In vitro antifungal activity of Desmodium intortum and D. uncinatum root extracts against growth of toxigenic Fusarium verticillioides and Aspergillus flavus

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Abstract

Maize grown under push-pull cropping system has been reported to contain lower concentrations of fumonisin and aflatoxin than maize monocrop. This study determined the inhibitory effect of desmodium root extracts on spore germination and radial growth of toxigenic Aspergillus flavus and Fusarium verticillioides. Aspergillus flavus and F. verticillioides were isolated from maize and soil and tested for ability to produce aflatoxin and fumonisin by inoculation on mycotoxin-free maize. Aflatoxin and fumonisin were detected and quantified by direct competitive ELISA. Desmodium roots were dried, ground to fine powder and extracted with methanol and dichloromethane and evaluated for anti-fungal activity by inhibition of spores of A. flavus and F. verticillioides potato dextrose agar (PDA) medium. Isolates of F. verticillioides produced up to 599,741 µg/kg fumonisin while isolates of A. flavus produced up to 199,184 µg/kg aflatoxin. Desmodium root extracts reduced germination of A. flavus spores and F. verticillioides by 9.6% and 43.8%, respectively and reduced their respective colony radial growth by 15% and 57%. The results suggested that desmodium roots contain chemical compounds that inhibit growth of A. flavus and F. verticillioides. This may explain the reduction in infection of maize with mycotoxin-producing fungi before harvest in push-pull cropping systems by reducing the pathogen inoculum in the soils. Determination of the active compounds in the root exudates is recommended.

Keywords: aflatoxin; Aspergillus flavus; Desmodium; fumonisin; Fusarium verticillioides; push-pull; root extracts.

Introduction

Mycotoxins are chemical compounds produced by fungi found in soil and crop residues (Miller, 2008; Negedu et al., 2011; WHO, 2018). The amount of mycotoxin produced by a fungus is dependent on environment conditions (moisture, humidity, temperature) and available nutrients (Miller, 2008). Aflatoxins and fumonisins are the most economically important mycotoxins of maize in sub-Saharan Africa (Lewis et al., 2005; Mutegi et al., 2018; Mutiga et al., 2015). Chronic exposure to food contaminated with aflatoxin is associated with suppressed immunity in humans, liver cancer, damage of vital organs and decreased productivity (IARC, 1972; Lewis et al., 2005). Fumonisins cause health and economic hazards and it is associated with apoptosis of the liver and kidneys, pulmonary edema, esophageal cancer, neural tube birth defects and toxicity of the nervous system (IARC, 1972; Olga, 2009; WHO, 2018).

Aspergillus flavus and F. verticillioides are the major producers of aflatoxin and fumonisin, respectively (IARC, 1972; Klich, 2007; Samson and Varga, 2009) and they are responsible for maize ear rots (Schmaile III and Munkvold, 2009). Aspergillus flavus is an opportunistic pathogen of maize and grows as yellow-green spore masses on maize grains (Schoeman, 2012) leading to deterioration of maize quality (Cotty and Jaime-Garcia, 2007). Strains of A. flavus differ in the amounts of aflatoxins produced, with A. flavus S strain (< 400 µm sclerotia diameter) producing higher levels of aflatoxin than A. flavus L strain (> 400 µm sclerotia diameter ) (Cotty, 1989). Fusarium verticillioides is a cosmopolitan pathogen of maize whose growth on maize is characterized by pinkish to violet moldiness on the grain (Leslie and Summerell, 2006). Although F. verticillioides can infect the maize plant systemically from infected seeds and through wounds caused by Lepidopteran larvae, the most efficient route of infection is through the silk (Sobek and Munkvold, 1999; Thompson et al., 2018). Fusarium verticillioides persists in host residues for up to 90 days depending on the prevailing climatic conditions (Fernandez et al., 2008).

