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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

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Full Length Research Paper

Behavior of fungus *Rhizoctonia solani* under faba bean cotyledons when away from host and the effect of its starvation on aggressiveness

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Behavior of *Rhizoctonia solani* on their hosts was carried out-in many studies when the pathogen was in close proximity with its host. The purpose of this study was focused on the behavior of this fungus on faba bean cotyledons when it was away from the host and the effect of nutritional status on its pathogenicity was also taken into consideration. Observations led to speculation that *R. solani* feels its host even when it was away from it and begins to assemble its strength to grow toward the host then start to form its pathogenic structures when it reach it. Scanning electron microscopic (SEM) observations which was carried out after 24, 48 and 72 h from exposing the pathogen to the host when the fungus was away from it revealed that when the hyphal pathogen feel the host, they swell and some hyphae begin to differentiate and appear very thick, forming likelihood mycelial strands and such hyphae grow vigorously and vertically toward the host. Starvation of the pathogen was found to be vital factor in *R. solani* pathogencity. Growing of the fungus on water ager led to dramatic increase in the pathogen. This strength was confirmed by visual determination of disease severity and by determination of polyphenol oxidase activity in infected cotyledons. These results exclude the role of host exudates in the attraction of the *R. solani* toward its host.

Key words: Scanning electron microscopic (SEM), polyphenol oxidase, disease severity, host exudates.

INTRODUCTION

Rhizoctonia solani (Kuhn) = *Thanetophorus cucumeris* (Frank) Donk is one of the most important soil borne pathogen all over the world. It causes very serious diseases of wide varieties of plants ranging from damping-off till stem canker led to dramatic effects on plant nutrition and its physiology (Baker, 1970; Hanounik, 1978; Anderson, 1982; Salt, 1982; Ogoshi, 1987, Wallwork, 1996; Hsiang et al., 2006, Simonetta et al., 2007; Kammerer and Harmon, 2008). Infection structure of this fungus was studied either under light microscopy or by using scanning electron microscopy (SEM). All studies revealed that the fungus produce infection cushions on the host surface and many infection pigs form on the underside of the cushions for penetrating the host (DeSilva and Wood, 1964; Dodman et al., 1968; Bassi et al., 1978; Marshall and Rush, 1980b; Armentrout and Downer, 1986; Kazuho Matsuura, 1986; Demirici and Doken, 1997; Zheng and Wang, 2011).

There is strong evidence which suggests that infection cushion formation by *R. solani* is induced by host exudates (Wyllie, 1962; Flentje et al., 1963; Martinson, 1965; Dodman and Flentje, 1970). However DeSilva and Wood (1964) were able to produce infection cushions-like structures on washed strips of host cuticle and epidermis without the addition of the exudates. Weinhold and

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Bowman (1974) induced repression of virulence of *R. solani* by glucose and 3-O-methylglucose. Moreover, Marshall and Rush (1980) have found that the exogenous supply of 3-O-methylglucose and glucose resulted in a decided reduction in lesion development on rice seedlings and they postulated that the nutritional status of *R. solani* hyphae prior to penetration had a decided effect on aggressiveness of the pathogen.

The purpose of this study was to investigate the behavior of *R. solani* (AG 4) under faba bean cotyledons when it was away from the fungus and test the effect of nutritional status of the fungus on its aggressiveness.

MATERIALS AND METHODS

Pathogen isolation and pathogenicity

R. solani isolates were isolated from the diseased plants of faba bean (*Vicia faba* L.) collected from farm of Faculty of Agriculture, Ain Shams University, Qualiobya Governorate, Egypt. Specimens were rinsed in tap water then cut into small pieces (2-5 mm), washed three times in sterile distilled water and blotted dry on sterile filter paper. Pieces were placed on 2% water agar (WA) and incubated at 25°C for 2 days. Emerging hyphal tips were transferred on potato sucrose agar (PSA: 200 g of potato, 20 g sucrose and 20 g agar) and pure culture was transferred to PSA slants.

Pathogenicity test of *R. solani* isolates was carried out by putting sterilized germinated faba bean seeds on fungal growth of tested isolates growing on PSA medium. The most aggressive isolate on faba bean cotyledons was chosen for further studies. This isolate was found to belong to AG4.

Scanning electron microscopic observation

The fungus *R. solani* was grown on PSA medium till the dishes were completed by fungal growth. Faba bean cotyledons were prepared as mentioned later, then the top of a plastic tip of micro-pippete was immersed in the central part of cotyledons and the base part was immersed in fungal growth. Six millimeter distance was left between fungal growth and cotyledon surface. After 24, 48 and 72 h fungal growth laid beneath cotyledons was taken for observation of SAM. Control fungal pieces were taken from the same dish in a wide distance from the site of cotyledons.

