Full Length Research Paper

Identification of micro-organisms associated with *jatropha curcas* and inhibition by selected natural plants extracts

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Studies were carried out to determine the microorganisms associated with *Jatropha curcas* and the effect of selected botanicals crude extracts (bitter leaf: *Vernonia amygdalina* and Uziza leaf: *Piper guineensis*) on the inhibition of these microorganisms. The experiment comprised three extract concentrations 20, 30 and 50% and was carried out in a completely randomized design with six replications. Results show that only *Rhizoctonia* sp., *Fusarium oxysporum*, *Septoria apii* and *Aspergillus* sp. were isolated from seeds and leaves of *J. curcas*. *P. guineensis* leaf extract, at 50% concentration, inhibited the growth of these organisms (94%) more than the bitter leaf (*V. amygdalina*) extract (80%) and similar trend was observed at all levels of concentrations. The high level of microbial inhibition obtained from the *P. guineensis* on *J. curcas* would be very useful information in the production of pesticides or fungicides combinations as their effectiveness is higher when combined than when singly applied. It was observed that higher percentage germination was recorded on healthier *Jatropha* seeds than diseased ones and only *Fusarium solani* and *S. apii* were isolated from leaf samples of *J. curcas* while *Rhizoctonia* spp. and *Aspergillus* spp. were isolated from the seed samples that showed characteristic rot appearance. However, few organisms observed in *J. curcas* showed that the crop does not provide conducive environment for the growth of many microorganisms, and as such possess fungicidal properties.

Key words: Micro-organisms, inhibition, selected, natural plants, *Jatropha curcas*.

INTRODUCTION

*Jatropha curcas*, a multipurpose, drought resistant, perennial plant belonging to the family Euphorbiaceae is gaining lot of importance for the production of biodiesel (Achten et al., 2007; Achten et al., 2008; Kumar and Sharma, 2008; http://www.worldauroforestv.or /af/treeb/NFTPDF&Jatrova curcas). It is used as lubricants, biofuel, soap making and cosmetics. It is a tropical plant that can be grown in low or high rainfall areas either in the farm as a commercial crop or on the boundaries as a hedge to protect fields from grazing animal and to prevent erosion (Oliver, 1960). The plant survives in tropical and subtropical ecosystem. It was found to be native to Central and South Americas.

The physical properties of *J. curcas* such as moisture content, 1000-unit mass fruit part fraction, dimensions geometric mean diameter, sphericity, bulk density, solid density, porosity, surface area, specific surface area, static friction coefficient on various surface and angle of
repose, as well as mechanical properties like rupture force, deformation at rupture point, and the hull of the fruits having very high moisture content as compared to nut shell and kernel had been investigated by researchers (Isawanmi, 1984; Achten et al., 2008; Barnett and Hunter, 1998). The whole fruit contained 77.03% w.b. moisture content. The sphericity values indicated that fruit shape (0.95) is close to a sphere as compared to nut (0.64) and kernel (0.68) both of which are close to an ellipsoid (Isawanmi, 1984). It has been observed that J. curcas contains important microbial groups involved in nutrient cycling (Mohanty et al., 2013) and antifungal properties of the seed oil and plant extract has been reported (Kumar and Nutan, 2013, Okwute, 1992).

_Piper guineensis_ belongs to the family Piperaceae and it is a climber on trees. The fruits, 5 mm in diameter, are reddish brown in colour when it is fully ripped and black when it is dry and _J. curcas _is widely distributed in many parts of tropics and sub tropics of the world and can be easily cultivated in low to high rainfall areas of saline and marshy lands (Openshaw, 2000). _P. guineensis_ possesses the principles chavicine and piperine, which contributes to its flavor (Barnett and Hunter, 1998). Oliver (1960) observed that the seeds yield 1 to 2.5% essential oil, comprising mainly terpene (phellandrene and dipentene) and the Nigerian black pepper has starch and low piperine content, and they have been used extensively as an insecticide against pests of stored maize and other crops (Verma et al., 2008). They can also be used in the preservation of fish (Achten et al., 2008).