Push-pull cropping system was reported to significantly reduces the levels of aflatoxin and fumonisin in pre-harvest maize (Njeru et al., 2019). The ‘push-pull’ technology involves intercropping cereals such as maize or sorghum with a fodder legume such as Desmodium and planting a fodder grass such as Napier or Brachiaria as a border around the maize-Desmodium intercrop (Cook et al., 2007; Khan et al., 2002). The Desmodium acts as the ‘push’ crop by producing chemicals that repel stemborer and fall armyworm moths while the Napier or Brachiaria border act as the ‘pull’ crop by
attracting stemborer and fall armyworm moths away from the maize (Khan et al., 2000; Midega et al., 2018). Plant root-exudates have been reported to influence the fungal community in the soil by maintaining the population of resident fungi and inhibiting existence of non-residence fungi (Broeckling et al., 2008; Yang et al., 2014). The relationship between soil microorganisms and plants are very specific (Boivin et al., 2016; Steinkellner et al., 2007; Sullia, 1973) and in *vitro* studies have demonstrated the inhibitory activity of plant extracts against fungal plant pathogens (Muthomi et al., 2017; Njoki et al., 2017; Okumu et al., 2019). The inhibitory activity could be attributed to presence of bioactive chemicals such as flavonoids and alkaloids, which suppress spore germination and modify the structure of the fungal mycelia (Tabassum and Vidyasagar, 2013). Roots of mature Desmodium produces C-glycosyl flavonoid exudates (Hooper et al., 2015). This study sought to determine the mechanisms involved in reduction of ear rots and mycotoxins in maize under push-pull cropping system. Extracts from Desmodium roots were evaluated for their activity against toxigenic *A. flavus* and *F. verticillioides* in *vitro*.

**Results**

**Aflatoxin and fumonisin production by Aspergillus flavus and Fusarium verticillioides**

Maize colonized by *A. flavus* and *F. verticillioides* isolates is shown in supplementary figure 1. Ninety-four (94) percent of *A. flavus* isolates from both push-pull and non-push-pull maize produced detectable levels of aflatoxin. There was no significant (P = 0.266) difference between the aflatoxin levels produced by isolates recovered from push-pull and those recovered from non-push-pull cropping systems. Aflatoxin production potential of the *A. flavus* isolates ranged from below lower limit of detection to 199,184 µg/kg (Table 1). On the contrary, all the *F. verticillioides* isolates produced high levels of fumonisin between 2804 and 599,741 µg/kg (Table 1). The fumonisin production potential did not differ between isolates from push-pull and those from non-push-pull (P = 0.757).

**Effect of desmodium root extracts on mycelial growth**

Crude extracts from both of *D. intortum* and *D. uncinatum* significantly (P < 0.001) reduced colony radial growth of *A. flavus* and *F. verticillioides* isolates (Supplementary figure 2, Table 2). The reduction in colony radial growth was also significant across days after incubation (P < 0.05). Colony radial growth was significantly (P < 0.05) influenced by the days of incubation. Reduction of radial growth of *A. flavus* was, however, not significantly different between the extracts of *D. intortum* and *D. uncinatum*. The root extracts reduced the radial growth of *F. verticillioides* and *A. flavus* colonies by 53-61% and 12 – 17%, respectively (Table 3). The percentage reduction in radial growth for most *F. verticillioides* colonies decreased with increased duration of incubation. Percentage reduction of *A. flavus* and *F. verticillioides* growth was significantly (P < 0.05) decreased across days of incubation. *Desmodium uncinatum* root extract was significantly (P < 0.05) more effective in reduction of *F. verticillioides* radial growth compared to *D. intortum* extract. Both *D. intortum* and *D. uncinatum* caused significantly (P < 0.001) more reduction in colony diameter of *F. verticillioides* than that of *A. flavus*.

