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International Journal of Current Research
Vol. 5, Issue, 03, pp.505-507, March, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

THE ANTIFUNGAL POTENTIALS OF *Jatropha curcas* L. ON MANAGEMENT OF *Fusarium* WILT OF TOMATO IN FADAMA LANDS OF SOKOTO, NORTH WESTERN NIGERIA (*Fusarium oxysporum* F. sp. *Lycopersici*)

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ARTICLE INFO

Article History:
Received 22nd December, 2012
Received in revised form
24th January, 2013
Accepted 18th February, 2013
Published online 19th March, 2013

Key words:
Fusarium oxysporum, Antifungal, *Jatropha curcas*, *in vitro*, *in vivo*.

ABSTRACT

In present study, *Fusarium oxysporum* F. *sp*. *Lycopersici* were isolated from infected tomato plants in Fadama lands of Sokoto metropolis and identified based on morphological and cultural characteristics. The *in vitro* efficacy of *Jatropha curcas* L. aqueous and ethanol of seed and leaf extracts at concentrations, 5, 10, 20, 40 and 160 mg/ml were tested against *F. oxysporum* using poison food techniques. The result showed growth impairment in all extracts. Highest inhibition were recorded in ethanol extracts of seed and leaf 92.2 and 90.2% followed by water extracts 71.7 and 58.3% at 160 mg/ml. Low inhibition were recorded at 5 and 10 mg/ml aqueous extracts. The *in vivo* experiment showed that treatment with *J. curcas* seed extract recorded 13.6% disease incidence compared with control 91.4%. A significant ($p<0.05$) in extract concentration activities were observed. The findings indicate promising potentials of *J. curcas* in management of fungal diseases.

INTRODUCTION

Fusarium wilts of tomato caused by *Fusarium oxysporum f.sp. lycopersici* (Sacc.) W. C. Snyder & Hansen 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 especially, in the savannah areas of Nigeria (Erinle, 1981). 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). Damages attributed by *F. oxysporum* includes stunting, yellowing of the lower leaves, formation of adventitios roots, wilting of leaves and young stems defoliation, marginal necrosis of remaining leaves and finally death of entire plants (Agrios, 2002). The initial symptoms appear as chlorosis and distortion of the lower leaves, often on one side of the plant, chlorotic wedges, necrosis and plant stunting become more pronounce as the disease progresses (Bowers, 2001).

The use of plants in management of plant disease is an aged practice in Africa largely due to their availability, affordability and cheapness (Nwosu and Okafor, 1995). The increasing incidences of fungal infections and resistance to conventional synthetic fungicides have necessitated the need to find a very effective antifungal agent from other sources especially from higher plants (Shu, 1998). Plants have been used as antifungal agents (Ruskin, 2002; Tripathy and Dubey, 2004; kumar *et al*., 2007; Bajpai *et al*., 2007) and have been reported to be safe, non toxic to mammals, biodegradable and effective against plant pathogens (Shivpuri *et al*., 1997). In Nigeria, Plant extracts have been used to control fungal diseases of many crop plants (Amadioha and Obi 1998; Okigbo and Emoghene, 2004; Okigbo and Nmeka, 2005). *Jatropha curcas* (physic nut, purging nut) is known in Nigeria by various local names as bini da zugu, lapapa, ologbo, it is a multipurpose hedge plants which are widely distributed all over the world, especially in the tropics. *J. curcas* (family *Euphobiaceae*) is a drought-resistant small tree or large shrub (up to 5 m tall), which probably originated from Mexico and Central America (Makkar and Becker, 2009). The plant is now cultivated in many locations in the tropics where it is known for traditional medicinal and industrial uses (Heller, 1996). Numerous investigations have been carried out to determine the antimicrobial and medicinal properties of various parts of these plants (Lans *et al*., 2001; Ogbebor *et al*., 2007).

Considering the availability and diversity of this plant in local community in Nigeria, the antifungal potentials of this hedge plant on management of *Fusarium oxysporum* have not been fully investigated and documented. This study attempted to evaluate the potentials of aqueous and ethanol extracts of *J. curcas* seed and leaves in the management of *Fusarium oxsporum in vitro* and *in vivo*.

