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Control of Tomato (*Solanum lycopersicum* L.) Wilt Caused by *Fusarium oxysporum* Schlecht using Plant Extracts and Ash Tamarind in Girei, Local Government Area, Adamawa State

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ABSTRACT

The studies on the control of *Fusarium* wilt of tomato was carried out in Girei Local Government Area Adamawa State. Using treatments combinations consisted of three levels each of jatropha, neem and tamarin ash assigned in Completely Randomized Design (CRD), replicated four times. Data collected was analysed using Statistical tool for Applied Sciences (SAS) and means that were significantly different were separated using the Least Significant Difference (LSD). Results of in-vitro control trials indicated that there were significant differences ($P \leq 0.05$) among the treatments with respect to the control of the *Fusarium oxysporum*. *Jatropha* leaf extracts produced the inhibition zone of 37.50%; neem gave 32.53% while ash had 24.86% inhibition. Concentration levels of 2.0 ml, 1.5 ml, and 1.0ml/20 ml of PDA were also significantly different, with increased efficacy as concentration of treatment increases. For in-vivo trial, the treatments were significantly different ($P \leq 0.05$) from the control, reducing the disease of tomato seedlings. Concentration of 3.0 ml/kg, 5.0 ml/kg and 10 ml/kg were also significantly different at $P \leq 0.05$, with increased efficacy as concentration of treatment increases. From the in-vitro findings and screen house study, *jatropha* leaf extracts gave the best control of the pathogen and produced the highest seedling growth followed by neem leaf extracts and ash whereas increase in concentration increases the control of the pathogen, therefore farmers are recommended to use *jatropha* at 5.0ml/kg.

Keywords: *Fusarium oxysporum,* jatropha, neem, ash

INTRODUCTION

Fusarium wilt of tomato is one of the most economically important and wide spread diseases of the cultivated tomato (*Solanum lycopersicum*). It is one of the most important diseases which are highly destructive to tomatoes grown in greenhouse and in the field in many warm regions of the world, where it causes 10-50 % yield loss (Larkin and Fravel, 1998; Borrero *et al.,* 2004). The disease is endemic in vegetable growing areas and has caused important yield losses in Nigeria (Erinle, 1981: Mes *et al.,* 1999/. The pathogen is a soil-inhabiting fungus which is resistant to chemical fungicide (Sibounnavong *et al* 2010). The management of *Fusarium* wilt pathogen is particularly complex because it lives in or near the dynamic environment of rhizosphere and can frequently survive long periods in soil through the formation of certain resistant structures of the pathogen (Blum and Rodriquez-Kabana, 2004). The disease caused by this fungus is characterized by wilted plants, yellowing of leaves and minimal or absent crop yield. The pathogen enters tomato through the roots and caused yellowing of the oldest leaves, often on only one side of the plant (Abdel-monaim 2012).

The risks involved in the use of synthetic fungicides to the environment are a measure concern to plant pathologist globally (Okigbo, 2009). Searching for harmless alternative methods of pathogen control is necessary (Ijato *et al.,* 2011). The control of *Fusarium* wilt of tomatoes is important in maintaining plant vigour and fruit quality and quantity (Agrios, 1988). The increasing public outcry against pesticide use has generated interest in the use of plant extracts (organic substances) to prevent and control plant diseases. The use of plant extract to control diseases in plants is an effective alternative that enables the use of pesticides to be reduced (Segarra *et al.,* 2009). The use of plant extracts in controlling soil-borne diseases has been reported by several researcher (Litterick and Hamler, 2004; Segarra *et al.,* 2009). However, there is scanty report indicating that control attempt with plant extracts on *Fusarium* wilt has been carried out, using *jatropha* leaf extract, neem leaf

extract and tamarind ash. Hence the objectives of the study was to investigate the effect of *jatropha* leaf extract, neem leaf extract and tamarind ash in vitro and in vivo as a method to control *Fusarium* wilt. This study hopped to provide an alternative control to synthetic chemicals for controlling *Fusarium* wilt responsible for Tomato diseases.

