

Full Length Research Paper

The effect of organic soil amendments on stalk rot of maize caused by *Fusarium verticillioides*

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The effect of 3 organic soil amendments viz., cassava peel (*Manihot esculenta*, Cranz), sawdust (*Gmelina arborea*, Roxb) and leaves of (*Cedrela odorata*, L) on the stalk rot of maize (*Zea mays* L.) caused by *Fusarium verticillioides* was investigated. Fourteen treatments made up of single or combined treatments with pathogenic or non-pathogenic inoculation of concentrations 3:1, 2:2 and 1:3 were added to 15.8 g sterilized soil. Growth parameters data on leaf numbers, stem girth, plant height and leaf area were collected biweekly. All treatments had significant effects on plant heights, number of leaves, leaves areas, stems girths and on disease indices and disease severities of the treated plants compared to controls. Concentration 2 (2:2) had the highest effect on all the growth parameters considered and gave the lowest disease index ($P= 0.05$, $R^2= 0.98$) and disease severity ($P= 0.05$, $R^2= 0.92$) in the treated plants. Plants treated with cassava peels combined with *C odorata* had significantly lowest disease index and severity thus, competing favourably with *F verticillioides*. Severity of stalk rot of maize can reduce significantly in amended soils compared to unamended soils.

Key word: Organic amendment, stalk rot, *F verticillioides*, toothpick inoculation, maize stem, *Gmelina arborea*, *Manihot esculenta*, *Cedrela odorata*.

INTRODUCTION

Maize is one of the most important cereal crops cultivated in Nigeria (Iken and Amusa, 2004). Maize is the most important cereal crop and staple food for about 1.2 billion people (Macauley, 2015). It belongs to the grass family Poaceae. Maize constitutes a major part of diet in Nigeria. It started as a subsistence crop and gradually became a major important crop in the commercial sector

on which many agro based industries depend on for raw materials (Iken and Amusa, 2004). In the last decade it registered as the highest growth rate among all food grain. The total production of maize in 2020 was estimated to be about 12 million metric tons (Doris Dokua Sasu, 2022).

However, the production of maize in the whole world

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including Nigeria is threatened by various constraints including its susceptibility to both pest and diseases that cause pre- and post-harvest losses in Nigeria. Pathogenic infections are considered as one of the most prevalence limiting factors that contribute to reduction in the cultivation of maize. Among the variety of pathogens, *Fusarium* is considered a devastating fungal menace in maize cultivation (Agoda et al., 2011).

Stalk rot of maize is the most destructive disease worldwide and reduced yield by 10.0% (Li et al., 2010) and may increase to 30.0 to 50.0% in serious cases (Yu et al., 2017) caused by *F. verticillioides*. The pathogen has been documented to be a major cause of stalk rot of maize (Akinbode et al., 2014; Christensen and Wilcoxson, 1996; Drepper and Renfro, 1990). *F. verticillioides* infect maize across the world, posing one of the greatest threats of toxin contamination especially fumonisin. The stalk rot caused on maize results in stalk breakage and lodging thereby making harvesting difficult and consequently reduces yield. The fungus *F. verticillioides*, penetrate stalk and root directly or spread systemically in the plant after infection that originated from seed borne inoculum (Akinbode et al., 2014; Olawuyi et al., 2011; Munkvold and Desjardins, 1997).

Organic soil amendments made from plant and animal remains are of more importance than inorganic fertilizer because it consists of relatively stable decomposed materials resulting from accelerated biological degradation of organic matter under controlled aerobic conditions (Epstein, 1997). Several studies have shown that organic amendments can be very effective in controlling diseases caused by pathogens such as *F* spp. (Lewis and Papavizas, 1977; Akanmu et al., 2013), since most subsistence farmers do not have the means to purchase synthetic fungicides.

Several methods have been employed in controlling various plant pathogens including: physical, chemical, biological and cultural methods. Synthetic pesticides are known to be highly efficient and effective in the management of crop diseases (Akanmu et al., 2013); however, the indiscriminate use of fungicides has posed a serious threat to human health and to the ozone layer. There is therefore a need for a more environmentally and human friendly approach of controlling plant diseases.