The material to be examined by SEM was fixed for 2-3 h in 5% glutaradehyde in 0.1 M phosphate buffer (pH 7), rinsed twice with the same buffer, and then dehydrated by immersing in increasing concentrations of ethanol (10-100% in 10% increments, 15 min in each concentration). Mycelia were attached with colloid on stubs and immediately immersed again in liquid nitrogen for 20 min before coating with 20 µm (200 A°) thick gold in Separating Coating Unit under vacuum (Matsuura, 1986). The material was then examined in stereo scan electron microscope at 28 KV. SEM was kindly carried out at Electron microscopy unit, Faculty of Science, Ain Shams University using (JEOL) JEM.1200EXII electron microscope.

Effect of nutritional status of *R. solani* on its aggressiveness

The fungus *R. solani* was grown in Petri dishes (9 cm in diameter) contained PSA medium at 25°C. When hyphal growth reached the edge of the dish, a piece (5 mm in diameter) was transferred to the center of another Petri dish contained water agar (2%) and incubated at the same temperature in the dark till the growth filled the dish (first transfer), a piece of fungal growth was taken from the edge of the first transfer and transferred to another Petri dish contained water agar and incubated till the growth reached the edge of the plate (second transfer).

Seeds of faba bean (Giza 2 cv.) were rinsed several times in tap water, then in sterilized distilled water. Seeds were dried on filter papers then rinsed in 2% sodium hypochlorite for 5 min for surface sterilization, finally, they were washed again in sterilized distilled water and left to germinate in plastic box in the dark. Apparently healthy germinated seeds have been selected then peeled. To avoid the mass effect of the pathogen on disease incidence, the tip of a plastic tip of micropipette (2 cm long) was immersed in the central part of cotyledons and the base part of the tip was immersed in fungal growth (either on PSA, first transfer or second transfer) leaving 6 mm distance from the surface of fungal growth (Figure 1a, b).

Dishes contained cotyledons were incubated at 25°C for three days in the dark, then they were photographed and disease incidence was estimated on cotyledons using adopted scale ranged from 1-5 where: 1: apparently healthy cotyledons; 2: very weak infection; 3: moderate infection; 4: very severe infection; 5: cotyledons completely covered by fungal mats and died. Three dishes were used as replicates for each treatment and five cotyledons were put in every dish. This experiment was repeated at least three times. Average of disease incidence was calculated and standard deviation(s) was estimated (Ghahramani, 2002). Sticky strips were used to make a finger print of hyphal growth and infection cushion on faba bean cotyledons and to observe them using light microscope (Leica DM2500) connected to computer in Histopathology Unit, Plant pathology Department, Faculty of Agriculture, Ain Shams University. In another experiment, *R. solani* was grown in PSA medium till the fungus fills the dish. Strips 1 cm width were carried in the growth then transferred to another sterilized Petri dish contained filter paper. Strips of fungal growth contained the medium were pasted on sides of the dish and filter paper was wetted by 1 ml of sterilized distilled water. Tow cm. apart from fungal growth sterilized cotyledons of faba bean were put on wetted filter paper, and tow cotyledons were put in every dish. Control dishes were carried out as mentioned without faba bean cotyledons. Dishes were incubated at 25°C in the dark and observed periodically and photographed after 3 days.

Determination of polyphenol oxidase activity (PPO)

PPO activity was determined in five seeds with the highest degrees of disease incidence for each particular treatment. Infected seeds were grinded in pistil and mortar at high speed in phosphate buffer solution (pH 5.5) (1:2 w/v). Suspension was centrifuged at 3000 g for 10 min at 4°C. Supernatant was taken for determination of PPO activity using catechol as substrate according to the method described by Arnnok et al., 2010. The absorbance at 410 nm was recorded continuously at 25°C for 15 s using Unico UV-2100 ultraviolet-visible spectrophotometer, USA.

RESULTS

Behavior of the fungus *R. solani* when away from host

As shown in Figure (1a) when the fungus was far from its host either vertically or horizontally (Figure 2a) the fungus try to reach it, where it gathering pace to reach the host.