**Effect of desmodium root extract on spore germination and germ tube growth**

Aspergillus flavus and *F. verticillioides* spores germinated after six hours of incubation and the germ tubes started developing into hyphae after 10 hours (Supplementary figure 3, Supplementary figure 4). The Desmodium root extracts significantly (P = 0.001) reduced the proportion of germinated spores of *A. flavus* after ten hours of incubation (Table 4). The number of germinated *A. flavus* spores was reduced by 3.2-11.4% while germ tube elongation was inhibited by 16.1-48.3% depending on the duration of incubation (Table 4). The extracts significantly (P < 0.05) reduced the proportion of germinated *F. verticillioides* spores and length of germ tubes by up to 50.0% and 43%, respectively, after six and eight hours of incubation (Supplementary figure 4, Table 4). After ten hours of incubation, over 95% of the spores in the plates treated with extracts had germinated but the germ-tubes were short and the hyphae had not started branching. However, in the control plates without extract, there was 100% spore germination after 10 hours and hyphae were fully formed and branched.

**Discussion**

The results of this study showed that all the *F. verticillioides* produced high amounts of fumonisin between 2804 and 599,741 µg/kg. This indicates that under favourable environmental conditions, high amounts of fumonisins produced by *F. verticillioides* may accumulate in maize maturing in the field (Leslie and Summerell, 2006). This poses risk of toxin exposure to consumers in the study area, where maize is the main staple food. The fumonisin levels recorded in this study could be due to the ideal incubation conditions of the maize under experimental conditions. In spite of being a field toxin, high populations of *F. verticillioides* in physiologically mature maize can also lead to high levels of fumonisin during post-harvest handling and storage, especially if the maize is exposed to temperature, humidity and moisture that is favorable for proliferation of the fungus and fumonisin production (Fountain et al., 2014; Miller, 2008). *Fusarium verticillioides* grow and produce fumonisin at temperatures between 20 and 25°C at moderate water activity (Camardo Leggieri et al., 2019). Fumonisin production under field conditions is also increased by drought stress (Leslie and Summerell, 2006; Samapundo et al., 2005). These conditions are prevalent in western Kenya, where average temperature and precipitation is 18.5 – 22.7°C and 1300 toover 2000 mm, respectively, annually (Jaetzold et al., 2009). Unlike *F. verticillioides*, not all tested *A. flavus* isolates produced aflatoxin. Six percent of *A. flavus* isolates did not produce aflatoxin, while 53% produced low levels of up to 10 µg/kg and 41% produced between 10 and 199,184 µg/kg of aflatoxin. Although *A. flavus* has been reported to occur in low frequency and abundance (Nyangi, 2016; Owuor et al., 2018), further infection and aflatoxin production could increase during handling and storage under high moisture, temperature
Table 1. Aflatoxin levels (µg/kg) produced in clean maize by A. flavus and fumonisin levels (µg/kg) produced by F. verticillioides isolated from maize and soil under push-pull and non-push-pull cropping systems.

| Range          | Aflatoxin | Fungal species | F. verticillioides |
|----------------|-----------|----------------|-------------------|
| Minimum        | 2.929.4   | 2.808.8        | < LOD             |
| Maximum        | 521,482.4 | 599,740.6      | 199,184.3         |
| Median         | 213,058.3 | 143,563.7      | 178,625.9         |

LSD = lower limit of detection (1 µg/kg for aflatoxin and 100 µg/kg for fumonisin)

Table 2. Colony diameter (cm) of A. flavus and F. verticillioides grown on PDA amended with root extracts from two desmodium species and PDA without extract for two, four and six days.

