



## Using *Trichoderma* Species in Combination with Cattle Dung as Soil Amendment Improves Yield and Reduces Pre-Harvest Aflatoxin Contamination in Groundnut

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### ABSTRACT

Aflatoxin contamination in groundnut constitutes a serious risk to human and animal health. The efficacy of combining five *Trichoderma* species and cattle dung in the management of aflatoxin-producing *Aspergillus flavus* under laboratory and field conditions was evaluated. *Trichoderma asperellum*, *T. hamatum*, *T. viride*, *T. harzianum* and *T. pseudokoningii* were bioassayed against *A. flavus* *in vitro*, while they were applied in combination with cattle dung in the field experiment. A randomized complete block design was used for field treatments with four replications. *Trichoderma* containing  $2.3 \times 10^8$  cfu/g was applied as seed treatment, while soil application was done using 2.5 kg/ha of sorghum grains as carrier and 5 t/ha cattle dung. Aflatoxin concentration was quantified using high-performance thin-layer chromatography. There was no significant difference among the treatments in the laboratory experiment. However, *T. harzianum* was the most effective with 72.3% mycelial inhibition of *A. flavus*. Seed treatment with combination of *T. harzianum* and cattle dung was most effective in reducing *A. flavus* soil population to  $1.1 \times 10^3$  cfu/g, while the combination of *T. hamatum* and cattle dung produced the highest pod yield of 2.7 t/ha, which was significantly higher than carbendazim fungicide. Therefore, integrated approach is recommended in managing aflatoxin contamination in pre-harvest groundnut.

### INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is a legume crop grown mainly for its edible seeds by both small-scale and commercial farmers in the tropics and subtropics. It has the potential to fix atmospheric nitrogen which improves the fertility of the soil and makes it a vital crop in the rotational cropping system. This capacity to fix nitrogen implies that groundnut requires less nitrogen-containing fertilizer. It contains about 25-30% protein and a significant amount of B vitamins and minerals such as calcium, iron, phosphorus, and potassium (Chiuraise, Yobo, & Laing, 2015). Pre-harvest infection of groundnut by *Aspergillus flavus* and aflatoxin contamination has been positively correlated with drought stress, especially towards the end of the cropping season in the last four weeks before the maturity of the crop (Guo, Chen, Lee, & Scully, 2008). The undesirable effects of

climate change in sub-Saharan Africa have led to the exposure of groundnut to late-season drought stress (Bankole, Schollenberger, & Drochner, 2006), which favours the production of aflatoxins by the toxigenic strains of *A. flavus*

Aflatoxins are toxic metabolites that are produced by *Aspergillus* section *Flavii* on many crop produce, especially cereals and legume kernels (Gachomo & Kotchoni, 2008; Parimi, Kotamraju, & Sudini, 2018). *Aspergillus flavus* produces aflatoxins B1 and B2, whereas *A. parasiticus* produces G1 and G2. Aflatoxin B1 is carcinogenic and the most potent toxin produced by *A. flavus* which is a saprophytic soil-borne fungus that infects and contaminates food crops in the field and after harvest (Guru Prasad, 2014). Aflatoxin contamination can occur before and after harvest and under poor storage conditions. It constitutes a serious constraint to international trade on

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agricultural produce due to the strict regulatory measures on aflatoxin contamination by importing countries (Shifa, Tasneem, Gopalakrishnan, & Velazhahan, 2016). About 25% of the world's food crops are contaminated by mycotoxins, of which the most dangerous is aflatoxin (Boutrif, 1997). Therefore, aflatoxins are a major threat to the marketing of plants and their products worldwide. The contamination of agricultural produce by aflatoxin has attracted world attention due to the adverse effects on human and animal health (Amaike & Keller, 2011; Aristil, Venturini, Maddalena, Toffolatti, & Spada, 2020). The use of susceptible groundnut cultivars, the occurrence of drought in the late season, *A. flavus* soil concentration and high soil temperature are some of the important predisposing factors to aflatoxin production (Hamidou, Rathore, Waliyar, & Vadez, 2014; Sudini et al., 2015; Waliyar et al., 2015). The control of the toxigenic strains of *Aspergillus flavus* will significantly increase the quality of groundnut production; improve human and animal nutrition, and better health. This will further boost the international market for groundnut and its allied products with a corresponding increase in revenue for the exporting countries.

Chemical control is costly and as a result, requisite fungicides are out of reach of poor resource farmers in addition to toxicological issues. Organic manure is cheap and constitutes a viable strategy of replenishing soil nutrients and increasing groundnut production (Parimi, Kotamraju, & Sudini, 2018). About 90% total aflatoxin contamination has been eradicated due to the application of farmyard manure under pre-harvest conditions (Waliyar, Osiru, Sudini, & Njoroge, 2013). The use of organic fertilizers has significantly reduced the incidence and severity of several plant diseases through the enhancement of resistance, alteration in pathogenicity of plant pathogens and disease escape. *Trichoderma* species have been widely used by several authors in the biological control of plant diseases (Dania, 2019; Kumar et al., 2014; Woo et al., 2014). Biological control constitutes an important constituent of integrated management of plant diseases. It is cheap, easily formulated, and makes use of resident antagonists in the rhizosphere. This study evaluated the efficacy of combining *Trichoderma* species and cattle dung as soil amendment in the integrated management of aflatoxin contamination in cultivated groundnut.