Application of organic amendments to the soil is emerging as an economically and environmentally acceptable alternative to disposal through landfill because of its agronomic benefits and ability to reduce predisposal to soil borne diseases. The use of organic amendments for the managements of plant disease has been documented to achieve several successes and the level of disease control has been documented to be consistent or predictable to an extent (Akamu et al., 2013). The aim of the study was to determine the effect of selected organic soil amendment on the survival of *F. verticillioides* in maize stalk and maize production, with regard to combination of amendments.

MATERIALS AND METHODS

Experimental site

The pot experiment was conducted in the screen house of the Department of Botany, University of Ibadan, Oyo state, Nigeria.

Source of materials used

Maize seeds, (genotype DMR-LSR-Y) were obtained from the Institute of Agricultural Research and Training (IAR and T), Ibadan.

Source of botanical samples

Fresh cassava peels were collected in bags from a cassava processing center at the outskirts of University of Ibadan in Agbowo area of Ibadan. Sawdust of *Gmelina arborea* were collected from a major sawmill at Bodija in the area of Ibadan, while fresh leaves of *Cedrela odorata* were collected from the botanical garden of the University. All materials were dried at room temperature for 2 to 3 weeks, and then milled to fine particles, with a 2 mm sieve. The infected stalks of maize were collected from the International Institute of Tropical Agriculture, Moniya, Ibadan, Oyo state Nigeria.

Isolation and identification of pathogen

The infected stalk samples were cut into small pieces up to about 1.5 to 2 cm and surface sterilized in a mixture of 1% sodium hypochlorite and placed on potato dextrose agar (PDA) media containing anti-bacterial (lactic acid) drops for 3 to 5 days in an incubator at a temperature of $30 \pm 1^\circ\text{C}$. The colonies of observed fungal growth were sub-cultured on agar media till pure cultures of suspected *F. verticillioides* isolates were obtained. The identification and taxonomic classification was aided by the use of *Fusarium* identification manuals "Fuskey" Seifert (1996) and pictorial atlas for identification of fungi by Watanabe (2002) for description of colony morphology.

Preparation of fungal spores' count of *F. verticillioides*

The mycelia growth of 5 days old culture of *F. verticillioides* were harvested by rinsing with sterile distilled water into a sterile bottle and the solution was sieved using a double folded cheese cloth to allow the passage of fungal spores which were later counted using the haemocytometer (BLAUBRAND: BRT17810-1EA) to approximately 3×10^5 spores/ml.

Screen house experiment

Top soil collected from the nursery of the Botany Department, University of Ibadan was sieved and sterilized using an electric soil sterilizer and left to cool. Six kilogram of soil were placed in several 15x11 cm polypots and 15.8 g of various treatment were added, two weeks prior planting in other to decompose, before the test crop (DMR-LSR-Y) was planted.

Experimental design

The pot experiment was arranged in a Completely Randomized Design (CRD) with three replications. The individual and combined effects of treatments were observed. The treatments consisted of:



Figure 1a. Inoculation of treated toothpick into the internode of the maize stalk.
Source: Authors

Treatment 1= cassava peels + *F.verticillioides*
 Treatment 2 = sawdust + *F.verticillioides*
 Treatment 3 = *Cedrela odorata* + *F.verticillioides*
 Treatment 4 = cassava peels + sawdust + *F.verticillioides*
 Treatment 5= cassava peels + *Cedrela odorata* + *F.verticillioides*
 Treatment 6= sawdust + *Cedrela odorata* + *F.verticillioides*
 Treatment 7 (Control 1) = *F.verticillioides*
 Treatment 8 (Control 2) = Control.
 Each of the sawdust and botanical treatments were combined in the ratio 3:1 (75/25), 2:2 (50/50) and 1:3 (25/75).

Planting and inoculation of maize

Maize seeds were planted in organic amended soil, three seeds per pot. Adequate management practices such as wetting, thinning and weeding were carried out. Maize plants were inoculated at tasseling stage (7 weeks after planting) with *F. verticillioides* using toothpick method (Figure 1a) as modified by Drepper and Renfro (1990); Hameed et al., (1997); Sobowale (2011).

Disease incidence rating and analysis

Fourteen weeks after planting (7weeks after inoculation) maize stems were observed for rot formation. The incidence and severity of disease were determined around the inserted toothpicks at harvest (7weeks after inoculation) as shown in Figure 1b. This was achieved by centrally splitting the stalk lengthwise and recording the extent of spread of rot on a modified form of the Hooker's scale (Iken and Amusa, 2004) which indicates the percentage of infection in the inoculated internode, using the scale below:

1 = 0 to 4% of internode rotten.
 2 = 5 to 25% of internode rotten.