| Fungal species | Isolate | Days incubated | D. intorrum | D. uncinatum |
|----------------|---------|----------------|-------------|--------------|
|                |         | Extract        | No extract  | Extract      | No extract  |
| A. flavus      | 81B     | Two            | 1.10±0.0    | 1.41±0.1    | 1.40±0.0    |
|                |         | Four           | 2.5±0.1     | 3.1±0.0     | 2.8±0.0     |
|                |         | Six            | 4.1±0.2     | 5.3±0.0     | 4.6±0.0     |
|                | 151D    | Two            | 1.10±0.1    | 1.21±0.0    | 1.3±0.1     |
|                |         | Four           | 2.5±0.2     | 2.9±0.0     | 2.4±0.1     |
|                |         | Six            | 4.0±0.3     | 5.0±0.0     | 3.9±0.0     |
|                | 105A    | Two            | 1.5±0.0     | 2.0±0.0     | 1.9±0.1     |
|                |         | Four           | 3.5±0.1     | 4.1±0.0     | 2.4±0.0     |
|                |         | Six            | 5.4±0.1     | 6.3±0.0     | 3.8±0.1     |
|                | 2M35E   | Two            | 1.2±0.0     | 2.1±0.0     | 1.3±0.1     |
|                |         | Four           | 3.8±0.0     | 4.3±0.0     | 2.8±0.0     |
|                |         | Six            | 4.6±0.0     | 6.5±0.1     | 4.6±0.0     |
|                | 379B    | Two            | 1.4±0.0     | 1.4±0.0     | 1.3±0.0     |
|                |         | Four           | 2.9±0.1     | 3.3±0.1     | 2.8±0.0     |
|                |         | Six            | 4.8±0.2     | 5.2±0.1     | 4.6±0.1     |
|                | 479D    | Two            | 1.0±0.0     | 1.2±0.0     | 1.1±0.0     |
|                |         | Four           | 2.4±0.0     | 2.8±0.0     | 2.2±0.0     |
|                |         | Six            | 3.9±0.0     | 4.5±0.0     | 3.7±0.0     |

LSD = 0.3

Table 3. Reduction (%) in colony diameter of A. flavus and F. verticillioides isolates grown on PDA amended with root extracts of two desmodium species and incubated for two, four and six days.

| Isolate | D. intorrum | D. uncinatum |
|---------|-------------|--------------|
| A. flavus | Two: | Four: | Six: | Mean: | Two: | Four: | Six: | Mean: |
| 81B     | 21.4        | 19.4        | 22.6  | 21.1±0.4 11 | 28.6 | 7.1  | 6.5  | 14.1±2.2 11 |
| 151D    | 8.3         | 13.8        | 20.0  | 14.0±2.0 11 | 23.1 | 4.2  | 5.1  | 10.8±2.1 11 |
| 105A    | 25.0        | 14.6        | 14.3  | 18.0±1.2 11 | 36.8 | 4.2  | 7.9  | 16.3±2.9 11 |
| 2M35E   | 42.9        | 11.6        | 29.2  | 27.9±2.4 11 | 7.7  | 7.1  | 8.7  | 7.8±0.3 11 |
| 379B    | 0.0         | 12.1        | 7.7   | 6.6±0.0 11  | 15.4 | 10.7 | 10.9 | 12.3±1.0 11 |
| 479D    | 16.7        | 14.3        | 13.3  | 14.8±0.4 11 | 9.1  | 4.5  | 10.8 | 8.1±1.1 11  |

LSD = 0.3

P value = <0.001

F. verticillioides

| Isolate | D. intorrum | D. uncinatum |
|---------|-------------|--------------|
| 561B    | 57.1        | 47.6        | 47.8  | 50.8±0.7 11 | 68.8 | 59.4 | 56.9 | 61.7±0.8 11 |
| 552A    | 55.6        | 50.0        | 48.1  | 51.2±0.5 11 | 68.8 | 57.6 | 53.8 | 60.1±0.5 11 |
| 581A    | 60.0        | 47.4        | 46.4  | 51.3±1.0 11 | 66.7 | 62.5 | 51.2 | 60.1±1.0 11 |
| 538A    | 60.0        | 50.0        | 47.4  | 52.5±0.9 11 | 68.8 | 60.0 | 56.8 | 61.9±0.7 11 |
| 519A    | 60.0        | 54.8        | 56.1  | 56.9±0.4 11 | 66.7 | 57.6 | 56.6 | 60.3±0.7 11 |
| 601A    | 60.0        | 53.7        | 54.5  | 56.1±0.4 11 | 68.8 | 57.6 | 57.4 | 61.2±0.4 11 |