## MATERIALS AND METHODS

### Experimental Location and Source of Materials

The research was conducted at the Plant Pathology Laboratory and crop field of the Department of Crop Protection and Environmental Biology (CPEB), respectively at the University of Ibadan, Nigeria between June and November, 2019. Aflatoxin analysis was carried out at the pathology laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan. The five *Trichoderma* species, *T. asperellum*, *T. hamatum*, *T. viride*, *T. harzianum* and *T. pseudokoningii* used in this study were obtained from the mycological culture collection of the pathology unit of CPEB, which had earlier been identified and shown inhibitory action against post-harvest pathogens of sweet potato in a previous study (Dania, 2019). The susceptible groundnut cultivar GN 23B and synthetic carbendazim fungicide were purchased from agro tropical and agrochemical stores, respectively, while cattle dung was obtained from the cattle rearing unit of the University of Ibadan Teaching and Research Farm.

### Isolation and Morphological Identification of *Aspergillus flavus*

*Aspergillus flavus* was isolated from the groundnut rhizosphere in the experimental field using the serial dilution method. Topsoil was collected from six representative points and bulked into a composite sample. Thereafter, 1 g of the soil was dissolved in a test tube containing 9 ml of sterile distilled water to obtain a stock solution ( $10^{-1}$ ) from which serial dilutions were made by dispensing 1 ml of the preceding medium into 9 ml of sterile distilled water contained in test tubes labelled as ( $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$ ). An aliquot of 0.1 ml of the dilution  $10^{-4}$  to  $10^{-7}$  was dispensed on prepared acidified potato dextrose agar (APDA) and incubated at 27-29°C for 4 days to allow growth of fungal species. *Aspergillus flavus* colonies were purified on APDA. Microscopic characteristics including the presence of conidiophores, vesicles, phialides, and conidia of isolates were assessed. Cultural features such as colony growth and texture colour were also examined. Prepared slides were examined under a light microscope using a 40× magnification and identification was done according to taxonomic manual and keys described by Diba, Kordbacheh, Mirhendi, Rezaie, & Mahmoudi (2007), Gams, Christensen, Onions, Pitt, & Samson (1986), and Klich (2002).

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### Laboratory Assay of *Trichoderma* Species against Mycelial Growth of *Aspergillus flavus*

Bioassay of *Trichoderma* species against mycelial growth was evaluated using the dual culture procedure. Each *Trichoderma* species was inoculated at four equidistant points at a 4.5 cm distance from the center of the Petri dishes on APDA. Thereafter, mycelial disk of *A. flavus* was inoculated at the center using a 3 mm-cork borer. The *in vitro* experiment was a completely randomized design with four replicates. Petri dishes that were inoculated with *A. flavus* only served as the control, while carbendazim 50WP at 0.25 g/l was the positive check. Inoculated Petri dishes were incubated at 27-29°C for five days. Mycelial growth of *A. flavus* toward the *Trichoderma* species was measured daily and the percent growth inhibition calculated according to Thathana, Murage, Abia, & Pillay (2017).

### Formulation and Field Delivery of *Trichoderma* Species

*Trichoderma* species obtained as previously described were purified using a single spore technique and maintained on APDA at room temperature in McCartney bottles. The biological control agents (BCAs) were mass-produced using the liquid fermentation technique (Kumar, Thakur, & Rani, 2014). They were grown in 250 ml Erlenmeyer flasks containing 100 ml of sterile potato dextrose broth in a rotary shaker at 150 rpm for 72 h and filtered through Whatman filter paper No 1. *Trichoderma* conidia concentration was adjusted to  $2.3 \times 10^8$  cfu/g and mixed with talc powder (1 kg of sterile talc powder, 15 g of CaCO<sub>3</sub> and 10 g carboxymethyl cellulose) in a ratio of 1:2. The mixture was dried under the shade to 10% moisture content and applied as seed coating at 10 g/kg of groundnut seeds. Seeds sprinkled with sterile distilled water served as the control, while the positive check comprised seeds treated with carbendazim at the rate of 2 g/kg (Shifa, Tasneem, Gopalakrishnan, & Velazhahan, 2016). Fresh cattle dung collected as described was cured by air drying at room temperature for 10 days. This was applied at 1 week before sowing at the rate of 5 t/ha which was equivalent to 1.50 kg N and 20 g per plant and mixed thoroughly with the soil.

Sorghum seeds that served as inoculum carrier in field application of the BCAs were soaked overnight and the water decanted after 24 h. Thereafter, jam bottles of 200 ml capacity were filled

with 100 g of moist sorghum grains and sealed with aluminum foil paper fitted with rubber bands. The bottles were sterilized at a pressure of 1.05 kg/cm<sup>2</sup> for 1 h to eliminate inherent seed-borne inocula. The grains were allowed to cool to room temperature before being inoculated with a 3 mm-mycelial disc taken from the edge of a 5-day-old culture of the BCAs. The inoculated bottles were incubated at room temperature for 10-15 days depending on when the grains were completely ramified.