3= 26 to 50% of internode rotten.
 4 = 51 to 75% of internode rotten.
 5 = 76 to 100% of internode rotten as modified by (Sobowale, 2011).

Grain yield was also recorded seven weeks after inoculation (14WAP); yield was estimated based on weight of grain at 15%moisture. Disease data on 0 to 5 scale were transformed to percentages for statistical analysis as follows (Hameed et al., 1997):

$$\text{Disease index} = \frac{\sum \text{Disease rating in plants examined}}{\text{Total number}}$$

$$\text{Disease severity}\% = \frac{\text{Disease index}}{5} \times 100$$

Data collection and analysis

Data on leaf number, plant height (cm), stem girth (mm) and leaf area (cm²) were obtained from two weeks after planting on a weekly basis till twelve weeks after planting. The results obtained were subjected to Analysis of Variance (ANOVA) using SAS package (1993) version and means were separated with Duncan Multiple Range Test (DMRT) at p=0.05

RESULTS

The mean comparisons of the treatments on growth parameters of the treated maize plants gave significant

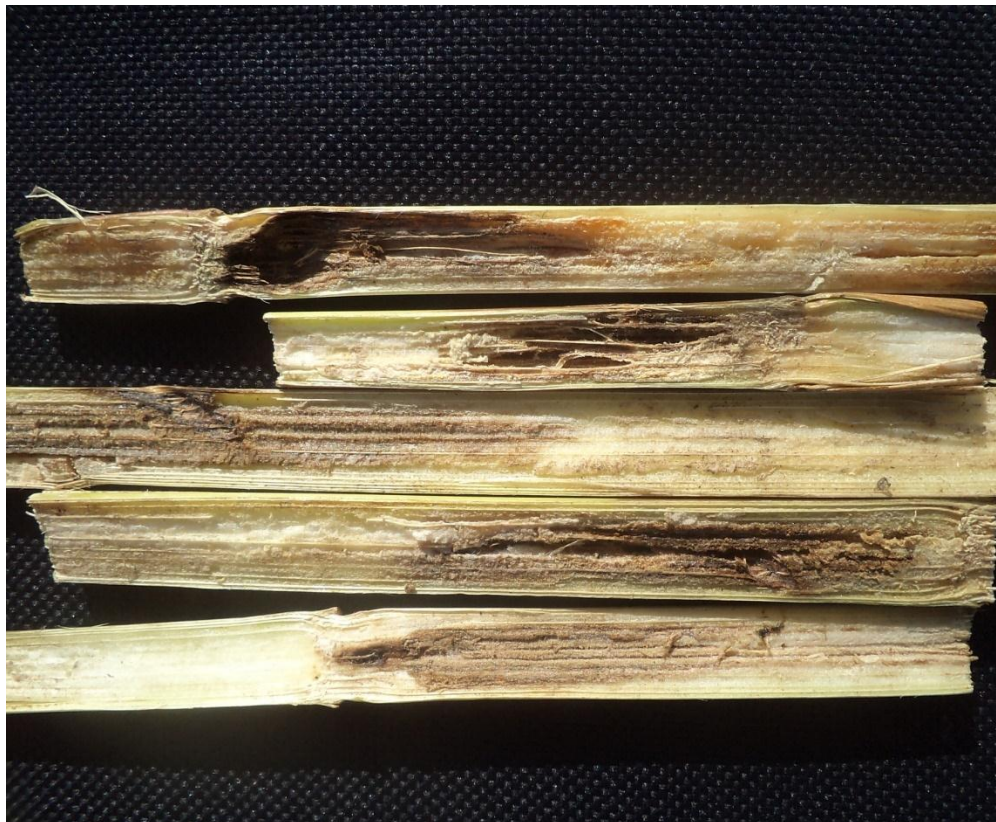


Figure 1b. Split treated maize stem (internode) with severe rot formation.
Source: Authors