MATERIALS AND METHODS

The control of tomato *(Solanum lycopersicum)* wilt was conducted in the field farms in Girei of Adamawa State and the laboratory of Plant Science Department, Modibbo Adama University of Technology (MAUTECH) Yola, located at coordinate 9°20′ N 12°30′ E/ 9.333° N 12.500° E (C-GIDD, 2008) where the isolation, identification and control trial were carried out.

Source of Sample and Isolation of the Pathogen

The isolation of the fungi *Fusarium* spp was done from diseased tomato plants and Soil collected from the fields in Girei, Adamawa State. The diseased plant stems base was cut into 5mm sections and then surface sterilized in 0.5% sodium hypochlorite for 30seconds. Then washed in three changes of sterile distilled water and dried between sterile filter papers. With a flamed and cooled pair of forceps, four sections were aseptically plated on 9cm diameter in the month of February 2015. Petri-dishes containing sterile solidified potato dextrose agar (PDA) and incubated at 33±2°C for 7 days. The inoculated plates were arranged on the bench at room temperature 33±2 °C under fluorescent light in the laboratory for seven days as described by (Mark *et al* 2015). Using a sterile forceps agar plugs was taken from the actively growing region of the mycelial growth for sub-culturing in other sterilized Petri dishes containing PDA and left for 7 days under fluorescent light at the room temperature. Sub-culturing was done and repeated until the pure culture of the pathogen was obtained and kept as slants in Mc-cartney bottles. They were loosely corked until they attain full growth then, tightly corked and stored at a temperature range of 0-4° C in a refrigerator to

serve as stock culture. Cultures were stored in Mc- Cartney bottle as pure culture for subsequent use.

Preparation of Jatropha, Neem Leafs Extracts

Jatropha and Neem leaf extracts were prepared according to method of Paul and Sharma (2002). Jatropha plants were obtained from the farms in Girei study area. Fresh weights (100g) of mature leaves of each of the plants were homogenized in a pre-chilled pestle and mortar using chilled, sterilized distilled water. The extract was filtered through four layers of a cheese cloth. The final volume was adjusted to 1000 ml with distilled water. The filtrate was centrifuged at 8000 rpm, 4 °C for 15 min. The supernatant thus obtained was designated as concentrated leaf extract.

Extraction of Powder Ash and Liquid Ash for Control

The ash was obtained by burning dry leaves of tamarind (*Tamaridus indica*). The weight of 100 grams of the powdered ash was placed in a beaker and then sterilized in an oven for one hour at 160° C. One hundred (100) grams portion of the powdered ash were mixed with 120mls of distilled water and then filtered (Mark *et al.*, 2015) using a cheese-cloth, gauze and Whatman filter paper to obtain filtrate of ash. The filtrate was collected in a conical flask. The filtrate of ash was used for the disease control trial.

Control of Mycelial Growth of Pathogen by Plant Extracts and Ash

In-vitro Control Trial Using Jatropha Leaf, Neem Leaf and Ash

Petri dishes containing PDA were incubated with jatropha, neem leave extracts and ash at three concentration levels (1.0, 1.5 and 2.0ml) per 20ml of PDA and replicated four time (poisoned food method) (Nene and Thalpiyal, 2000). Approximately 2.5mgs of chlorophenicol was dissolved in 2mls of sterilized distilled water and then added to each 250mls of PDA to prevent bacterial growth. The media containing the extract and chlorophenicol were gently agitated by hand for 2 minutes for proper mixing of the content before pouring into the plates. This was done by

creating four equal sections on each plate by drawing two perpendicular lines at the bottom of the plate. The centre of the plates indicated the point of intersection of the inoculums. Up to 20mls of the mixed media was dispensed into 9cm Petri-dishes. Approximately 0.1ml from *Fusarium oxysporum* spore suspensions (conc.1 × 106 spores/ml) were dispensed at the center of the amended PDA media slightly opened. The inoculated plates were then sealed with a masking tape and then incubated at 33±2°C for 24 to 72 hours in the month of May.
The Petri-dishes without the plant extracts and ash served as control. The experiment was performed under aseptic conditions and replicated four times.

