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Evaluation of efficacy of some botanicals and bioagents against stalk and ear rot pathogen of maize, *Fusarium verticillioides*

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The efficacy of some botanicals and bio-agents were assessed *in vitro* as the potential biofungicides against *Fusarium verticillioides*, the causal organism of stalk rot disease of maize plant. The botanicals used in this study were *Morinda lucida* (Oruwo) and *Tithonia diversifolia* (Mexican Sunflower) and the bio-agents used were *Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma pseudokoningii* and *Bacillus subtilis*. All the plant extracts and antagonistic organisms significantly inhibited the growth of *Fusarium verticillioides*. However, out of the antagonists, *T. viride* provided the highest inhibitory effect followed by *T. pseudokoningii*, *T. harzianum*, and *B. subtilis* which was the lowest suppressive antagonist. The extract from *T. diversifolia*, provided the highest inhibitory effect followed by *M. lucida*.

Key words: Maize, ear rot, botanicals, bioagents, *Fusarium verticillioides*.

INTRODUCTION

Maize (*Zea mays* L.) is the third most important cereal crop in the tropical countries (CIMMYT, 2004). It belongs to the grass family *Poaceae*. It yields economically under the marginal production conditions of low fertility and management (FAO, 2005). Maize has been diversely used in several aspects including being food for human, animal feeds and industrial uses Fajemisin JM (1992).

All parts of the maize plant have economic values ranging from stalk, leaves and the cobs (Fajemisin, 1992). Maize can be eaten either boiled, roasted or processed into corn flakes or used as corn meal (Ubalua

and Oti, 2007; Iwena, 2008). It is also an important raw material in pharmaceutical, baby food, soft drink and brewing industries. Oil obtained from maize is used to make soap and glycerine or is refined for cooking and salad making (Adesimi, 1982).

The production of maize in the whole world at large including Nigeria is being threatened by various constraints including its susceptibility to both pests and diseases that causes pre and post harvest losses which cause great economic losses in Nigeria (Agoda et al., 2011). Moreover, the limiting factors that reduce the yield

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of maize plants are pathogen infection. *Fusarium* ear and stalk rot of maize is among the destructive diseases in many areas of Nigeria. This maize fungus produces mycotoxin (fumonisins) which is associated with serious animal and human diseases. Ear rot reduces maize production by destroying the grains and, causing food contamination which is poisonous to human and livestock. Stalk rot causes stalk breakage and lodging thereby making harvesting difficult and consequently reduce yield. Ear and stalk rot of maize is caused by *Fusarium verticillioides*. The fungus *F. verticillioides* lives in soil, penetrate stalks and root directly or spread systemically in the plant after infection that originated from seed-borne inoculums (Soonthornpoch et al., 2000). Also *F. verticillioides* produces phytotoxin fusaric acid (FA) in naturally infected plants (Nadubinska et al., 2002). Maize seeds infected by *F. verticillioides* enter the food chain of animal and human with the potential of producing a mycotoxin that adversely affect the consumers. The mycotoxin occurs anywhere this fungus proliferates, causing chemical pollution of biological origin and setting (Bacon et al., 1992a, b, c; Merrill et al., 1996a, b). The level of damages caused by this pathogen to maize production calls for the control measures that are cheaper, economically sound and environmentally safe to eliminate or reduce the incidence of this pathogen.

In Nigeria, the public is generally unaware of the hazard posed by this fungus on any infected seed. There is little information on how to reduce or eradicate its presence in maize either at pre - and post - harvest. The approaches to control pathogens have taken various from such as the use of chemicals, cultural control strategies and integrated methods (Macdonald and Chapman, 1997). The control measures sometimes have various setbacks even at the farmers' level of operation in Nigeria. This has raised the interest in developing alternative method of control from nature. This of course requires little or no skill; it is a cheap and readily available control measure, an environmentally friendly strategy for disease management which could inactivate the pathogen on maize.