### Effect of *Trichoderma* Species and Cattle Dung on Growth, Yield and Aflatoxin Contamination in Groundnut Under Natural Conditions

Based on the promising results obtained in the *in vitro* trial, the BCAs were further evaluated for their biocontrol ability in combination with cattle dung for management of pre-harvest aflatoxin contamination in groundnut under field conditions. The experiment was conducted under rainfed conditions between June and November, 2019 at the crop field of the Department of Crop Protection and Environmental Biology, the University of Ibadan using a susceptible groundnut cultivar GN 23B in naturally infected soil. Groundnut seeds were treated with the talc formulation of *Trichoderma* species at the rate of 10 g/kg as previously described before planting. The field experiment was a randomized complete block design with four replicate blocks. Each plot size was 5 × 3 m with a spacing of 70 cm between rows and 20 cm between plants. The *Trichoderma*-impregnated sorghum seeds were broadcast along the furrows at six weeks after sowing (Le et al., 2019). The control consisted of plants that were untreated, while treatment with carbendazim 50WP served as a positive check. Cattle dung was prepared as previously described and applied at 5 t/ha (Waliyar, Osiru, Sudini, & Njoroge, 2013). The manure was worked into the soil using a hoe before planting, while manual weeding was done as and when required. The treatments are presented in Table 1. Pods were harvested by digging with a hoe and dried to 10% moisture content using Gallenkamp oven at 45°C for 3 days.

### Determination of *Aspergillus flavus* Soil Population, Seed Infection and Pre-Harvest Aflatoxin Contamination in Groundnut

The initial *Aspergillus flavus* population in the soil was determined before planting and subsequently at 80, 90, 100, and 120 days after sowing (DAS) and final spore count at harvest using

a 10-fold serial dilution. Soil samples were collected from each treatment plot at 1-10 cm depth with the aid of a hand trowel. One gram of soil was added to 10 ml of sterile distilled water in a 20 ml test tube and shaken vigorously to allow the soil components dissolve properly. Spores were quantified using a hemocytometer and concentration was adjusted to  $2.1 \times 10^6$  conidia/ml. An aliquot of 1 ml of the dilution was dispensed into sterilized Petri dishes and mixed with molten APDA. The inoculated Petri dishes were incubated at room temperature for 4-6 days and developing colonies were enumerated. Harvested pods were air-dried under shade for one week. The dried pods were shelled and nine seeds were placed aseptically on malt extract agar amended with 10% NaCl in sterile Petri dishes to enhance sporulation and incubated at 28-29°C for one week. Spore count per ml was done using a hemocytometer. A sample of 3 plants was taken randomly from the inner rows of each treatment at 80, 90, 100, and 120 DAS to determine *A. flavus* infectivity in pods. Data were collected on plant height, number of branches per plant, number of pods per plant, plant fresh weight, plant dry weight, and seeds per pod.

**Aflatoxin Analysis and Quantification**

Groundnut samples were ground with an electric coffee miller model 220-150 USA and 20 g of the sample was weighed for extraction. It was blended with 100 ml of 80% methanol for 3 minutes using a waring blender and poured into a 250 ml Pyrex conical flask sealed with paraffin. The sample was mixed

using an orbit shaker at 400 rpm for 30 minutes and filtered into a clean conical flask using quantitative Whatman filter paper No. 1. The filtrate was mixed with 40 ml of 10% NaCl and 25 ml of hexane in a separating flask and shaken vigorously. The extract (bottom phase) was drained into a 250 ml conical flask, while the filtrate was poured into the separating flask and 25 ml of dichloromethane was added. The mixture was thoroughly shaken and then allowed to separate to the top and bottom phases. The bottom phase was drained through a bed of 20 g anhydrous sodium sulfate into a 150 ml white plastic beaker. An aliquot of 10 ml of dichloromethane was added to the remaining mixture in the separating flask. The extract obtained from this mixture was drained through a bed of 20 g anhydrous sodium sulfate into a 150 ml white plastic beaker and allowed to dry overnight (IITA, 2018). High-performance thin-layer chromatography (HPTLC) was employed for the quantification assay of extracts (Wacoo, Wendi, Vuzi, & Hawumba, 2014). The dried extract was reconstituted with 1 ml dichloromethane and vortexed to homogenize the mixture (Patil et al., 2013). Four micro liters of each sample extract was carefully spotted as marked on the plate and an air-dried plate was developed in a solution of diethyl ether, methanol and distilled water in ratio 96:3:1. Each isolate was qualitatively scored and compared with the standards, depending on the intensity of the fluorescence. The developed plates were viewed and read under the ultraviolet light-box (wavelength of 365 nm) using a Camag TLC scanner.

**Table 1.** List of treatments used in the field experiment

| Treatment | Description   |
|-----------|---|
| 1         | Plants inoculated with <i>A. flavus</i> and treated with <i>T. asperellum</i>                   |
| 2         | Plants inoculated with <i>A. flavus</i> and treated with <i>T. hamatum</i>                      |
| 3         | Plants inoculated with <i>A. flavus</i> and treated with <i>T. viride</i>                       |
| 4         | Plants inoculated with <i>A. flavus</i> and treated with <i>T. harzianum</i>                    |
| 5         | Plants inoculated with <i>A. flavus</i> and treated with <i>T. pseudokoningii</i>               |
| 6         | Plants inoculated with <i>A. flavus</i> and treated with <i>T. asperellum</i> + cattle dung     |
| 7         | Plants inoculated with <i>A. flavus</i> and treated with <i>T. hamatum</i> + cattle dung        |
| 8         | Plants inoculated with <i>A. flavus</i> and treated with <i>T. viride</i> + cattle dung         |
| 9         | Plants inoculated with <i>A. flavus</i> and treated with <i>T. harzianum</i> + cattle dung      |
| 10        | Plants inoculated with <i>A. flavus</i> and treated with <i>T. pseudokoningii</i> + cattle dung |
| 11        | Plants inoculated with <i>A. flavus</i> and treated with cattle dung                            |
| 12        | Plants inoculated with <i>A. flavus</i> and treated with carbendazim                            |
| 13        | Untreated control   |



### Data Analysis

The *in vitro* experiment was laid out in a completely randomized design, while the field trial was a randomized complete block design and both experiments were replicated thrice. Data were subjected to analysis of variance (ANOVA) using SAS (2009) ver. 9.2 and means were separated with the Duncan's Multiple Range Test (DMRT) at 5% level of significance (Gomez & Gomez, 1984).