differences at $P=0.05$ (Table 1). The number of leaves of the maize plants treated with T5 (cassava peel+*C. odorata*+*F. verticillioides*) and T6 (sawdust+leaf+ *F. verticillioides*) were significantly different ($p=0.05$, $R^2=0.54$) from Control 1 (T7) maize + *F. verticillioides*. The number of leaves of the maize plants treated with T1 (cassava peels + *F. verticillioides*) was significantly different from Control 2 (T8). However, the number of leaves of maize that received other treatments were not significantly different from controls 1 and 2 ($P=0.05$). The stem girth and plant height of the maize treated with T1, T2 (sawdust + *F. verticillioides*), T3 (*C.odorata* + *F.verticillioides*), T4 (cassava peels + sawdust + *F. verticillioides*), T5 and T6 were less than controls (1 and 2) ($P=0.05$, $R^2=0.85$) compared to other treatments. The leaf area of the maize treated with T1 to T4 were significantly different from the controls 1 and 2 ($P=0.05$, $R^2=0.77$). However, the leaf area of maize that received treatments T5 and T6 were not significantly different from control ($P=0.05$).

Table 2 show the effect of different concentrations of treatment on some growth parameters of the maize after treatment. The number of leaves of maize affected were significantly higher and similar ($P=0.05$, $R^2=0.54$) in groups treated with concentration 1 (3:1) and 2 (2:2) than

those that received concentration 3 (1:3). The plant height of maize treated with concentration 2 were significantly higher ($P=0.05$, $R^2=0.95$) than those that received concentrations 1 and 3. The leaf area of maize treated with concentration 2 was significantly higher ($P=0.05$, $R^2=0.77$) than those that received concentrations 1 and 3.

The summary of mean ($P=0.05$, $R^2=0.95$) rot formation and rot rating for maize stem that received different treatments are presented in Table 3. Treatments 1 to 8 had moderate rot formation within the inoculated internode ranging from as low as 27% in treatment 3 has no ratio, treatment 4 (2:2), treatment 5 (1:3 and 3:1), treatment 6 (1:3 and 2:2) to slightly higher rot formation of 47% in treatment 1:40% treatment 2:33% in treatment 4 (1:3 and 3:1) and treatment 6 (3:1). However, severe rot formation was recorded within inoculated internodes of control 1 (T7) 93% and control 2 (T8) with 67%.

Table 4 gives means comparison of disease index and disease severity of maize plants after receiving different treatments. The disease indices and disease severity recorded in plants that received T1 to T6 were significantly lower than Controls 1 and 2 ($P=0.05$, $R^2=0.98$).

The effect of different treatment combinations on shoot and root weight of the treated maize plants are presented

Table 1. Means comparison of various treatments on growth parameters of maize plants.

Treatment	No. of leaves	Stem girth (mm)	Plant height (cm)	Leaf area (cm ²)
T1	7.15 ^{ba}	3.97 ^{cb}	102.45 ^a	221.34 ^a
T2	7.09 ^{bc}	3.89 ^{cb}	101.25 ^{ab}	206.21 ^b
T3	6.70 ^{cd}	3.92 ^{cb}	95.86 ^{cde}	200.22 ^{cb}
T4	6.93 ^{bc}	3.98 ^{cb}	94.26 ^{cde}	192.43 ^{cd}
T5	6.52 ^d	3.89 ^{cb}	84.09 ^f	171.26 ^f
T6	6.54 ^d	3.62 ^d	84.42 ^f	145.12 ^f
T7	7.00 ^{bc}	2.75 ^e	75.49 ^g	152.61 ^f
T8	6.67 ^{cd}	2.86 ^e	78.46 ^g	148.18 ^f
LSD _{0.05}	0.10	0.05	1.59	3.95
R ²	0.54	0.85	0.95	0.77

Means followed by the same letter(s) in each column are not significantly different at ($p \geq 0.05$).
Source: Authors

Table 2. Effect of treatment concentrations on some growth parameters of treated maize plants.

Concentration	Number of leaves	Stem girth (mm)	Plant height (cm)	Leaf area (cm ²)
1 (3:1)	7.02 ^a	3.47 ^a	87.03 ^b	175.47 ^b
2 (2:2)	6.92 ^a	3.50 ^a	89.69 ^a	184.43 ^a
3 (1:3)	6.71 ^b	3.48 ^a	87.17 ^b	174.07 ^b
LSD _{0.05}	0.12	0.05	1.95	4.84
R ²	0.54	0.85	0.95	0.77

Means followed by same letter(s) in the same column are not significantly different ($p \geq 0.05$)
Source: Authors

Table 3. Mean percentage rot formation and rating within inoculated internode of maize stem after different treatments.