Mycelial growth diameter of the isolate was measured and recorded when the growths of the isolate were completed in the control treatment. Each treatment was repeated four times. Mean radial mycelial growth of the isolate was recorded and data were transformed into inhibition percentage by using the following formula (Naz *et al.,* 2006):

$$\text{Inhibition percentage (\%)} = \frac{\text{DC} - \text{DT}}{\text{DC}} \times 100$$

Where: DC - Average diameter of fungal spore in control.
DT - Average diameter of fungal spore with treatment.
Data were taken every day for the period of (7) days with caliper and rule with the aid of hand lens. Results were analyzed with Statistical Analysis (SAS) Version 9.1.

Invivo Screen House Trial

Tomato seeds (*Solanum Lycopersicum* UC, 82B) were sterilized in hydrochloric acid and sodium hyphochlorite for 5min, washed 3 times with sterile distilled water and dried between the filter papers then plated in seed trays containing sterilized sandy loamy soil (2: 1 w/w) and then placed on benches in a greenhouse until transplanting. Four weeks old healthy tomato seedlings were selected and transplanted into polythene, containing sterilized sandy loamy soil (2: 1 w/w 2 seedlings for each polythene bag) Songs *et al.* (2004).

Fusarium oxysporum f.sp. lycopersica strain for inoculation was obtained from naturally infected diseased plants from the surveyed farms in Girei study area. Fungal suspension was prepared according to Leslie and Summerell (2006). Cooked rice (100g) were inoculated with *Fusarium oxysporum f.sp. Lycopersica* and incubated at 33+2°C for 14 days. The inoculated cooked rice medium was crushed and suspended in a volume of sterile distilled water. Spores were obtained and used for infection of tomato seedlings by adding 10 ml of fungal spores suspension in the soil close to tomato seedlings roots in polythene.

After 3 days of inoculation drenched in soil different treatments, were administer at different concentrations levels of 3ml/kg, 5ml/kg, and 10ml/kg and the one without the treatment served as control, using the method of (Mark *et al.,* 2015).

Disease assessment based on disease incidence, disease severity was calculated using the following formula recommended by Masood *et al.* (2010).

$$Disease\ incidence\ \% = \frac{Number\ of\ infected\ plants}{\text{Total number of plants assessed}} \times 100$$

Disease severity was scored based on the modified disease severity scale of Silva and Bettiol (2005). They were as follows: 1 = no symptom; 2 = plant showed yellowing leaves and wilting 1 -20%, 3 = plant showed yellowing leaves and wilting 21 - 40%, 4 =plant showed yellowing leaves and wilting 41 - 60%, 5 = plant showed yellowing leaves and wilting 61 - 80% and 6 = plant showed yellowing leaves and wilting 81-100%.
Assessments of growth characters were done after seventh day. Growth characters included;

I. Ruler measurement of seedlings height (cm).
II. Measurement of stem girth (cm)
III. Count of leaf number
IV. Count of leaf area (cm^2)

Data Analysis

All the data were analyzed using analysis of variance (ANOVA) as adopted by Gomez and Gomez (1984) using Statically Analysis System (SAS) program version 9.1 and the least significant difference (LSD) at P=0.05% level according to Scheffer (1953) was used in separating significant means.

RESULTS

Effect of Plant Leaf Extracts and Ash on Fusarium oxysporum In-vitro effect of Leaf Extracts of Jatropha and Neem and Ash ofTamarind on the Colony Growth and Percentage Inhibition of Fusarium oxysporum

Analysis of variance for effect of *jatropha* leaf extracts, neem leaf extracts and ash on the colony growth and inhibition of *Fusarium oxysporum* were significant at P<0.05 as shown in Table 2. *Jatropha* leaf extracts had best efficacy with least colony growth of *Fusarium oxysporum* (6.92mm) thus the highest inhibition zone (37.50%), followed by neem having the colony diameter of 7.25 mm with inhibition zone of 32.53%. Ash gave the colony diameter of 9.08mm and inhibition zone of 24.86%. The least (with the highest colony diameter) was observed in control (11.58mm) with zero inhibition zones there was however no significant difference between *jatropha* and neem leaf extracts.