## RESULTS AND DISCUSSION

### Isolation and Morphological Identification of *Aspergillus flavus*

Cultural characteristics of *A. flavus* showed initial white mycelial colour, which later turned olive green, while dark green conidia dominated colony appearance (Fig. 1a). Microscopic characteristics of *A. flavus* showed conidiophores bearing vesicles that were colourless, thick-walled, and coarsely textured (Fig. 1b). The phialides were borne on uniseriate cells, which were directly attached to the vesicles. The conidia were globose with thin walls, which were slightly roughened. These distinguishing features used in the identification of *A. flavus* are consistent with previous reports of several authors that colony colour, diameter, production of exudates, the structure of conidiophore, vesicle, phialides and conidia are employed in morphological identification of *A. flavus* (McClenny, 2005; Diba, Kordbacheh, Mirhendi, Rezaie, & Mahmoudi, 2007; Shifa, Tasneem, Gopalakrishnan, & Velazhahan, 2016). Admittedly, morphological identification may be less effective than the molecular approach, the affordability of requisite diagnostic tools and technical

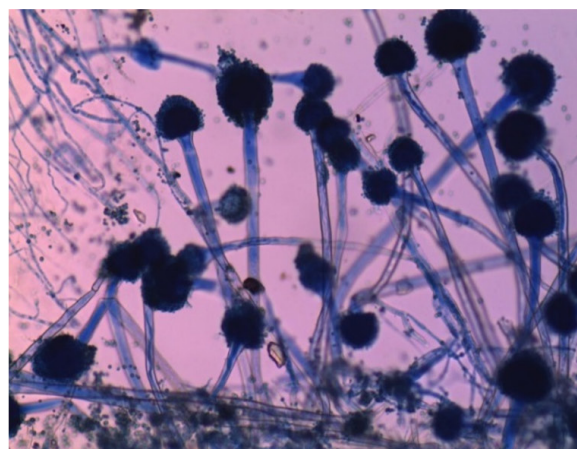
know-how, especially in the developing countries still make the former a relevant identification tool for fungi.

### Laboratory Assay of *Trichoderma* Species against Mycelial Growth of *Aspergillus flavus*

The BCAs were statistically significant in their efficacy against *A. flavus* in the *in vitro* trial ( $F = 3.724$ ,  $p < 0.028$ ). *Trichoderma asperellum* had the highest mycelial inhibition at 2 days after inoculation (DAI) with a 46.3% reduction in the growth of the test pathogen, which was significantly higher than other treatments including the positive check (Table 2). The control treatment that was untreated recorded the highest mycelial growth of 13.6 cm with no inhibition at 2 DAI. All the BCAs significantly reduced the growth of *A. flavus* relative to the control with inhibition that varied between 61.6 and 72.3%. *Trichoderma harzianum* was, however, the most effective with 72.3% mycelial inhibition of *A. flavus* at 5 DAI. This was closely followed by treatment with carbendazim fungicide (71.8%) inhibition. The control had complete mycelial growth with no inhibition. *In vitro* antagonism of *Trichoderma* species have been reported by several authors. Karthikeyan, Sankaralingam, & Nakkeeran (2006) had reported the inhibitory effect of *T. viride* strains and *T. harzianum* on mycelial growth and reduction in sclerotia size of *Macrophomina phaseolina* causing root rot disease in groundnut. Similarly, Dania (2019) found that *Trichoderma asperellum*, *T. hamatum* and *T. pseudokoningii* also effectively reduced the *in vitro* growth of post-harvest rot pathogens of sweet potato tubers.



(a)



(b)

Fig. 1. (a) Culture and (b) micrograph of *Aspergillus flavus*

The efficacy of *Trichoderma* isolates had been reported against soil-borne pathogens (Redda et al., 2018; Tomaha, Abd Alamer, Li, & Zhang, 2020). The reduced mycelial growth of *A. flavus* could be attributed to the production of volatile organic compounds (VOCs) by the BCAs. Many existing research findings have implicated *Trichoderma* species in the production of toxic metabolites and antibiotics in dixerenic culture (Kumar et al., 2014; Moya, Girotti, Toledo, & Sisterna, 2018) and *Trichoderma* species are well endowed with several biocontrol mechanisms such as competition,

hyperparasitism and antibiosis (Sekhar, Ahammed, Prasad, & Devi, 2017; Shang, Liu, & Xua, 2020).

Many atoxigenic species of *A. flavus* are also known to have biocontrol potential and competitive capacity to inhibit the toxigenic strains that are naturally present in the groundnut rhizosphere causing aflatoxin contamination (Divakara, Aiyaz, Hariprasad, Nayaka, & Niranjana, 2014; Woo et al., 2014). The competitive ability of *A. flavus*, therefore, explains the reason why the *Trichoderma* species applied singly may be unable to achieve absolute inhibition of the pathogen either *in vitro* or in the field.

