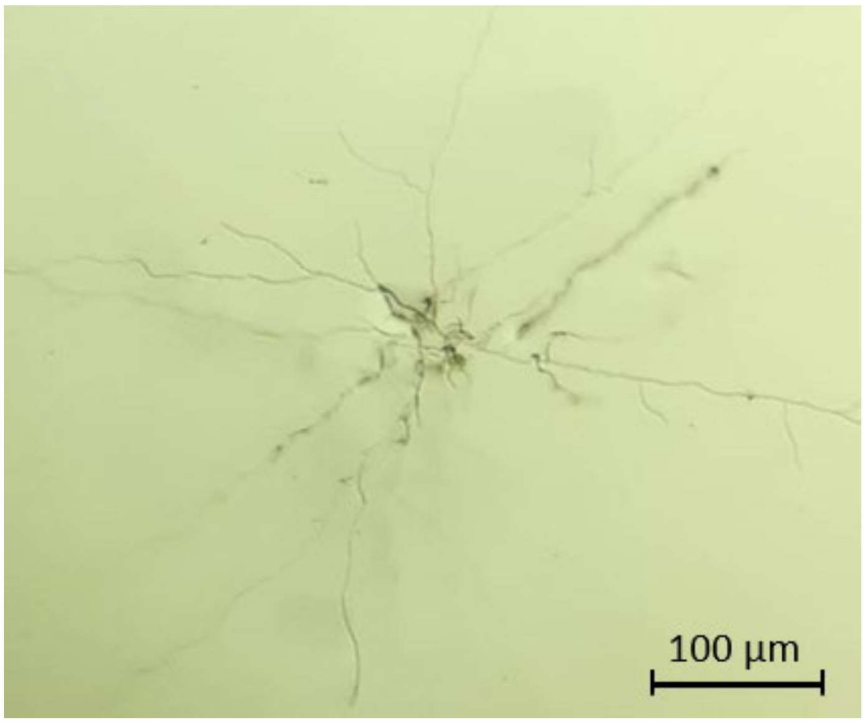

Figure 1. Fungal colony of *F. oxysporum* on PDA 24 h after plating

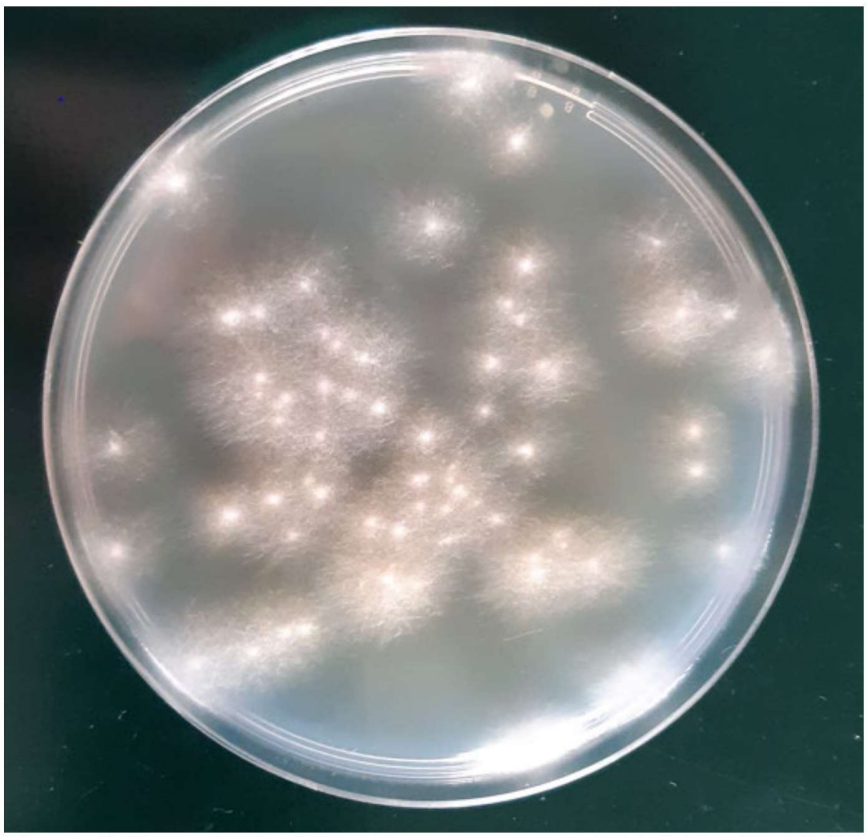

Figure 2. Fungal colonies of *F. oxysporum* on PDA 64 h after plating

Figure 3. Maize seed treated with *F. oxysporum* spore powder germinating on PDA after 7 days

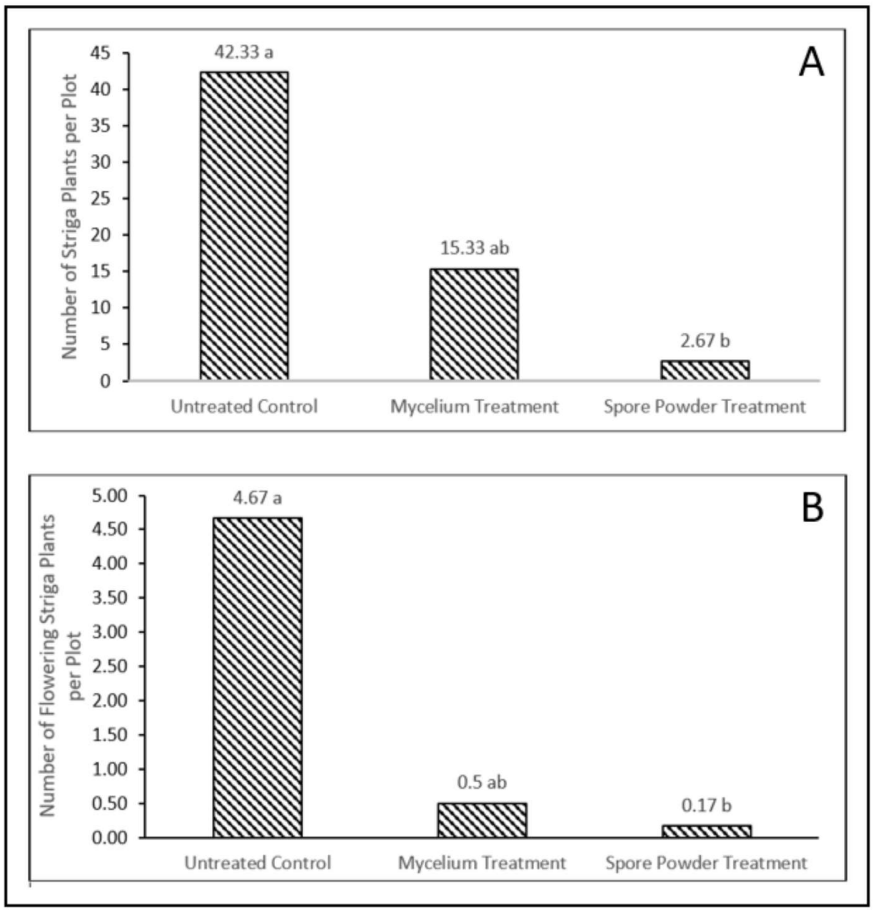

Figure 4. Number of striga plants per plot.
A: Number of emerged striga plants 12 weeks after planting maize.
B: Number of flowering striga plants 12 weeks after planting maize.
Means on top of the columns followed by the same letter are not significantly different at $p < 0.05$ by t-test.

PS_7522_Bari Graphical Abstract Image.JPG

A microbial bioherbicide for *Striga hermonthica* control: production, development, and effectiveness of a seed coating agent

P. Lüth*, H.S. Nzioki, C.S. Baker and D.C. Sands

Fusarium oxysporum f. sp. *strigae* formulated as a spore powder and applied as a seed coating agent effectively controls the parasitic weed *Striga hermonthica* in maize production.