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

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ABSTRACT
A survey was conducted to find and control *Fusarium* wilt of tomato at Labundo, Gyariyo and Battare all located in Girei Local Government Area of Adamawa State. The treatments combinations consisted of three levels each of jatropha leaf extracts, neem leaf extracts and tamarind ash assigned in s 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). Surveyed result of naturally infected tomato plant identified *Fusarium oxysporum* in all the surveyed locations as the wilt pathogen. The highest percentage incidence of tomato wilt was recorded in Gyariyo with 85.07% followed by Labundo with 26.69% and Battare with 26.06%. The result also showed significant difference ($P \leq 0.05$) with respect to disease severity. Gyariyo had the highest severity of 88 %, Labundo 36 % and Battare having the least severity of 34 %. 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 but no significant different between 5.0 ml/kg and 10 ml/kg. 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 advised to use jatropha leaf extracts at 5.0ml/kg.

Keywords: Fusarium wilt, jatropha, neem, ash, extract, concentration, in-vivo and in-vitro.

INTRODUCTION
Fusarium wilts of tomato is one of the most economically important and widespread diseases of the cultivated tomato (*Solanum lycopersicum* L). 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 vigor 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 objective 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.

MATERIAL AND METHODS

The survey and control of tomato *(Solanum lycopersicum L)* 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.

Survey and Sampling

Three different locations in Girei Local Government Area of Adamawa State were surveyed and samples of plants along with the soil were collected from tomato fields. Collection of the plant and soil samples was done from 3 fields (i.e. Labundo, Gyeriyo and Battare). Each field represented five hundred square metres. Soil and all the plants part samples from every field (five samples collected from each corner and the centre of field) were taken, pooled and mixed well into a single composite sample and kept in a plastic bag. Moist soil samples were immediately stored in sealed plastic bags at 4 °C. Fungal isolation was done within one week of sampling (Joshi *et al.,* 2013).

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. These were placed in 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 mycelia 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 McCartney 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 McCartney bottle as pure culture for subsequent use

Preparation of Jatropha and Neem Leaves 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 120 ml 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 2.0 ml) 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 250 ml 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 20 mls of the mixed media was dispensed into 9 cm 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.

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

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

Where: DC - Average diameter of fungal spore in control.
DT - Average diameter of fungal spore with treatment.

In-vivo Screen House Trial

Tomato seeds (*Solanum lycopersicum* var. UC, 82B) were sterilized in hydrochloric acid and sodium hypochlorite for 5 min, 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) was 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 3 ml/kg, 5 ml/kg, and 10 ml/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; Seedlings height (cm), stem girth (cm), leaf number and leaf area.

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 AND DISCUSSION

Survey

The survey of *Fusarium* wilt of tomato seedlings showed the incidence of wilt from the 3 locations sampled was significance difference ($P \leq 0.05$). The study recovered *Fusarium oxysporum* as the fungal isolate associated with *Fusarium* wilt of Tomato plant in Girei study area. The highest percentage of disease incidence was recorded in Gyariyo with 85.07% followed by Labundo with 26.69% and Battare with 26.05%. Likewise, severity with 88.00% recorded in Gyariyo and 36.00% in Labundo and 34.00% in Battare (Table 1). Similar reported by Joshi *et al.* (2013) showed that thirty nine isolates were confirmed as *F. oxysporum.* And the disease incidence was found within the range of 0%-78.7%.

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 mycelia growth of *F. oxysporum in-vitro.* The effect of jatropha leaf extracts, neem leaf extracts and ash on the colony growth and inhibition of *F. oxysporum* were significant at $P<0.05$ as shown in Table 2. Leaf extract of jatropha showed best control of disease caused by the *F.*

oxysporum with least colony growth of 6.92 mm 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 9.08 mm and inhibition zone of 24.86%. The highest colony diameter of 11.58 mm 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 leafs extracts contained more of the active ingredients that are effective in controlling the mycelia development of *Fusarium* wilt. This study also showed that increase in the concentration of jatropha leaf extracts reduces the mycelia growth of *F. oxysporum* (Table 2). 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.* More so, 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/20 ml of PDA.