MATTERIALS AND METHODS

The Study Area

The study was conducted during the months of September to March, 2012 in the Department of Biological Sciences, Usmanu Danfodiyo University Sokoto. Sokoto State is one of the northern states where a large proportion of tomato production and storage take place annually. The area is located in the north – western Nigeria (Longitude 3 – 9° East; latitude 10 – 14° North). It is characterized by long dry season (October to April) and a short rainy season (May to September). Average monthly temperature ranges from 21 to 35℃ and is lowest in December and January. Heat is more severe in March and April, the mean annual temperature is 27°C (Ojanuga, 2006).

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Plant materials and extract preparation

The leaves and seeds of *J. curcas* were collected from the permanent site of the Usmanu Danfodiyo University Sokoto, and identified in the herbarium of the institution. The seeds were sun dried and leaves were dried in an ambient laboratory conditions. The dried seeds and leaves were separately ground into powder in a blender (Philips, Mexico City, Mexico). The resultant powders were sieved in a fine sterile mesh (0.2 mm) to obtain the finest powder. One hundred gram (100 g) portion of each of the seeds and leaves powder were extracted separately with 500 ml of water and ethanol for 48 hours at room temperature. The extracts were filtered using a sterile muslin cloth. The filtrates were evaporated to dryness using a water bath at 40^{o}C. The residues were stored at 4^{o}C for subsequent use.

Collection of Samples

Tomato plants showing symptoms of wilting and soil samples from three different Fadama areas: Kwalkwalawa, Dundaye and Kofar fari were collected for microbial analysis.

Isolation and identification of pathogen

Fusarium oxysporum f. sp. lycopersici was isolated from the stem and roots of naturally infected tomato plants in Fadama lands of Sokoto. They were surface-sterilized by soaking in 0.5 % bleach solution (sodium hypochlorite) for five minutes, washed in three changes of sterile distilled water. The small sections of disease tissues were plated directly on sterile potato dextrose agar (PDA) using flamed inoculating pins; replicates were made. Isolation of fungi in soil samples was done on (PDA) by pour plate technique (Cheesbrough, 2000). Samples of appropriate dilutions were transferred to sterile Petri dishes, swirled to distribute the inoculums evenly, left to solidify on laboratory bench. The inoculated plates were incubated at (28 ± 2^{o} C) for 7 days. Developed fungal colonies were sub – cultured continuously on fresh PDA plates to obtain pure culture of the isolate. Fungal isolates were identified based on cultural and morphological characteristics (Barnett and Hunter, 1998; Koneman *et al.*, 2006).

Antifungal testing

Potato dextrose agar (PDA) was prepared and autoclaved before the addition of extracts. Seed and leaves extracts were mixed with the molten agar (at 45^{o}C) to final concentrations of 5, 10, 20, 40 and 160 mg/ml and poured into Petri dishes. Each plate was swirled carefully until the agar began to set. Blank plates containing only PDA served as control. The prepared plates were inoculated with plugs obtained from actively growing margin of fungi plates and incubated at 25^{o}C for 7 days (Aliero *et al*., 2006). The diameter of the fungal growth was measured and expressed as percentage inhibition of three replicates (Baretto *et al*., 1997; Quiroga *et al*., 2001):

$$\% \text{ Inhibition} = \frac{\text{Growth of fungal colony in control- growth of fungal colony in extract}}{\text{Growth of fungal colony in control}} \times 100$$

Determination of % disease incidence

The seedlings of Solanum lycopersicum L. raised in a sterile soil were arranged in space away from direct sunlight were transplanted into sterile soil filled polythene bags in triplicates after 2 weeks, these were watered twice daily. The sporangia of stock culture of *Fusarium oxysporum* were dislodged with camel hair brush in 20 ml sterile distilled water per culture plates. The inoculums were sprayed on seedlings and aqueous seed and leaf extracts were applied as drench each 5 mg/ml, 40 mg/ml and 160 mg/ml as treatments, control experiment was set with seedlings and inoculums only. Disease incidence was observed at 8th week after transplanting using standard method (Wheeler, 1969).

$$\% \text{ Diseases incidence} = \frac{\text{No of infected plants}}{\text{Total No. of Plants}} \times \frac{100}{1}$$

All data collected were statistically analyzed for significant difference ($P<0.05$) by analysis of variance (ANOVA) and means were separated using Duncan Multiple Range Test (DMRT). (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

The results of bio-efficacy of aqueous extracts of *J. curcas* seed and leaf extracts evaluated against *Fusarium oxysporum* were depicted in Table 1. Highest percentage inhibition was recorded at160 mg/ml aqueous leaf 71.7% and 58.3% seed extracts. Over 50% inhibition on growth of *F. oxysporum* was achieved above 40 mg/ml in leaf extracts. At 5 and 10 mg/ml very low inhibition sensitivity were obtained in this investigation.