**Table 2.** Efficacy of *Trichoderma* species against mycelial growth of *Aspergillus flavus*

| Treatment                         | Mycelial growth (mm) |        |        |        | Percent inhibition |        |        |        |
|-----------------------------------|----------------------|--------|--------|--------|--------------------|--------|--------|--------|
|                                   | 2DAI                 | 3DAI   | 4DAI   | 5DAI   | 2DAI               | 3DAI   | 4DAI   | 5DAI   |
| <i>Trichoderma asperellum</i>     | 7.3ab                | 13.5ab | 16.2ab | 17.3ab | 46.3a              | 58.3b  | 61.2b  | 61.6ab |
| <i>Trichoderma hamatum</i>        | 7.8ab                | 12.2ab | 15.6ab | 18.9ab | 42.1ab             | 62.3ab | 63.3b  | 63.3ab |
| <i>Trichoderma viride</i>         | 10.2ab               | 13.9ab | 15.8ab | 16.4ab | 25.0bc             | 57.1b  | 62.3b  | 63.5ab |
| <i>Trichoderma harzianum</i>      | 8.7ab                | 10.5ab | 12.1b  | 12.3b  | 36.0bc             | 67.6a  | 71.0a  | 72.3a  |
| <i>Trichoderma pseudokoningii</i> | 10.7ab               | 12.3ab | 14.1ab | 15.3ab | 21.3c              | 62.0ab | 66.2ab | 66.2ab |
| Carbendazim                       | 8.3ab                | 11.4ab | 11.8b  | 11.4b  | 39.0b              | 64.8ab | 71.8a  | 71.8a  |
| Control                           | 13.6a                | 32.4a  | 41.8a  | 45.0a  | 0cd                | 0bc    | 0bc    | 0b     |

Remarks: Values are means of three replicates. Means with same letter along the column are not significantly different ( $p < 0.05$ ), using Duncan Multiple Range Test (DMRT). DAI = Days after inoculation.

**Table 3.** Effect of *Trichoderma* spp. and poultry manure on growth performance of groundnut inoculated with *A. flavus*

| Treatment                                    | Plant height (cm) | Number of branches | Plant width (cm) | Fresh plant weight (g) | Dry plant weight (g) |
|--|-------------------|--------------------|------------------|------------------------|----------------------|
| T1 = <i>T. asperellum</i>                    | 27.4b             | 10.2a              | 24.6ab           | 129.4c                 | 53.1cd               |
| T2 = <i>T. hamatum</i>                       | 32.7ab            | 10.8a              | 22.1ab           | 132.8c                 | 61.2bc               |
| T3 = <i>T. viride</i>                        | 33.6ab            | 12.3a              | 27.2a            | 140.3c                 | 58.2c                |
| T4 = <i>T. harzianum</i>                     | 30.7ab            | 11.1a              | 18.4b            | 148.7b                 | 55.5c                |
| T5 = <i>T. pseudokoningii</i>                | 33.9ab            | 13.5a              | 27.2a            | 151.3b                 | 64.4b                |
| T6 = <i>T. asperellum</i> + cattle dung      | 36.9a             | 12.2a              | 21.1ab           | 133.4bc                | 60.7bc               |
| T7 = <i>T. hamatum</i> + cattle dung         | 29.6ab            | 9.8a               | 22.7ab           | 148.4b                 | 73.2a                |
| T8 = <i>T. viride</i> + cattle dung          | 33.7ab            | 14.5a              | 26.8a            | 154.1ab                | 68.3ab               |
| T9 = <i>T. harzianum</i> + cattle dung       | 28.3ab            | 13.7a              | 19.2b            | 158.4a                 | 74.3a                |
| T10 = <i>T. pseudokoningii</i> + cattle dung | 37.6a             | 13.1a              | 23.3ab           | 154.8ab                | 75.6a                |
| T11 = Cattle dung                            | 31.1ab            | 11.4a              | 24.5ab           | 160.3a                 | 69.1ab               |
| T12 = Carbendazim                            | 30.3ab            | 12.3a              | 21.6ab           | 155.5ab                | 73.4a                |
| T13 = Control                                | 35.6a             | 12.6a              | 24.4ab           | 150.3b                 | 65.7b                |

Remarks: Values are means of three replicates. Means with same letter along the column are not significantly different ( $p < 0.05$ ), using Duncan Multiple Range Test (DMRT).

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### **Effect of *Trichoderma* Species and Cattle Dung on Growth Performance of Groundnut in Field Naturally Infected with *Aspergillus flavus***

Plants that were treated with a combination of *T. pseudokoningii* and cattle dung reached the highest height of 37.6 cm, which was not significantly ( $p > 0.05$ ) higher than the untreated control (Table 3). Also, plant width among the treatments was not significantly higher than the control treatment. The application of *T. harzianum* in combination with cattle dung produced the highest fresh weight of 154 g per plant, which was significantly higher than the other treatments, including the control and positive check ( $F = 5.77$ ,  $p = 0.038$ ). The efficacy of the single application of the BCAs was generally lower on the growth parameters of the plants compared to their combined application with the cattle dung amendment.

Groundnut has been reported as a compatible host crop with endophytic *Trichoderma*. The rapid colonization of roots by *Trichoderma* spp. is an indication of the BCAs inherent ability of prolonged protection of the plant from *A. flavus* infection (Hermosa, Viterbo, Chet, & Monte, 2012; Khalid, 2017). Siddiquee, Cheong, Taslima, Kausar, & Hasan (2012) found that root colonization by *Trichoderma*, promotes the absorption of essential plant nutrients, which stimulates the production of plant hormones that enhance plant growth. Kifle, Yobo, & Laing (2017) had shown a kd strain of *T. harzianum* to be mycoparasitic on *A. flavus* under scanned electron microscopy and its capacity to penetrate and degrade the fungal mycelia.