Nature has a lot of plants and micro-organisms that can be used for disease control which is cleaner and safer in the environment, this is the use of botanicals and antagonistic organisms. In recent year, much attention has been given to non-chemical production against plant diseases due to environment pollution and health hazards caused by these chemicals, and various setbacks at farmers' level. This has raised the interest in developing alternative methods of control from nature. Moreover, some plant materials are found to be biocidal (Gurib-Fakim et al., 1996) among which are *Azadirachta indica* (Neem) and *Vernonia amygdalina* for the control of *Fusarium moniliforme* (now *F. verticillioides*) (Macdonald and Chapman, 1997; Owolade et al., 2002). The abundance of plant on the earth surface has led to an increasing interest in the investigation of different extracts

obtained from traditional medicinal plants as potential sources of new antimicrobial agents (Bonar and Forrokha, 2004). This will help in reducing the health hazards and contaminations due to the use of synthetic fungicides. Efforts has been made to investigate the method, time of application of the botanicals and antagonistic microorganisms on maize stalk, as well as the toxin inactivation in maize using this control strategy especially in Nigeria (Sobowale et al., 2007). It was reported that the extracts prepared from plants have variety of insecticidal properties against pests such as toxicity to nematode, mites and other agricultural pests. They also have antifungal, antiviral and antibacterial properties against pathogens (Charleston, 2002; Bushra and Genga, 2003).

However, since most farmers do not have resource to purchase nor apply expensive synthetic pesticides, using biological control in form of locally natural enemies together with the preparation of plant extracts from tree growing in the surrounding area, have little to no cost and are therefore uniquely suited for integrated pest management (Charleston, 2002).

The chemical analysis of extract from leaf of *Tithonia* showed that they contain sesquiterpene lactones (Achakzai, 2006; Gu, 2002), diversifolia methyl ether and Tirodindin as active component against inflammatory activity (Rungeler et al., 1998; Kuo and Chen, 1997). Methanol extracts of *M. lucida* showed anagelsic antipyretic effects and potentiated phenobarbitone sleeping time (Awe et al., 1998).

Trichoderma species are fungi that are present in nearly all soils; they frequently are the most prevalent cultivable fungi (Kubreek et al., 1998, Ubalua and Oti 2007). They are common inhabitants of the rhizosphere and are well recognized as biological agents of soil-borne plant pathogens (Chet, 1987). The efficient use of rhizosphere micro-organisms to plant pathogens has been reported worldwide in different plants (Cook, 1993). *Bacillus subtilis* is used as soil inoculants in horticulture and agriculture, it is used as a model organism for laboratory studies (Baker et al., 1983), and it has a natural fungicidal activity (Enzeby, 2008; Mazza, 1994). Due to these potentials of these plants and microorganisms, their efficiencies were also tested on *F. verticillioides* pathogen of maize. The objective of this work was to evaluate the antifungal potentials of two (2) botanicals and antagonistic of four (4) microbial agents on *Fusarium* stalk rot pathogen of maize.

MATERIALS AND METHODS

Isolation of *F. verticillioides* and antagonistic organisms

Maize sample that was infected with *F. verticillioides* was collected from farm and was taken to the laboratory. The infected samples were cut into pieces and surface sterilized for 2 min in 10% hypochlorite solution and rinsed in five changes of sterilized distilled water before planting them on PDA in Petri plates. Morphological

Table 1. Effect of antagonistic organisms on the growth of *Fusarium verticillioides*.

Treatment	Mean growth (cm)	Inhibition of the pathogen (%)
<i>B. subtilis</i>	2.22b	0.89
<i>T. pseudokoningii</i>	2.14c	4.46
<i>T. harzianum</i>	2.22b	0.89
<i>T. viride</i>	2.29a	-2.23
Control	2.24ab	

Mean with the same letter are not significant at ($P < 0.05$).

identification of the pathogen, *F. verticillioides* was done using the criteria of Gerlach and Nirenberg (1982) and Leslie and Summerell (2006). Methods employed in isolating antagonistic organisms were the soil plate method (Warcup, 1950) and soil dilution plate method (Tuite, 1969). Identifications were performed by using the keys provided by Rifai (1969) and Bissett (1991) for the *Trichoderma* species, and *B. subtilis* was identified using Zheng et al. (2008) method.