Table 1. Inhibition Percentage of *Fusarium oxysporum* by Water Extracts of *J.curcas*

Conc. (mg/ml)	Seed extracts	Leaf extracts
5	$20.7^{c} \pm 1.2$	$25.7^{d} \pm 4.5$
10	$25.4^{c} \pm 9.3$	$34.6^{cd} \pm 9.3$
20	$30.7^{bc} \pm 7.4$	$44.8^{bc} \pm 6.4$
40	$48.9^{ab} \pm 8.4$	$55.7^{ab} \pm 2.4$
160	$58.3^{a} \pm 3.8$	$71.7^{a} \pm 2.5$

^{a,b,c} means in a column with different superscripts are significantly different ($P<0.05$) Values are means ± standard error of 3 replications

Table 2. Percentage Inhibition of *Fusarium oxysporum* (Ethanol Extracts)

Conc. (mg/ml)	Seed extracts	Leaf extracts
5	$45.6^{c} \pm 5.1$	$43.7^{d} \pm 5.0$
10	$48.9^{c} \pm 4.0$	$56.7^{c} \pm 3.1$
20	$61.9^{b} \pm 3.8$	$63.1^{c} \pm 3.0$
40	$69.8^{b} \pm 2.3$	$77.8^{b} \pm 1.2$
160	$90.2^{a} \pm 1.3$	$92.2^{a} \pm 1.4$

^{a,b,c} means in a column with different superscripts are significantly different ($P<0.05$) Values are means ± standard error of 3 replications

Antifungal efficacy of *J. curcas* seed and leaf ethanol extracts were depicted in Table 2. A close study of the percentage inhibition of *J. curcas* extracts reveals that ethanol extract of the leaf is the most sensitive and effective against the tested pathogenic fungi *in vitro*, the ethanol extracts significantly reduces growth of the tested fungi up to 92.2% and 90.2% at 160 mg/ml leaf and seed. The degree of inhibition of test isolate at different extracts concentration varies, which shows that they are more fungitoxic at higher concentrations, the result of study correspond with investigation by (Amah and Aliero, 2009) on extract *of J. curcas* against *F. oxysporum* were by 54% inhibition was recorded at 80 mg/ml and low inhibition were obtained at 10 and 20 mg/ml. Other investigations showcasing the antimicrobial activities of *J. curcas* that corroborates with this study include (Atindehou *et al*., 2002; Oskouein *et al*., 2011; Makun *et al*., 2011 and Muklesur *et al*., 2011). The result of percentage disease incidence on tomato seedlings were depicted in Table 3. Leaf extracts at 160 mg/ml significantly reduces disease incidence to 13.6% as compared with control 91.4% .Notably there are variations in extracts concentrations activity. Plant extracts and essential oils are effective antimicrobial agents of soil borne fungi, food storage fungi, foliar pathogens and nematodes and do not produce any residual effects because they are non pollutant, cost effective non harzadous, readily available and do not disturb ecological balance (Babu *et al.*, 2009). Significant ($P<0.05$) in extract concentrations activity were observed.

Table 3. Percentage Disease Incidence due to Application of *Jatropha* Plant Extracts on Tomato Seedlings

Conc. (mg/ml)	Seed extracts	Leaf extracts
5	$53.1^{b} \pm 3.3$	$23.5^{c} \pm 3.3$
40	$27.2^{c} \pm 8.9$	$12.1^{c} \pm 2.1$
160	$13.6^{c} \pm 4.5$	$67.9^{b} \pm 9.6$
Control	$91.4^{a} \pm 4.5$	$91.4^{a} \pm 6.9$

^{a,b,c} means in a column with different superscripts are significantly different ($P<0.05$)Values are means ± standard error of 3 replications

Conclusions

The result of the study demonstrates the potential use of *J. curcas* as biofungicide. The extract of *J. curcas* seed and leaf demonstrated significant antifungal properties on phytopathogenic fungi tested. The observed antifungal sensitivity was highly significant ($p<0.05$) on the test isolates, *Fusarium oxysporum* in the *in vitro* experiment. Reduced diseases incidence was also observed with increase in concentration for the *in vivo* study. The findings of the study showed extract of leaf and seed of *J. curcas* would serve as natural fungicide for agricultural application at low cost. A further step towards isolation and characterization of the antifungal properties and its evaluation for plant protection strategies is desirable.

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