LSD = 1.3

P value = <0.001

P = Calculated 95% probability value, LSD = least significant difference, hours plates inoculated plates were incubated before counting the number of germinated spores

Means followed by the same letters along the same column are not significantly different.
and humidity (Fountain et al., 2014). *Aspergillus flavus* is favored by hot and dry climatic conditions characterized by temperatures of 25-42°C and low moisture content, but the optimum temperature for aflatoxin production is 28-30°C at high water activity (> 0.95) (Camardo Leggieri et al., 2019; Sanchis and Magan, 2004). Under field conditions, *A. flavus* is an opportunistic pathogen that infects maize weakened by factors such as drought stress, nutrient stress and physical damage (Abbass et al., 2006; Pitt and Hocking, 2006; Klich, 2007).

Root extracts of both *D. intortum* and *D. uncinatum* exhibited antifungal activity against *F. verticillioides* and *A. flavus* as demonstrated by the significant reduction in radial growth of the fungal colonies. The effect was, however, significantly higher against *F. verticillioides* compared to *A. flavus*. Previous studies reported reduced incidence of ear rots in maize under push-pull cropping (Owuor et al., 2018) and lower levels of mycotoxins, especially aflatoxin and fumonisin (Njeru et al., 2019). Therefore, the higher activity against *F. verticillioides* could be responsible for the observed higher reduction in the amounts of fumonisin produced on maize compared to aflatoxin as reported by (Njeru et al., 2019). However, the difference in activity between the extracts of *Desmodium D. intortum* and *D. uncinatum* cannot be attributed to the extracts were used in crude state. The difference in activity between the extracts of *Desmodium D. intortum* and *D. uncinatum* cannot be attributed to inhibition of radial growth and mycelial growth of toxigenic *F. verticillioides* and *A. flavus*. The observed reduction in radial growth of the fungal colony could be attributed to inhibition of the fungal mycelia and subsequently mycelial growth of toxigenic *F. verticillioides*.

### Table 4

| Treatment                  | Germinated spores (%) | Germ tube length (µm) |
|----------------------------|-----------------------|-----------------------|
|                            | Six       | Eight    | Ten      | Six       | Eight    | Ten      |
| *A. flavus*                |           |          |          |           |          |          |
| Extract                    | 8.2±1.6   | 32.3±2.9 | 86.2±3.0 | 3.3±0.3   | 9.4±0.6  | 23.0±3.3 |
| Reduction (%)              | 3.2       | 14.2     | 11.4     | 16.1      | 17.6     | 48.3     |
| P value                    | 0.913     | 0.392    | 0.001    | 0.219     | 0.099    | 0.017    |
| *F. verticillioides*       |           |          |          |           |          |          |
| Extract                    | 5.9±0.9   | 42.3±3.9 |          | 10.7±1.2  | 21.3±1.4 |
| No extract                 | 9.4±2.2   | 86.4±2.5 |          | 14.1±1.2  | 37.4±2.1 |
| Reduction (%)              | 37.5      | 50.0     |          | 24.1      | 43.1     |          |
| P value                    | 0.021     | < 0.001  |          | 0.044     | < 0.001  |          |

**Mean ± standard error of mean;** — hours plates inoculated plates were incubated before counting the number of germinated spores.

Algal colonies y oil as the mechanism by which the mycotoxin producing fungi such as *A. flavus* and *F. verticillioides* from the leaves and foliage that falls and decompose, thus inhibiting proliferation of *A. flavus* and *F. verticillioides*. The decomposing residues also encourage the multiplication of beneficial microorganisms that

*References*
may also contribute to the suppression of the pathogenic fungi.