Analysis of variance for different concentration levels of *jatropha* leaf extracts, neem leaf extracts and ash on colony growth of *Fusarium oxysporum* showed a significant difference among all the concentrations levels (P<0.05). Increase in concentrations of both *jatropha* leaf extracts, neem leaf extracts as well as ash resulted in decrease in colony diameter of the pathogen up to 1.5ml/20ml of PDA. Concentration of 2.0ml gave the least control with the colony growth of 23.08mm, and 1.0ml concentration level gave 20.33mm colony growth and also treatment with 1.5ml produced the highest control of the pathogen with colony growth of 17.42mm. And the results of the percentage inhibition showed significant difference among the concentration levels of 1.5ml, 1.0ml and 2.0ml but differs significantly with 0.0ml. The concentration of 1.5ml produced the

highest percentage inhibition zone of 53.35%, followed by 1.0ml with 43.92% and 2.0ml produces the percentage inhibition zone of 32.88% (Table 2) there was significant difference in inhibition between 1.0ml and 2.0ml/20ml of PDA. Implying that high concentrations of extracts pose an adverse effect.

Invivo Effect of Jatropha Leaf Extracts, Neem Leaf Extracts and Ash on Growth of Tomato Infected with *Fusarium oxysporum*

The growth characters of tomato seedlings analyzed were; stem height of tomato seedlings (cm), stem girth of tomato seedlings (cm), tomato seedling leaf number and tomato seedling leaf area (cm^2). Analysis of variance in Table 4 indicated that no significant difference at $P<0.05$ among *jatropha* leaf extracts, neem leaf extracts and ash with respect to seedling height. All the treatments have the mean height of 39.25mm, 35.25mm, and 34.62mm respectively, but the mean heights differs significantly with control which has the least seedling mean height of 26.33mm.

For stem girth, the results in Table 3 showed no significant difference at $P<0.05$ among *jatropha* leaf extracts, neem leaf extracts and ash but differ significantly with control. Control trial result showed that stem girth of untreated seedlings (control) was lower (8.00mm) than for *jatropha* leaf extracts, neem leaf extracts and ash with the mean stem girth of 13.44, 12.25 and 12.13 respectively. Analysis of variance in Table 4 indicated that the seedling leaf number obtained from the plants treated with *jatropha* leaf extracts, neem leaf extracts and ash, had no significant difference ($P<0.05$) with respect to the seedling leaf number as affected by neem leaf extracts and ash. However, there was a significant difference ($P<0.05$) with the applied *jatropha* leaf extracts having the highest leaf number of 69.38mm and control with lower leaf number of 35.00.

For seedling leaf size, as shown in Table 4 revealed no significant difference ($P<0.05$) among *jatropha* leaf extracts, neem leaf extracts

and ash which are recorded as 10.94mm, 11.06mm and 11.44mm respectively, but differs significantly with control at $P<0.05$, which had the least seedlings leaf size of 8.25mm.

Based on the concentration level, 10.0ml/kg of treatment concentrations produced the highest seedling height (42.08mm) of tomato followed by 5.0ml/kg with seedling height of 40.43mm and the least seedling growth (36.67mm) was observed in 3.0ml/kg level of the treatments. It is observed that seedlings treated with high concentration of the treatments have more vigor and height compared to lower concentration of the treatments. This is to show that increase in concentration increases the height of tomato seedlings. However, different concentration levels on seedling height, showed no significant difference at $P<0.05$ among the concentration levels, but differs significantly with control having the least seedling height of 26.33mm.

Analysis of variance showed that there was no significant difference at $P<0.05$ among the different concentration levels on seedlings stem girth (Table 4). But, then 3.0 ml/Kg had the highest stem girth of 14.08mm, followed by 5.0 ml/Kg with mean stem girth of 13.33mm and the least stem girth was obtained by the concentration level of 10.0 ml/Kg with mean of 13.00mm.

DISCUSSION

In-vitro

The result from the study has shown that extracts from *jatropha* leaf extracts, neem leaf extracts and ash are capable of controlling the mycelial growth of *Fusarium oxysporum in-vitro.* The effect of *jatropha* leaf extracts, neem leaf extracts and ash on the colony growth and inhibition of *Fusarium oxysporum* were significant at $P<0.05$ as shown in Table 2. *Jatropha* leaf extract of *jatropha curcas* showed best control of disease caused by the *Fusarium oxysporum* with least colony growth of6.92mm thus the highest inhibition zone of 37.50%, followed by neem

having the colony diameter of 7.25 mm with inhibition zone of 32.53%. Ash gave the colony diameter of 9.08mm and inhibition zone of 24.86%.