_VLANIA amygdalina_ is common in West Africa and its leaves have a sweet and bitter taste. It is an erect shrub (Perennial) 1 to 2 m tall, cultivated in open bushes and old bushes. It is nearly glabrous in stem and screaming white flowers. It possesses a quadrangular stem which branches profusely from the ground. The leaves are opposite, simple, estipulate, pubescent and ovate in shape with serrated edges (Wilson et al., 1991). The extracts of the plant can be used in treating diabetes and fever. It also contains oils that are used in flavouring in both pharmaceutical and other industries. The plant has repellant attributes to some insects (Okiobo et al., 2009).

Hence the objectives of the research were to identify the micro-organisms associated with _J. curcas_ and to determine the growth inhibition effect of _V. amygdalina_ and _P. guineensis_, leaf extract on the observed micro-organisms.

**MATERIALS AND METHODS**

The work was carried out at the Crop Science Laboratory of the School of Agriculture and Agricultural Technology, Federal University of Technology, Owerri, Imo State, Nigeria. It was carried out in a randomized complete block design (RCBD), with three levels of concentrations at 20, 30 and 50% using two treatments: bitter leaf, _V. amygdalina_ and _Uziza_ (P. guineensis) leaf extracts, the set-ups were replicated six times.

Diseased _Jatropha_ leaves and seeds were collected from the farm at FUTO research farm. They were washed, sterilized and stored inside the laboratory (in container) for analysis. _Jatropha _seeds were randomly selected and taken to the laboratory and their percentage germination was determined. Ten seeds of each of the crops from different markets were randomly selected and placed in each Petri dish with a Whatman filter paper. They were watered and monitored for seed germination and growth after 10 days as follows:

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\text{Percentage germination} = \frac{\text{Number of seeds germinated}}{\text{Total seeds planted}} \times 100
\]

**Preparation of the leaf extract**

_V. amygdalina_ and _P. guineensis_ leaves were harvested, air-dried separately and ground into powdery form and stored in the laboratory. Exactly 20 g of each of the ground leaves was weighed and soaked in 100 ml of distilled water (for each plant extract) for 20% concentration, 30 g for 30% concentration and 50 g for 50% concentration. Decantation method was used to collect the extract and stored in a plastic bottle.

All equipment used in any stage of the laboratory work was sterilized carefully using ethanol and water at a ratio of 7:3. The glassware used were first washed with clean water and packed inside the oven at 160°C for 2 h. The inoculating needle, spatula, forceps, autoclave and incubator were sterilized also.

**Preparation of the medium**

The medium used for the research work was potato dextrose agar (PDA). Exactly, 250 g of Irish potato was weighed, washed with distilled water and peeled and chopped into tiny pieces with knife and boiled for 30 min in a large beaker. The potato filtrate was collected with a clean muslin cloth and poured into a conical flask with the aid of a funnel. Exactly 20 g agar and 20 g of glucose were added to the Irish potato filtrate and shaken thoroughly to ensure a homogenous mixture. The PDA was corked with cotton wool and sealed with aluminium foil and autoclaved for about 15 min at 121°C and 1.1 kg cm\(^{-2}\) for proper sterilization and allowed to cool to 45°C.

**Isolation and incubation**

At 45°C, the medium was poured into already sterilized Petri dishes and covered. The _Jatropha _sample diseased leaves and seeds were washed and small portion of the diseased samples were cut and introduced into the Petri dishes containing the PDA and covered with polyethylene bag and labelled accordingly and was transferred into the incubator for 2 days. At the third day, growth was observed and sub-cultured to obtain a pure culture.

**Preparation of potato dextrose broth (PDB)**

After the pure culture was obtained, exactly 20 ml broth medium was prepared using only the potato which was washed, chopped and boiled for 30 to 45 min and the filtrate was collected with a muslin cloth. Thereafter, 20 g glucose was added to the potato filtrate and autoclaved for 45 min at 121°C and 1.1 kg cm\(^{-2}\), and then allowed to cool. The broth was poured into test tubes using a measuring cylinder. The pure culture was introduced into the broth using a cork borer. Then 1 ml of bitter leaf extract _V. amygdalina_ and _P. guineensis_ leaf extract each were added to the test tubes according to their percentages and labelled accordingly, and
RESULTS

The result of the investigation shows that, the microorganisms associated with J. curcas were Fusarium oxysporum, Aspergillus spp., Septoria spp. and Rhizoctonia spp. F. oxysporum had highest occurrence of 54% followed by Rhizoctonia solani, 36% (Table 1).