Treatment	Scale	Mean rot formation (%)
T1= CP + SD + <i>F. verticillioides</i>	3	47
T2= CP + Leaf + <i>F. verticillioides</i>	3	40
T3= SD + Leaf + <i>F. verticillioides</i>	3	27
T4C1= CP + SD + <i>F. verticillioides</i>	3	33
T4C2= CP + SD + <i>F. verticillioides</i>	3	27
T4C3= CP + SD + <i>F. verticillioides</i>	3	33
T5C1= CP + Leaf + <i>F. verticillioides</i>	3	27
T5C2= Maize + CP + Leaf + <i>F. verticillioides</i>	2	20
T5C5= Maize + CP + Leaf + <i>F. verticillioides</i>	3	27
T6C1= Maize + SD + Leaf + <i>F. verticillioides</i>	3	27
T6C2= Maize + SD + Leaf + <i>F. verticillioides</i>	3	27
T16C3= Maize + SD + Leaf + <i>F. verticillioides</i>	3	33
T7= Maize + <i>F. Verticillioides</i>	5	93
T8= Maize only	4	67

CP= Cassava peels, SD= Saw dust, Leaf= *Cedrela odorata*, C1= concentration 1(1:3) C2= concentration 2(2:2) C3= concentration 3(3:1).
Source: Authors

in Table 5. The shoot weights of plants that received treatment combinations 1 and 2 were significantly different

Table 4. Means comparison of disease severity and disease Index of the maize plants after receiving different treatments.

Treatment	Disease index	Disease severity
1	2.33 ^c	3.00 ^c
2	2.00 ^d	3.00 ^c
3	1.33 ^g	3.00 ^c
4	1.55 ^e	3.00 ^c
5	1.22 ^h	2.67 ^d
6	1.44 ^f	3.00 ^c
7 (Control 1)	4.67 ^a	5.00 ^a
8 (Control 2)	3.33 ^b	4.00 ^b
R ²	0.98	0.92

Means followed by same letter(s) in the same column are not significantly different ($p \geq 0.05$). 1=cassava peels + *F. verticillioides*, 2 = sawdust + *F. verticillioides*, 3 = leaf + *F. verticillioides*, 4=cassava peels + sawdust + *F. verticillioides*, 5=cassava peels+ *Cederela odorata* + *F. verticillioides*, 6=sawdust+ *Cederela odorata*+ *F. verticillioides*, 7(Control1) = *F. verticillioides*, 8(Control 2) = control. Source: Authors

Table 5. Effect of different treatment combinations on shoot and root weight of infected maize plants.

Treatment combination	Shoot weight (g)	Root weight (g)
1	20.02 ^b	3.52 ^a
2	21.71 ^a	4.08 ^a
LSD _{0.05}	1.65	0.76
R ²	0.79	0.48

Means followed by same letter(s) in the same column are not significantly different ($p \geq 0.05$). 1= single treatment + *Fusarium verticillioides*, 2= combined + *Fusarium verticillioides*. Source: Authors

from each other ($P=0.05$, $R^2= 0.79$) while root weights were similar ($P=0.05$, $R^2= 0.48$).

Table 6 shows the effect of different concentrations of treatments on Disease severity, Disease index and cob yield after treatments. The disease index and disease severity of plants treated with concentration 2 (2:2) were significantly lower ($P= 0.05$, $R^2= 0.98$) than those that received concentrations 1(3:1) and 3 (1:3). The cob yield of plants treated with concentration 2(2:2) were significantly higher ($P= 0.05$, $R^2= 0.98$) than those that received concentrations 1 (3:1) and 3 (1:3).

DISCUSSION

The observed changes (increase) in height and stem girth of maize plants that received all the treatments compared to control indicates that these amendments stimulated plant growth even in the presence of a pathogen. This agrees with the reports of Chilimba (2002)

and Ajanga et al. (2003) who opined that plant diameter and shoot length was significantly higher in amended soil as compared to controls; an indication that the amendments stimulated plant growth.