The effect of antagonistic microorganisms on target organism was done by pairing the antagonists with the target organism. Cork borer of 5 mm diameter was used to make 5 holes in the solidified PDA, where the target pathogen was placed in four holes at the side of the plate and antagonist was placed at the middle hole. Also, all the materials were incubated at room temperature. The measurement of rate of growth of the fungus was taken at 24 h interval until there was no further growth.

Preparation of plant extracts

The leaves of *T. diversifolia* and *M. lucida* were oven dried at 60°C for 48 h and after which they were blended, using Binatone Blender. Twenty grams of each blended leaves were measured and poured in Elemeyer flasks. Sterile distilled water of 200 ml was added to each of flask, soaked for 2 h, and then filtered with a white muslin cloth into a 250 ml beaker. 5 and 3 ml of the filtrate were released into the plate with the molten 10 and 12 ml of PDA respectively, and allowed to solidify.

In vitro study of *F. verticillioides*' growth suppression

The potency of two plant extracts on *F. verticillioides* growth and inhibition was investigated. Inoculating needle was used to take *F. verticillioides* into the extract-containing (poisoned) PDA in Petri dishes to check for its growth and PDA without extract served as the control. The extracts were then incorporated as 5 and 3 ml into the agar. Each treatment was replicated 3 times.

All plates were incubated at room temperature ($28 \pm 2^\circ\text{C}$) and the measurement of the growth of the fungus was taken with a metre ruler, at every 24 h interval until there was no further growth. The fungal growth rate on extract incorporated agar plates (using a metre rule to measure the radial growth) were compared with the control that is non extract incorporated agar plates. The difference in growth rate represented the activities of the extract in inhibiting the growth of the fungus. Laid out in a 2 x 5 factorial experiment in a Completely Randomized Design (CRD) for the antagonists (2 locations and 5 treatments: 4 antagonistic organisms + control) and 2 locations x 2 botanicals, factorial experiment for the botanicals were used. Data collected was subjected to statistical analysis using SAS, 2001 and means were separated by least significant difference (LSD).

RESULTS AND DISCUSSION

Effect of treatments and location on the growth of *F. verticillioides*

The mycelium of *F. verticillioides* appeared pinkish in colour with white fluffy at the top. The antagonistic organisms such as *B. subtilis*, *T. harzianum*, *T. pseudokoningii*, *T. viride* were inoculated at the middle and at the side of the media culture. *B. subtilis* was inoculated at the middle of the growth medium and the growth of *F. verticillioides* was 0.44 cm on the second day and increased to 1.85 cm on the seventh day. The observation showed that the *B. subtilis* grew slowly compared with the growth on the control plate (Table 1); had 0.89% inhibition on the growth of the pathogen, which is similar in action to the effect of *T. harzianum*, *T. harzianum* that had 0.89% growth inhibition of *F. verticillioides*. *T. pseudokoningii* had 4.46% inhibition of the pathogen.

The growth of target organism, *F. verticillioides* was inhibited by *T. pseudokoningii*. The mycelium growth of *F. verticillioides* paired with *T. pseudokoningii* on the second day was 1.23 cm and on the seventh day it grew to 2.48 cm, which was significantly different from the control plates. The mycelium of *T. pseudokoningii* appeared greenish in colour and fully occupies the plate, covering up the pathogen. (Table 1)

For *T. harzianum*, the mycelium appeared green with white fluffy parches, and suppressed the growth of *F. verticillioides*. The mycelium growth of *F. verticillioides* was 1.50 cm second day and increased to 2.47 cm seventh day. There was significant difference compared with the control plate which showed inhibition of *F. verticillioides*' growth at these times (Table 1).

For *T. viride*, the mycelium appeared in ring shape, greenish at the tip and white at the centre. *T. viride* fully occupy the plate at 7 days of incubation. It inhibited the growth of target organism, *F. verticillioides*. The growth of *F. verticillioides* on the second day was 1.81 cm and seventh day 2.46 cm; there were significant differences when compared with the control plate, which had higher growth of the pathogen (Table 1).