### **Determination of *Aspergillus flavus* Soil Population, Seed Infection and Pre-Harvest Aflatoxin Contamination in Groundnut**

Pre-field soil test showed that all the treatment plots were infected with *Aspergillus flavus* under natural conditions with initial soil population that varied between  $4.1 \times 10^3$  and  $4.7 \times 10^3$  cfu/g (Table 4). The inoculum population of the treatments was not significantly higher than the control ( $F = 12.66$ ,  $p > 0.078$ ) at the onset of the field experiment in June 2019. However, soil test at 80 days after sowing (DAS) showed that seed treatment and the soil application of *Trichoderma* formulation using sorghum grains as carrier significantly reduced the soil population of *A. flavus* relative to control. The BCAs had undergone rapid proliferation in the soil and significantly inhibited *A. flavus* soil population at 100 to 120 DAS. The untreated control had an inoculum concentration

of  $4.7 \times 10^3$  cfu/g which was not significantly lower than the initial population before sowing ( $F = 13.21$ ,  $p > 0.084$ ) but was significantly higher than other treatments ( $F = 7.855$ ,  $p < 0.033$ ). Seed treatment and soil application of *T. asperellum* significantly reduced soil population of *A. flavus* to  $1.5 \times 10^3$  cfu/g of soil, which was not significantly higher than the other BCAs. However, seed treatment with *T. harzianum* and cattle dung in combination was most effective in the reduction of the pathogen soil population to  $1.1 \times 10^3$  cfu/g. Similarly, the infectivity potential of *A. flavus* and rate of aflatoxin contamination was lowest in treatment with *T. harzianum* and cattle manure amendments. *Aspergillus* infection increased with the length of days after sowing and the concentration of aflatoxin B<sub>1</sub> was significantly higher than B<sub>2</sub> in all treatments. Final soil population of *A. flavus*, infection rate and aflatoxin contamination were significantly higher in the untreated control relative to other treatments. The detection of *A. flavus* and aflatoxin in groundnut seeds that were harvested from uninoculated soil may be attributed to the ubiquitous nature of the pathogen occurring as a saprophyte naturally in many soils (Chiuraise, Yobo, & Laing, 2015). Cultivated groundnut is prone to aflatoxin contamination because of the natural occurrence of several species of *Aspergillus* on farmlands, which are the main source of infection of field crops (Torres, Barros, Palacios, Chulze, & Battilani, 2014; Yang et al., 2020).

Shifa, Tasneem, Gopalakrishnan, & Velazhahan (2016) found that *A. flavus* spores were significantly higher in the groundnut pod rhizosphere than in the adjacent field soil, which reached a peak at maturity and also established a positive correlation between *A. flavus* soil population and aflatoxin concentration in harvested groundnut. However, it was observed in this study that the soil population of *A. flavus*, infectivity rate, and aflatoxin contamination decreased with the crop maturation. This may be attributed to the treatment combinations that were applied which significantly reduced field inoculum at maturation of the groundnut plants, with a low final soil population. Although organic manure inoculates the soil with microorganisms (Gaiotti et al., 2017), the increased microbial activity reduced pod infection by *A. flavus*, which causes aflatoxin contamination in groundnut seeds. Similarly, a 42% reduction in cumulative aflatoxin content has been reported after soil treatment with 2.5 metric t/ha of farmyard manure (Waliyar, Osiru, Sudini, & Njoroge, 2013).

**Table 4.** Determination of *Aspergillus flavus* soil population, seed infection and pre-harvest aflatoxin contamination in groundnut