The higher plant growth as a result of organic amendment could be associated with the release of nitrogen for plant use which support the result of Akanbi (2002) while Adebayo et al. (2012) who stated that the increase in plant growth was as a result of nitrogen released which is essential for chlorophyll and protoplasm formation.

The rot formation in the stem of maize plants on unamended soil was significantly higher than those on amended soils; this showed the impact of the amendments on rot formation within the maize stem. It is possible that the amendments adversely affected the rot forming fungi within the soil. This supports the report of Ajanga et al. (2003) who reported high recovery of *F. moniliforme* in unamended soil. The significant reduction in disease severity (stalk rot) of plants that received (C.

Table 6. Effect of different treatment concentrations on Disease Index, Severity and cob yield.

Concentration	Disease index	Disease severity	Cob yield
1 (3:1)	2.41 ^a	3.40 ^a	23.04 ^a
2 (2:2)	2.18 ^b	3.10 ^b	23.69 ^b
3 (1:3)	2.43 ^a	3.45 ^a	23.03 ^a
R ²	0.98	0.92	0.98

Means followed by same letter(s) in the same column are not significantly different ($p \geq 0.05$).

Source: Authors

odorata at ratio 2:2) could be due to the biological activity of the treatment against the rot forming pathogen (*F. verticillioides*). However, the suppression of the pathogen by the soil amendment cannot be said to be absolute or consistent. The effective result generally obtained with *C. odorata* (2:2) and combined treatments of cassava peel and *C. odorata* (2:2) supports the result of (Akamu et al., 2013). In their work, Akanmu et al. (2013) concluded that 5 g/ml of cassava peels extracts had a significant effect on *F. anthophilum* and that the efficacy of peels was an effective biocontrol agent for the management of Fusarium disease in millet seedlings. Falade et al. (2006) affirmed the efficacy of cassava seed extracts in the control of *Scelerotium rolfsii* and *F. oxysporium* in Southwestern Nigeria. Akanmu et al. (2020) in their studies revealed that organic amendments are effective in controlling diseases caused by pathogens such as *F. sp.*

The observed disease severities of plants that received different treatments showed significant impacts of different treatments on the rot forming fungus (*F. verticillioides*). Treatments with cassava peels + *C. odorata*, had the highest effect on disease severity compared to other treatments, and can thus be said to be preferred above others. The results obtained in the study could be attributed to differences in mode of action of all treatments considered. This agrees with the findings of Elad et al. (2010) who reported that the differences in effect of organic amendments on *Fusarium verticillioides* at two recovery intervals from ear rot of maize could be attributed to the rate and mode of disease depression such as antagonism, antibiosis and lysis.

The general reduction in disease severity of maize by *C. odorata* (leaves) and cassava peels compared to control could also be due to increased population of other more competitive fungi and bacteria in amended soil leading to decline in disease severity. This agrees with the reports of Saravanan et al. (2008) and Ajanga et al. (2003). They reported that soils high in organic matter support huge populations of diverse micro-organisms and because of this plant diseases may be suppressed by the activities of plant-associated micro-organisms. *C. odorata* has been reported by Asekun et al. (2013) to contain flavanoids.

The variations recorded in organic amendments used could be as a result of variation in the materials used. This agrees with the findings of Flores et al. (2006) who concluded that not only do amendments from different materials vary in disease suppression, but those from different batches of the same material are also variable. The high rot formation recorded with saw dust could be because of the greater surface area found in wood materials limiting the availability of nitrogen needed by soil microorganisms to break down the material.

The combined treatments were more effective than single treatments against disease severity supports findings (Akanmu et al., 2020), which stated that organic amendments can improve physical and chemical properties of soil.

Generally, this study revealed that *C. odorata*, cassava peels and sawdust have the potential to suppress the causal pathogen (*F. verticillioides*) with a resultant enhanced growth and yield of maize. Different soil amendments used are of benefits to maize production but may be effective in different proportions. *C. odorata* and cassava peels are appropriate as soil amendments for maize cultivation. Severity of stalk rot of maize can be said to reduce significantly in amended soils compared to unamended soils. The soil amendments may also have adverse effect on some soil borne pathogens such as *F. verticillioides* serving as an alternative treatment (Kenganal et al., 2017). Conclusively, the use of combined treatments such as cassava peels and *C. odorata* which competed favourably with *F. verticillioides* can thus be suggested as a good soil amendment against such fungi in the control of stalk rot of maize.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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