There is significant difference in the location effect irrespective of the antagonistic organisms paired with the

Table 2. Effect of location pairing on the *Fusarium verticillioides* with the antagonistic organisms.

Location	Mean growth (cm)
Centre	1.29 ^b
Side	2.75 ^a

Mean with the same letter are not significant at (P<0.05)

Table 3. Effect of location and bioagents on growth of *Fusarium verticillioides* at 120 h of incubation.

Location	Treatment	Mean growth (cm)	Percentage inhibition (%)
Centre	<i>B. subtilis</i>	1.83	63.27
	<i>T. pseudokoningii</i>	0.72	68.14
	<i>T. harzianum</i>	0.81	64.15
	<i>T. viride</i>	0.83	63.27
	Control	2.26	
Side	<i>B. subtilis</i>	0.61	73.01
	<i>T. pseudokoningii</i>	3.56	13.27
	<i>T. harzianum</i>	3.64	16.81
	<i>T. viride</i>	3.75	21.68
	Control	2.26	
L X T		*	

L, location; T, treatment; *, significant at P = 0.05.

pathogen (Table 2). When *F. verticillioides* was placed at the centre of the antagonistic organism, there was significant difference compared with when it was placed at the edge of the plate. The mean value for centre and side were 1.29 and 2.75 cm respectively. The centre pairing had higher inhibitory effect on *F. verticillioides* than the side pairing (Table 2).

The mean for location by the treatment, had significant difference between location and treatment (Table 3) at 120 h of incubation. When *T. harzianum* and *T. viride* were placed at the centre of the plates paired with *F. verticillioides*, the mean growth were 0.81 and 0.83 cm of *F. verticillioides* respectively which gave percentage inhibition of 64.15 and 63.27% respectively while *B. subtilis* and *T. viride* were not significantly different from each other. When *F. verticillioides* was placed at the side of the plates, paired with the antagonists, its growth was more than when placed at the centre. *B. subtilis* at this location gave the 73.01% highest inhibitory effect on the pathogen of all the antagonists tested and the least effective was *T. pseudokoningii* with 13.27% inhibition. The effect of time shows that there was significant difference at the times the observation was done in all the treatments. When the targeted organism, *F. verticillioides* was at the centre of the plate, *T. pseudokoningii* had the

highest inhibitory effect and *B. subtilis* had the lowest inhibitory effect. Also, when the *F. verticillioides* was placed at the side *B. subtilis* have lowest growth (0.60 cm) and *T. viride* had highest growth of 3.75 cm.

The result shows that there was significant difference between treatment and the time taken (Table 4). *B. subtilis* had the mean value of 0.44 and 1.85 cm during the second and the seventh days. *F. verticillioides* alone had 1.05 and 3.39 cm and *T. pseudokoningii*, *T. harzianum* and *T. viride* had 1.05 and 3.39 cm, 1.23 and 2.48 cm, 1.50 and 2.47 cm, 1.81 and 2.46 cm respectively during the second and seventh days.

Effects of plant extracts on *F. verticillioides*

Different concentrations of extract of *M. lucida* and *T. diversifolia* were incorporated into agar at different volumes of 3 and 5 ml and the poisoned agar was inoculated with 0.5 cm of *F. verticillioides*. After two days of inoculation, the growth of the organism on the concentrations of *M. lucida* extract were 1.93 and 1.80 cm respectively. By the fifth day, the growth were 3.26 and 3.16 cm respectively. The percentage inhibition of *F. verticillioides* due to the 3 and 5 ml of *M. lucida* extract

Table 4. Effect of location, time and bioagents on the growth of *Fusarium verticillioides*.