| Treatment                                    | Soil population of <i>Aspergillus flavus</i> (cfu/g) |                       |                       |                       | Aspergillus <i>flavus</i> infection (%) |                   |                    |                    | Aflatoxin ( $\mu\text{g}/\text{kg}$ ) |                   |
|--|--|-----------------------|-----------------------|-----------------------|---|-------------------|--------------------|--------------------|---------------------------------------|-------------------|
|  | Initial count  | 80 DAS                | 100DAS                | 120 DAS               | Final count                             | 80 DAS            | 100DAS             | 120 DAS            | B1                                    | B2                |
| T1 = <i>Trichoderma asperellum</i>           | 4.4x10 <sup>3a</sup>                                 | 3.8x10 <sup>3b</sup>  | 2.2x10 <sup>3bc</sup> | 1.7x10 <sup>3c</sup>  | 1.5x10 <sup>3b</sup>                    | 2.3 <sup>ab</sup> | 7.6 <sup>c</sup>   | 25.4 <sup>b</sup>  | 24.6 <sup>b</sup>                     | 2.1 <sup>ab</sup> |
| T2 = <i>T. hamatum</i>                       | 4.5x10 <sup>3a</sup>                                 | 4.1x10 <sup>3ab</sup> | 3.4x10 <sup>3ab</sup> | 2.5x10 <sup>3b</sup>  | 1.8x10 <sup>3b</sup>                    | 1.7 <sup>ab</sup> | 14.8 <sup>b</sup>  | 32.9 <sup>ab</sup> | 27.4 <sup>ab</sup>                    | 2.8 <sup>ab</sup> |
| T3 = <i>T. viride</i>                        | 4.3x10 <sup>3a</sup>                                 | 3.5x10 <sup>3b</sup>  | 2.7x10 <sup>3b</sup>  | 2.4x10 <sup>3b</sup>  | 1.7x10 <sup>3b</sup>                    | 2.8 <sup>s</sup>  | 7.1 <sup>c</sup>   | 28.5 <sup>ab</sup> | 23.3 <sup>b</sup>                     | 2.6 <sup>ab</sup> |
| T4 = <i>T. harzianum</i>                     | 4.2x10 <sup>3a</sup>                                 | 3.5x10 <sup>3b</sup>  | 2.6x10 <sup>3b</sup>  | 2.1x10 <sup>3bc</sup> | 1.4x10 <sup>3b</sup>                    | 1.2 <sup>ab</sup> | 5.2 <sup>c</sup>   | 25.7 <sup>b</sup>  | 18.5 <sup>c</sup>                     | 1.5 <sup>ab</sup> |
| T5 = <i>T. pseudokoningii</i>                | 4.1x10 <sup>3a</sup>                                 | 3.7x10 <sup>3b</sup>  | 2.9x10 <sup>3b</sup>  | 2.1x10 <sup>3bc</sup> | 1.7x10 <sup>3b</sup>                    | 1.5 <sup>ab</sup> | 18.8 <sup>ab</sup> | 30.8 <sup>ab</sup> | 20.8 <sup>bc</sup>                    | 2.3 <sup>ab</sup> |
| T6 = <i>T. asperellum</i> + cattle dung      | 4.3x10 <sup>3a</sup>                                 | 3.5x10 <sup>3b</sup>  | 2.9x10 <sup>3b</sup>  | 2.3x10 <sup>3b</sup>  | 1.3x10 <sup>3b</sup>                    | 2.3 <sup>ab</sup> | 10.3 <sup>bc</sup> | 18.8 <sup>c</sup>  | 15.3 <sup>c</sup>                     | 1.3 <sup>ab</sup> |
| T7 = <i>T. hamatum</i> + cattle dung         | 4.3x10 <sup>3a</sup>                                 | 3.3x10 <sup>3b</sup>  | 2.0x10 <sup>3bc</sup> | 1.6x10 <sup>3c</sup>  | 1.4x10 <sup>3b</sup>                    | 1.1 <sup>ab</sup> | 13.7 <sup>b</sup>  | 20.7 <sup>bc</sup> | 16.3 <sup>c</sup>                     | 1.4 <sup>ab</sup> |
| T8 = <i>T. viride</i> + cattle dung          | 4.5x10 <sup>3a</sup>                                 | 3.9x10 <sup>3b</sup>  | 2.5x10 <sup>3b</sup>  | 2.0x10 <sup>3bc</sup> | 1.5x10 <sup>3b</sup>                    | 1.3 <sup>ab</sup> | 12.6 <sup>b</sup>  | 22.8 <sup>bc</sup> | 17.2 <sup>c</sup>                     | 1.3 <sup>ab</sup> |
| T9 = <i>T. harzianum</i> + cattle dung       | 4.7x10 <sup>3a</sup>                                 | 4.2x10 <sup>3ab</sup> | 3.5x10 <sup>3ab</sup> | 2.7x10 <sup>3b</sup>  | 1.1x10 <sup>3bc</sup>                   | 2.0 <sup>ab</sup> | 9.5 <sup>bc</sup>  | 17.9 <sup>c</sup>  | 10.3 <sup>cd</sup>                    | 1.7 <sup>ab</sup> |
| T10 = <i>T. pseudokoningii</i> + cattle dung | 4.1x10 <sup>3a</sup>                                 | 3.8x10 <sup>3</sup>   | 3.1x10 <sup>3ab</sup> | 2.3x10 <sup>3bc</sup> | 1.6x10 <sup>3b</sup>                    | 1.3 <sup>ab</sup> | 13.1 <sup>b</sup>  | 23.7 <sup>bc</sup> | 15.5 <sup>c</sup>                     | 1.8 <sup>ab</sup> |
| T11 = Cattle dung                            | 4.5x10 <sup>3a</sup>                                 | 4.1x10 <sup>3ab</sup> | 3.3x10 <sup>3ab</sup> | 2.5x10 <sup>3b</sup>  | 1.5x10 <sup>3b</sup>                    | 1.8 <sup>ab</sup> | 15.3 <sup>b</sup>  | 22.3 <sup>bc</sup> | 17.3 <sup>c</sup>                     | 1.6 <sup>ab</sup> |
| T12 = Carbendazim                            | 4.4x10 <sup>3a</sup>                                 | 3.8x10 <sup>3b</sup>  | 3.4x10 <sup>3ab</sup> | 3.1x10 <sup>3ab</sup> | 2.7x10 <sup>3ab</sup>                   | 1.0 <sup>ab</sup> | 14.4 <sup>b</sup>  | 24.5 <sup>b</sup>  | 14.5 <sup>c</sup>                     | 1.3 <sup>ab</sup> |
| T13 = Control                                | 4.6x10 <sup>3a</sup>                                 | 4.7x10 <sup>3a</sup>  | 4.8x10 <sup>3a</sup>  | 4.7x10 <sup>3a</sup>  | 4.6x10 <sup>3a</sup>                    | 7.9 <sup>a</sup>  | 39.6 <sup>a</sup>  | 48.4 <sup>a</sup>  | 43.7 <sup>a</sup>                     | 6.3 <sup>a</sup>  |

Remarks: Values are means of three replicates. Means with same letter along the column are not significantly different ( $p < 0.05$ ), using Duncan Multiple Range Test (DMRT). DAS=Days after sowing.