The highest colony diameter of 11.58mm was observed in control with zero inhibition zones. There was however no significant difference between *jatropha* and neem leaf extracts. The result therefore indicated that *jatropha curcas* leafs extracts contained more of the active ingredients that are effective in controlling the mycelial development of *Fusarium* wilt. This study also showed that increase in the concentration of *jatropha* leaf extracts reduces the mycelial growth of *Fusarium oxysporum* (Table 1).This was in accordance with the report of Silva *et al.* (2008) in which *jatropha curcas* was reported for its high inhibitory effect on *F. oxysporum.* Moreso, in the report of Falade *et al.* (2006), the extracts of *Jatropha gossypifolia* effectively controlled *Sclerotium rolfsii* (*Corticium rolfsii*) and *Fusarium oxysporum* which were isolated from tomato. Increase in concentrations of both *jatropha* leaf extracts, neem leaf extracts as well as ash resulted in decrease in colony diameter of the pathogen up to 1.5ml/20ml of PDA.

Concentration of 2.0ml gave the least control with the colony growth of 23.08mm, and 1.0ml concentration level gave 20.33mm colony growth and also treatment with 1.5ml produced the highest control of the pathogen with colony growth of 17.42mm. And the results of the percentage inhibition showed significant difference among the concentration levels of 1.5ml, 1.0ml and 2.0ml but differs significantly with 0.0ml. The concentration of 1.5ml produced the highest percentage inhibition zone of 53.35%, followed by 1.0ml with 43.92% and 2.0ml produces the percentage inhibition zone of 32.88% (Table 2) there was significant difference in inhibition between 1.0ml and 2.0ml/20ml of PDA. Implying that high concentrations of extracts pose an adverse effect.
Also the phytochemical properties of *jatropha curcas* plant which attributed to several components, including saponins, lectin (curcin),phytates, protease inhibitors, and curcalonic acid and

phorbolesters (Adolf *et al.,* 1984; Makkar and Becker, 1997), as well as secondary metabolites alkaloids, tannins, flavonoids, phenols and saponins (Martinez-Herrera *etal.,* 2006). All this may be responsible for the antifungal properties of *jatropha curcas* which has been effective against the pathogen (*Fusarium oxysporum).*

The *in-vitro* bio-efficacy of plant extracts of *Azardirachta indica* was tested to control *Fusarium oxysporum)* wilt pathogen by 32.53% inhibition zone. Similar findings were obtained against *Fusarium* wilt of Carnation (Chandel and Tomar, 2008). The bio-efficacy of neem extract over pathogens can be attributed to the fact that neem has active compounds such as azadirachtin, nimbin, nimbidin, nimbinene and azadirone which are antifungal, antibactrial and anti-insecticidal in nature (Bohra *et al.,* 2006).

The ash extract also reduces the mycelial growth of pathogen *Fusarium oxysporum* by providing a reasonable inhibition zone of 24.86%, as it was similarly observed by Channya (1991) who demonstrated the control of fungal rot of plantain in South West Nigeria using ash.In this study, result showed that increase in concentrations of ash at the pH of 10.3, decreases the mycelial growth of *Fusarium oxysporum.* Effectiveness of ash as control for fungi was attributed to the potash nature that is highly alkaline which can control *in-vitro* mycelial growth of *Fusarium oxysporum.* The ash extract of *Tamarind* (*Tamaridus indica)* used in this study has some potentials to be effective antifungal agent against *Fusarium* wilt of tomato seedlings.

In-vivo Test

The present study has also shown that plant height of tomato seedlings was observed on the 21 days of transplanting; treatment with *jatropha* leaf extract produced the highest plant height of tomato seedlings compared to other treatments, while control has the least plant height. This shows that *jatropha* leaf extract support the growth of the tomato

seedlings after transplanting compared to the rest of the treatments. This is in line with the findings of (Glick *et al.,* 2007) who reported that, generally it is presumed qualitatively that *jatropha curcas* contain some antifungal compounds which may be utilized as phytofungicide and phytofertilizer to control the pathogenic fungi and enhance the growth of various economically important food crops.