Location	Time	<i>T. harzianum</i>	<i>T. pseudokoningii</i>	<i>T. viride</i>	<i>B. subtilis</i>	Control
Centre	48	7.58	9.00	5.83	5.20	10.50
	72	9.25	10.25	6.66	11.00	13.33
	96	9.83	9.25	8.16	15.62	19.33
	120	8.41	8.16	7.91	18.62	23.75
	144	7.25	7.33	7.41	22.62	26.83
	168	7.16	7.08	7.58	25.62	31.08
	192	7.16	7.08	7.33	30.00	33.91
Side	48	22.41	27.33	18.91	3.62	10.50
	72	24.41	31.41	21.58	5.75	13.33
	96	39.33	36.16	40.25	6.25	19.33
	120	41.75	41.41	41.83	6.25	20.00
	144	41.91	41.83	42.25	6.75	26.83
	168	42.25	42.25	42.41	7.00	31.08
	192	42.25	42.25	42.41	7.00	33.91
Sign.		S	S	S	S	S

S, Significant at 5%.

Table 5. Growth of *F. verticillioides* on PDA incorporated with two different concentrations of two plant extracts at 144 h of incubation.

Treatment	Concentration (ml)	48 h	72 h	96 h	120 h	Inhibition (%) at 120 h
<i>M. lucida</i>	3	1.93 ^d	2.46 ^d	2.90 ^d	3.26 ^c	56.14
	5	1.80 ^d	3.06 ^c	3.13 ^c	3.16 ^c	57.49
<i>T. diversifolia</i>	3	2.53 ^d	2.96 ^d	3.20 ^c	3.90 ^c	47.65
	5	2.76 ^d	3.63 ^c	3.63 ^c	3.63 ^c	51.24
Control	-	3.35 ^c	4.20 ^b	4.90 ^b	7.45 ^a	

Mean (in cm) with the same letters are not significantly different P<0.05.

incorporated into the growth media were 56.14 and 57.49% respectively at 5 days of incubation.

This observation shows that the growth of target organism on poisoned agar plate was reduced compared with the growth on the control plates which were not poison with extract of *M. lucida* (Table 5). There was significant difference in the growth of *F. verticillioides* on different concentration of *M. lucida* extracts. The 5 ml was observed to be the most effective and 3 ml was the least effective (Table 5).

After 48 h of inoculation (two days), the mycelium growth of the organism on different concentration of 3 and 5ml of *T. diversifolia* were 2.53 and 2.76 cm respectively. By the fifth day, the growths of target organism on the concentrations of *T. diversifolia* were 3.90 and 3.63 cm respectively. The percentage inhibition of *F. verticillioides* due to the 3 and 5 ml of *T. diversifolia* extract incorporated into the growth media; were 47.65 and 51.24% respectively at 5 days of incubation.

This shows that the growth of target organism was

reduced when compared with the growth on the control plate which was not poisoned with extract of *T. diversifolia*. In past researches, some of the bioactive compounds that have been isolated from the leaves of *T. diversifolia* included sesquiterpenes, saponins and alkaloids (Rurgler, 1998; Tona et al., 2000; Liasu and Achakzai, 2000) and this made *T. diversifolia* active in inhibiting mycelia growth of *C. lunata*. Methanol extracts of *M. lucida* shows analgesic, antipyretic effects and potentiated phenobarbitone sleeping time (Awe et al., 1998). Kemabonta and Okogbue (2000) reported efficacy of *M. lucida* in causing mortality of adult *C. maculatus* and reduction of oviposition and first filia generation emergence. The major constituents of *M. lucida* as reported by Nweze et al. (2004) and Akinyemi et al. (2005) are anthraquinones and anthraquinols. The most effective of the plant extracts was *T. diversifolia*, followed by extract of *M. lucida*. Extract of *M. lucida* also had inhibitory effect but not as much as the extracts of *T. diversifolia*.

The aim of finding alternative to synthetic fungicide use in Agriculture has led to the search for plant materials with biocidal properties and the use of antagonistic organisms. Therefore, the results of this investigation indicated that antagonistic organisms such as *T. viride*, *T. harzianum*, *T. pseudokoningii* and *B. subtilis* are toxic to *F. verticillioides*. The extract of *M. lucida* and *T. diversifolia* are also effective in the control of *Fusarium verticillioides* by inhibiting its growth *in vitro*. These plant extracts and the antagonistic organisms caused significant differences on the growth of *F. verticillioides*.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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