Concentration of 2.0 ml gave the least control with the colony growth of 23.08 mm, and 1.0ml concentration level gave 20.33 mm colony growth and also treatment with 1.5 ml produced the highest control of the pathogen with colony growth of 17.42 mm. And the results of the percentage inhibition showed significant difference among the concentration levels of 1.5 ml, 1.0ml and 2.0ml but differs significantly with 0.0 ml. The concentration of 1.5 ml 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 3). There was significant difference in inhibition between 1.0ml and 2.0ml/20 ml of PDA. This implies that high concentrations of extracts pose an adverse effect.

Also the phytochemical properties of jatropha plant which attributed to several components, including saponins, lectin (curcin), phytates, protease inhibitors, and curcalonic acid and phorbol esters (Adolf *et al.*, 1984; Makkar and Becker, 1997), as well as secondary metabolites alkaloids, tannins, flavonoids, phenols and saponins (Martinez-Herrera *et al.*, 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 (Table 3). Similar findings were obtained against *Fusarium* wilt of Carnation (Chandel and Tomar, 2008). The bioefficacy of neem extract on pathogens can be attributed to the fact that neem has active compounds such as azadirachtin, nimbin, nimbidin, nimbinin and azadirone which are antifungal, antibacterial 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% (Table 3), 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* mycelia 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 results as in Table 4 has 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 (39.25 cm) of tomato seedlings compared to other treatments, while the control treatment has the least plant height (26.33 cm) (Table 4). 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 phyto fungicide and phyto fertilizer to control the pathogenic fungi and enhance the growth of various economically important food crops.

The result in Table 5 revealed that the highest concentration of *Jatropha curcas* leaf extracts, neem leaf extracts and ash produced higher growth of tomato seedling. As the concentrations increases the plant height of tomato seedlings increases. Applications of botanical substances such as jatropha leaf extracts, neem leaf 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 vigor compared to low 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 (Table 4) 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 was observed in plants under control, 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, 5.0 ml/kg and 3.0 ml/kg (Table 5). All the concentrations showed increase in

stem girth of tomato seedlings. Similar finding was made by Mark *et al.* (2015)

The Screen house findings also revealed that there was significant difference between treated seedlings and the control in all the parameters measured (Table 4 and 5). Result on leaf number showed that plant treated with *jatropha curcas* leaf extracts, neem leaf extracts and ash gave higher leaf numbers of 69.38, 52.81 and 51.88 respectively, than the control with leaf number of 35.00. 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 jatropha leaf extracts, neem leaf extracts and ash showed a significant difference with the control plant (Table 4). The treated plants show higher leaf areas of 10.94, 11.06 and 11.44 respectively, than the control with leaf area of 8.25. As such, the control gave the lowest leaf size, this may be due to the fact that phytochemicals present in jatropha leaf extracts, neem leaf extracts and ash are capable of suppressing the pathogen thereby giving the plants room for absorbing water and nutrient for growth. There was no significant difference among the concentrations on leaf size of tomato seedlings. All concentrations show similar leaf area (Table 5).

CONCLUSION

The experimental trial to study the control on *Fusarium oxysporum using* jatropha leaf extracts, neem leaf extracts and ash showed an effective control *in-vitro* and *in-vivo*. The result of i*n-vitro* trial showed that there is inhibition on colony growth of *Fusarium oxysporum*. For the *in-vivo* trials, an increase in growth character is observed as the plant extracts (treatments) are administered. Therefore jatropha leaf extracts, neem leaf extracts and ash have the potential to replace the hazardous and environmentally unfriendly fungicides. They are also known to increase soil fertility since they had increase effect on plant growth characters.

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Table 1. Disease Incidence and Severity of Tomato Wilt Pathogen (*Fusarium oxysporum*) Surveyed at Different Farms Locations in Girei of Adamawa State

Location	Diseases Incidence (%)	Disease Severity (%)
Labundo	26.69	36.00
Gyeriyo	85.07	88.00
Battare	26.05	34.00
LSD (0.05)	6.99	52.00

Table 2. 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 3. Colony Diameter and Percentage Inhibition of *Fusarium oxysporum* at Various Concentrations of Jatropha, Neem Extract 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 4. Growth Character of Infected Tomato Seedlings Treated with Jatropha, Neem leaf Extracts 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 5. Growth Character of Infected Tomato Seedlings Treated with Jatropha, Neem leaf extracts 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