Victor Ohilebo Dania and Stephen Ejike Eze: *Pre-harvest Management of Groundnut Aflatoxin* .....Table 5. Effect of *Trichoderma* species and poultry manure on groundnut yield

| Treatment                                    | Number of pods | Pod weight (g) | Pod length (cm) | 100 seed weight (g) | Seeds per pod | Yield t/ha |
|--|----------------|----------------|-----------------|---------------------|---------------|------------|
| T1 = <i>Trichoderma asperellum</i>           | 10.67ab        | 1.56a          | 3.27a           | 56.11b              | 2.61ab        | 1.03bc     |
| T2 = <i>T. hamatum</i>                       | 11.33ab        | 1.82a          | 3.43a           | 59.01b              | 2.53ab        | 1.3bc      |
| T3 = <i>T. viride</i>                        | 10.22ab        | 1.78a          | 3.43a           | 63.33ab             | 2.78ab        | 1.8b       |
| T4 = <i>T. harzianum</i>                     | 11.33ab        | 1.73a          | 3.47a           | 63.33ab             | 21.60ab       | 1.2bc      |
| T5 = <i>T. pseudokoningii</i>                | 13.05a         | 1.54a          | 3.20a           | 44.50cd             | 2.51ab        | 1.9b       |
| T6 = <i>T. asperellum</i> + cattle dung      | 14.33a         | 2.03a          | 3.67a           | 59.40b              | 3.01a         | 2.2b       |
| T7 = <i>T. hamatum</i> + cattle dung         | 15.67a         | 1.92a          | 3.27a           | 54.50bc             | 3.07a         | 2.8ab      |
| T8 = <i>T. viride</i> + cattle dung          | 13.67a         | 1.19a          | 3.02a           | 57.51b              | 2.93ab        | 1.4bc      |
| T9 = <i>T. harzianum</i> + cattle dung       | 14.02a         | 1.64a          | 2.80a           | 49.53c              | 3.01a         | 2.7ab      |
| T10 = <i>T. pseudokoningii</i> + cattle dung | 15.31a         | 1.35a          | 3.27a           | 58.90b              | 2.80ab        | 3.3a       |
| T11 = Cattle dung                            | 11.42ab        | 1.65a          | 3.33a           | 58.91b              | 2.79ab        | 2.6ab      |
| T12 = Carbazim                               | 10.67ab        | 1.78a          | 3.47a           | 66.14a              | 2.67ab        | 1.9b       |
| T13 = Control                                | 9.67ab         | 1.48a          | 3.13a           | 53.79bc             | 2.72ab        | 1.2bc      |

Remarks: Values are means of three replicates. Means with same letter along the column are not significantly different ( $p < 0.05$ ), using Duncan Multiple Range Test (DMRT)

### Effect of *Trichoderma* Species and Cattle Dung on Groundnut Yield

Treatment with a combination of *T. hamatum* and cattle dung produced the highest number of 15.67 pods per plant (Table 5). This was closely followed by the application of *T. pseudokoningii* and cattle dung with 15.31 pods. There was no significant difference among the treatments in pod weight and length and were not significantly higher than the untreated control ( $F = 17.44, p > 0.061$ ). The highest number of 3.07 pods and the corresponding overall yield of 2.7 t/ha were recorded in treatment with combination of *T. hamatum* and cattle dung, which were significantly higher than the control and the application of the synthetic fungicide alone. Plants that were treated with either carbendazim alone or the untreated control produced the lowest groundnut yield per hectare in the field experiment.

Soil amendment with organic manure helps in the improvement of the fertility of the soil and crop yield. Brady & Weil (2010) reported that the incorporation of cattle dung into the soil promotes crop growth through the supply of available nutrients and the enhancement of nutrient recycling process. The use of cattle dung as compost manure has been associated with the supply of essential plant nutrients, especially potassium and magnesium, and also facilitates an efficient cation exchange capacity (CEC) with ultimate increment in groundnut yield per hectare (Chalwe et al., 2019). A higher CEC promotes water-retaining ability of the soil and nutrient availability to plants by helping to dissolve nutrient elements in soil solution for absorption by plants (McClintock & Diop, 2005).

### CONCLUSION AND SUGGESTION

The laboratory experiment showed that there was no significant difference among the five *Trichoderma* species in their inhibitory effect on *A. flavus*, though *T. harzianum* was most effective. Hence all the *Trichoderma* species were used in the treatment combinations for the integrated management of *A. flavus* and aflatoxin contamination under field conditions. The application of *Trichoderma* species in combination with cattle dung as soil amendment significantly increased the growth and yield performance of groundnut. Treatment with a combined dosage of *T. hamatum* and cattle dung produced the highest overall yield, which was significantly higher than the control and the application of the synthetic fungicide alone.

Seed treatment with *T. harzianum* and cattle dung in combination was most effective in reducing the pathogen soil population. The infectivity potential of *A. flavus* and rate of aflatoxin contamination was lowest in groundnut treated with the combined amendments, which was more effective than the application of synthetic carbendazim. Although the combined application of *Trichoderma* species and cattle dung was largely effective in this study, the regulation of the crop and soil water relations through irrigation, especially during adverse conditions of drought and water stress is strongly recommended, because of the rapid proliferation of *A. flavus* during moisture stress.

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