**Materials and Methods**

**Determination of aflatoxin and fumonisin production potential of Aspergillus flavus and Fusarium verticillioides**

*Aspergillus flavus* and *F. verticillioides* were isolated from maize and soils in push-pull and non-push-pull farms. Fifty three (53) isolates of *A. flavus* were from push-pull farms while 52 isolates were from non-push-pull farms. *Fusarium verticillioides* isolates tested comprised of 49 isolates from push-pull and 45 isolates from non-push-pull farms. Of the *A. flavus* tested, 21 isolates were from soil and 84 were from maize, while all the *F. verticillioides* isolates were from maize. The isolates were tested for mycotoxin production on autoclaved mycotoxin free maize grains. The maize had been tested for aflatoxin and fumonisin by direct competitive Enzyme Linked Immuno-Sorbent Assay (ELISA) (Helica Biosystems Inc., Santa Ana, USA) following manufacturer's instructions. Inoculum of each of the *Fusarium* and *A. flavus* isolates was separately inoculated on 20 g of the sterile maize grain in 40 ml glass vials by adding 500 µl of fungal suspension adjusted to 10³ spores per ml under aseptic conditions. The cultures were incubated at 25°C for seven days. The colonized maize grain was blended with 100 ml of 70% methanol for *A. flavus* and 40 ml of 90% methanol for *F. verticillioides* isolates. After settling, the supernatant was collected and the filtrate was used for detection and quantification of aflatoxin and fumonisin by direct competitive ELISA for the *A. flavus* and *F. verticillioides* isolates, respectively. The lower and the upper limits of detection for the aflatoxin kits were 1 and 20 µg/kg, respectively, while the corresponding limits for fumonisin kits were 100 and 6000 µg/kg, respectively. Samples with mycotoxin levels above the limit of detection were diluted and additional dilution factor in the interpretation of the results.

**Preparation of desmodium root extracts**

Fresh roots of *D. intortum* and *D. uncinatum* were collected from push-pull cropping system demonstration plots at the International Centre of Insect Physiology and Ecology (icipe) at Thomas Odhiambo Campus Mbita campus. The roots were washed under running tap water, chopped into one-centimeter pieces, followed by surface sterilization in 1.3% sodium hypochlorite and rinsed with distilled water. The roots were air-dried in the laboratory for one week and finely ground in a blender (Mika MNB1001 - Nutriblast Blender, 900W – Black). Two hundred grams of the ground desmodium roots was extracted with 1000 ml of dichloromethane: methanol (1:1 v/v) by soaking and frequently shaking the mixture for 48 hours. The extract was passed through cotton wool and filtered through Whatman No.1 filter paper. The filtrate was concentrated in vacuum rotary evaporator (Stuart, RE400/CO, SA) to 100 ml and the concentrated extract was stored in a refrigerator at 8°C until use.

**Determination of effect of the desmodium root extracts on mycelial growth of toxigenic Aspergillus flavus and Fusarium verticillioides**

Six of each of the aflatoxin and fumonisin producing isolates from push-pull and non-push-pull farms were randomly selected and purified on PDA medium and incubating for 48 hours at 25°C. Spores from seven-day old cultures were used to test the antifungal activity of desmodium root extracts. The ability of the desmodium root extract to inhibit fungal growth was tested using poisoned food technique (Al-Samarrai et al., 2012) with modifications. The crude root extract was mixed with molten PDA cooled to 45°C in the ratio of one ml of methanol extract to 20 ml media (v/v). Control PDA plates were incorporated with methanol. The PDA medium amended with root extracts was aseptically dispensed into sterile Petri-dishes and the plates were point inoculated at the center with spores from seven-day old purified cultures of toxigenic *A. flavus* and *F. verticillioides* and incubated at 25°C. Each treatment was replicated thrice and the experiment was repeated twice. Diameters of the fungal colonies were measured at the second, fourth and sixth day after incubation. Antifungal activity was determined as inhibition of radial mycelial growth of the fungal colonies by calculating the percentage reduction in fungal radial growth as follows:

\[
\text{% Inhibition} = \frac{(d_c - d_t)}{d_c} \times 100
\]

Where *dc* was the diameter of the fungal colony without extract, and *dt* was diameter of fungal colony in the plates with extract

**Determination of effect of desmodium root extracts on Aspergillus flavus and Fusarium verticillioides spore germination and germ tube growth**