It was also observed that only Fusarium solani and Septoria apii were isolated from the leaves of J. curcas while Rhizoctonia spp. and Aspergillus spp. were isolated from the seed sample that showed characteristic rot appearance.

Investigation revealed that F. oxysporum recorded the highest occurrence of 54% when Rhizoctonia spp. 36%, Aspergillus spp. 35% and Septoria apii 30% were low (Table 1). On the percentage germination, healthier seed samples recorded higher percentage germination in comparison with seed samples that showed characteristic rot appearance (Table 2). The V. amygdalina and P. guineensis leaf extracts were statistically significant on the inhibition of fungal organisms at 5% probability level.

Result in Table 3 shows that at 50% concentration, P. guineensis leaf extract, recorded higher inhibition (94%) than leaf extract of V. amygdalina (80%). At 20% concentration, P. guineensis, also had higher percentage inhibition of 83.6%, when leaf extracts of V. amygdalina (66.9%) was lower. However, at 30%, P. guineensis still maintained higher percentage inhibition of 89.8% when leaf extract of V. amygdalina was lower (77.4%). This showed that P. guineensis had higher potentiality in the inhibition of the fungi irrespective of the concentration applied (Table 2).

Investigation revealed that the condition of seed was highly significant on the percentage germination of J. curcas at 5% probability level. At 7 days of sowing, fairly healthy seeds recorded significantly high percentage of germination (80%) while the unhealthy ones were lower (40%). Highest percentage germination was recorded at 9 days of sowing and fairly healthy seeds recorded high percentage of germination (90%) while unhealthy ones recorded lower 55% (Table 3).

DISCUSSION

Fungi isolated from the leaves and seed samples of J. curcas include F. oxysporum, S. apii, Rhizoctonia spp. and Aspergillus spp. This showed that J. curcas though effectively fungitoxic, still create ambient condition for the growth of these microorganisms. This is in agreement with Isawanumi (1984) who reported Aspergillus spp. and Fusarium spp. as common rot causal agents. It also agrees with the proposals of Oji and Madubuike (1992), as well as Levingston and Zarnora (2006); Bitter and uziza leaf extracts showed some inhibitory activities on the fungi isolated from the leaves and seed of J. curcas. The
extracts are significant in the inhibition of fungal organisms isolated at 5% probability level. This showed that they are fungicide to F. oxysporum, Aspergillus spp., Rhizoctonia spp. and S. apii. The highest inhibition was recorded for P. guineensis leaf extract at 50% level of concentration. These inhibiting agents have been reported by other researchers to be toxicologically safe, environment friendly, easy to use and have wide range of insecticidal activity. This report was in Conformity with that of Levington and Zarnora (2006); as well as Amusan and Okorie (2002), who reported that P. guineensis leaf extract recorded the highest inhibition of Penicillium oxalicum, Botryodiplodia and Collectotrichum undemuthianum. Higher percentage germination was observed on healthy Jatropha seeds than unhealthy ones. This may be attributed to the fact that microorganisms in their process of obtaining nutrient devastate the seed tissues, thereby destroy the components of the seed and lower it germinability. They also destroy the quantity and quality of the seeds, reducing their nutritional and biochemical constituents resulting to the reduction in their viability. This will go a long way to affirm the outcomes of many researchers, such as Gadekar (2006) as well as Abdelmonem and Rasmy (2000).

The potentials of V. amygdalina and P. guineensis leaf extract on the inhibition of diseases of J. curcas cannot be over emphasized. However, P. guineensis leaf extract, performed better than that of V. amygdalina at all levels of concentration. Only very few microorganisms were identified to be associated with Jatropha showing that this crop does not encourage growth of many microorganisms and as such it can effectively be used against pests and diseases. This will not only improve the yield of J. curcas, but will also provide much economic benefits from Jatropha cultivation and production. For good seed germination and development, healthy Jatropha seed should be used as planting material, to ensure good seed development and high yield. This approach to plant disease management is economically viable and poses little environmental risk and the treatments are available to farmers in Nigeria locally.

REFERENCES


Jatropha curcas L. Eumorphiaceae (PDF). Agroforestry Database 4.0. World AgroforestryCentre (IPGRI), in “Assessment of the potential of Jatropha curcas, (biodiesel tree) for energy production and other uses in developing countries.” Mike Benge (Bengemike at aol dot com), Senior Agroforestry Officer, USAID (Ret.).