From this study, highest concentration of *Jatropha. curcas* leaf extracts, neem leaf extracts and ash produced higher growth of tomato seedling plant height. As the concentrations increases the plant height of tomato seedlings increases. Applications of treatments such as *jatropha. curcas* leaf extracts neem leafs extracts and ash can served as an alternative to the use of chemical fertilizer, pesticide and fungicide that are employed for facilitating plant growth such seedlings that received the highest concentrations of the extract were noted to have more vigour compared to low level concentration of the treatments. Which confirmed the report of Jogdande (2000) who affirmed that shoots cultivars contains naturally occurring growth promoters.

Result on stem girth of tomato treated with *jatropha curcas* leaf extracts, neem leaf extracts and ash produced increase in stem girth compared to the control. This was probably due to fungus inhibiting chemical present in *jatropha* leaf extracts, neem leaf extracts and ash which control the effect of *Fusarium oxysporum.* The least stem girth is observed in control plant this is likely due to attack of *Fusarium oxysporum.* Which affect the water and nutrient absorption surfaces of tomato seedlings. Effect of concentration on tomato seedling stem girth also showed no significant difference among 10ml/kg, 5.0ml/kg and 3.0ml/kg. All the concentrations showed increase in stem girth of tomato seedlings. It was in the vein with Mark *et al.* (2015)
Screen house findings also reveal that there is significant difference between treated seedlings and the control in all the parameters measured. Result on leaf number showed that plant treated with

jatropha curcas leaf extracts, neem leaf extracts and ash gave higher leaf number than control, and this may be attributed to the effect of the extracts on *Fusarium oxysporum.* Which control the pathogen as a result of the presence of phyto-chemicals. The plants were able to utilize the available water and nutrients present in soil for their growth. It was in the same vein that Muhammed *et al.* (2004) reported that extract of neem leaves (*Azadirachta indica*), *Calotropis procera* and datura *(Datura alba)* reduced soil borne pathogens of citrus and enhanced growth variability in the plant. The least plant leaf number was observed in the control plant. Stunted growth, wilting and yellowing leaves in the above ground level (chlorosis), reveal the symptom of *Fusarium oxysporum.*

Result also on leaf area treated with ash shows a significant difference with the control plant. The treated plants show higher leave area than the control. The control gave the lowest leaf size, this may be due to the fact that phyto-chemicals present in *jatrophacurcas* leaf extracts, neem leaf extracts and ash are capable of suppressing the pathogen there by giving the plants room for absorbing water and nutrient for growth. There was no significant difference among the concentrations and control treatments on leaf size of tomato seedlings. All concentrations show the same leaf area.

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Table 1 Colony Diameter and Percentage Inhibition of *Fusarium oxysporum* Treated with Jatropha, Neem Extract and Ash

Treatment	Colony Diameter (mm)	Inhibition (%)
Jatropha	6.92	37.50
Neem	7.25	32.53
Ash	9.08	24.86
Control	11.58	0.00
LSD (0.05)	1.73	13.82

Table 2 Colony Diameter and Percentage Inhibition of *Fusarium oxysporum* at Various Concentrations of Jatropha, Neem Extra and Ash

Concentration (ml)	Colony diameter (mm)	Inhibition (%)
1.0	20.33	42.92
1.5	17.42	53.35
2.0	23.08	30.88
LSD (0.05)	6.88	15.95

Table 3 Growth Character of Infected Tomato Seedlings Treated with Jatropha, Neem Extract and Ash

Treatment	Height (cm)	Stem girth (cm)	Leaf no.	Leaf size (cm^2)
Jatropha	39.25	13.44	69.38	10.94
Neem	35.25	12.25	52.81	11.06
Ash	34.63	12.13	51.88	11.44
Control	26.33	10.00	35.00	8.25
LSD (0.05)	8.20	1.23	17.50	1.82

Table 4 Growth Character of Infected Tomato Seedlings Treated with Jatropha, Neem and Ash at Different Concentration in ml/kg

Concentration (ml)	Height (cm)	Stem girth (cm)	Leaves no.	Leaves size (cm^2)
10	42.08	13.00	68.75	11.93
5.0	40.43	13.33	67.50	11.83
3.0	36.69	14.08	60.83	12.83
LSD (0.05)	9.46	1.40	2.20	2.10