Spores of *A. flavus* and *F. verticillioides* cultured on PDA were harvested from seven-day old cultures by adding five ml of 0.1% Tween 80 (v/v) solution into the plates. The spore suspension was serially diluted to 10⁻⁷/ml spore concentration and 100 µl of the diluted spore suspension was spread on PDA plates amended with crude desmodium root extract in the ratio of one ml extract to 20 ml media (v/v). Control plates contained PDA amended with methanol only and each treatment was replicated thrice. The media was allowed to set and the methanol to evaporate in the biosafety cabinet overnight. The plates were incubated at 25°C and observations made four, six, eight and ten hours after plating. A drop of lactophenol cotton blue dye was placed on the surface of the inoculated media and the number of germinated spores counted by observation under a compound microscope at ×400 magnification in 10 fields of view. The length of germ tube of the germinated spores was measured using a calibrated ocular micrometer. The hyphae and germ tubes were observed for growth and presence of deformities. The proportion of germinated spores was calculated using the formula

\[
\text{Germination} \% = \frac{\text{No.of germinated spores}}{\text{Total number of spores}} \times 100
\]

Percentage inhibition of spore germination was calculated using the formula: 1946
Germination inhibition (%) = \frac{\text{Germinated spores without extract} - \text{Germinated spores with extract}}{\text{Germinated spores without extract}} \times 100

Percentage inhibition of germ tube elongation was calculated using the formula:
\text{Inhibition of elongation (%) =} \frac{\text{Germination length without extract} - \text{Germination length with extract}}{\text{Germination length without extract}} \times 100

### Data analyses

Data on aflatoxin and fumonisin levels produced by test isolates was described using measures of central tendency in descriptive statistics procedure in SPSS version 22 (IBM Corp, 2013, New York, USA). Aflatoxin levels were categorized into below limit of detection, less than 10 and greater than 10, and association among the levels produced by isolate and cropping system from which they were isolated was determined by Chi-square test. Means of colony diameter were compared between treatments at different incubation time by Analysis of Variance (ANOVA) tested at 5% probability in R Studio version 3.5.3. The inhibition of spore germination, germ tube elongation and aerial growth of the fungal colonies were calculated as a percentage and compared between treatments after different hours of incubation by ANOVA. Percentage data that was not normally distributed was transformed before analysis by first changing the percentages to proportions and then using the arcsine transformation.

### Conclusions

Extracts of both *D. intortum* and *D. uncinatum* inhibited germination and radial growth of toxigenic *A. flavus* and *F. verticillioides*. This implies that desmodium root exudates have potential to reduce the inocula of *A. flavus* and *F. verticillioides* in the soil, thereby preventing proliferation of the pathogens in push-pull cropping systems. The effectiveness of the root extracts was significantly higher against *F. verticillioides* than *A. flavus*, suggesting that desmodium would be more active in reducing *Fusarium* ear rots on maize. Through reduction of fungal inocula in the soil, the populations of the fungi infecting maize during growth would be reduced and consequently the levels of aflatoxin and fumonisin in maize at harvest. The findings of this study suggest that suppression of fungal inoculum in the soil is one of the mechanisms by which push-pull cropping system reduces the occurrence of maize ear rots and the associated mycotoxins. Further research on the determining the identity of the specific active compounds in the desmodium root extracts and their effect on beneficial microorganisms in soils under push-pull cropping systems is recommended.

### Acknowledgments

We thank the German Academic Exchange Service (DAAD) for providing NKN with a doctoral scholarship through the ARRPIS-DAAD scholarship programme. We gratefully acknowledge the financial support for this research by the following organizations and agencies: UK’s Department for International Development (DFID); European Union (EU); Biovision Foundation, Swedish International Development Cooperation Agency (Sida); the Swiss Agency for Development and Cooperation (SDC); Norwegian Agency for Development Cooperation (Norad), Federal Democratic Republic of Ethiopia; and the Kenyan Government. The views expressed herein do not necessarily reflect the official opinion of the donors.

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