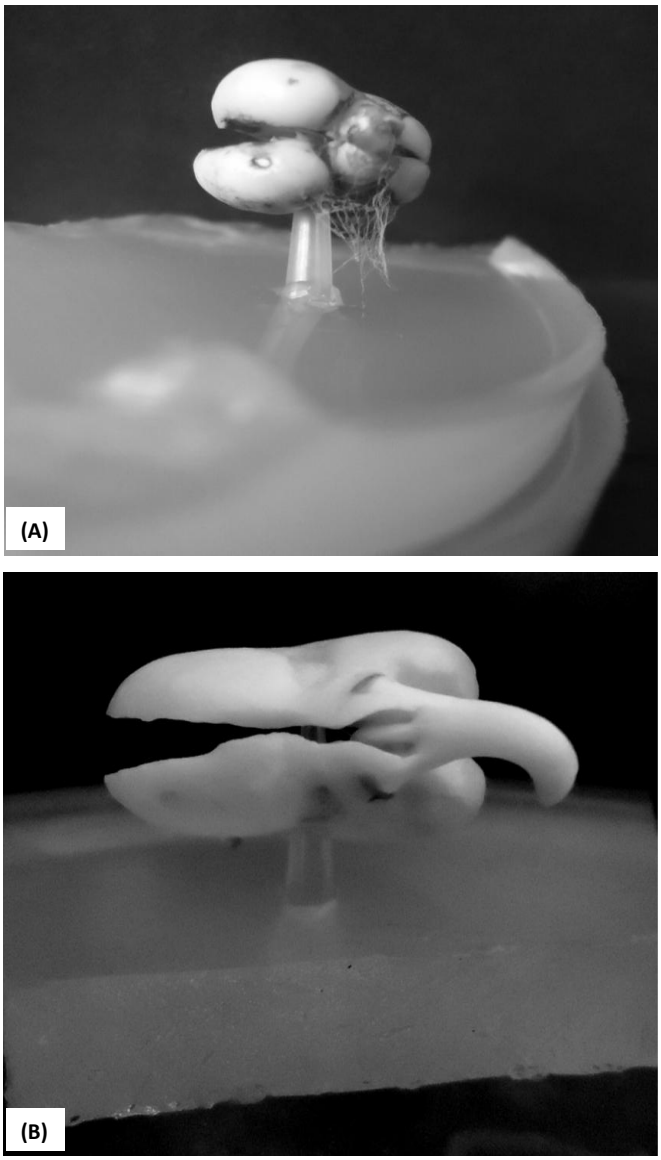

Figure 1. Hyphae of Rhizoctonia solani growing up, reached the cotyledons, invade it forming disease symptoms. A) Cotyledons fixed on fungal growth on water agar, b) water agar free from fungal growth.

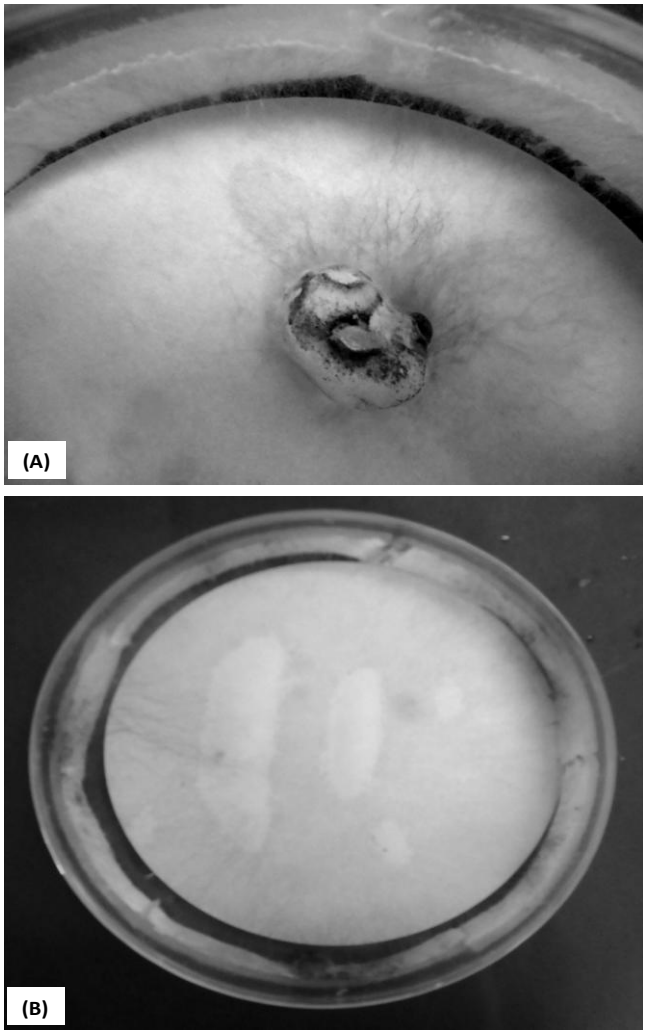

Figure 2. Hyphae of Rhizoctonia solani grow horizontally to reach the cotyledons. A) In the presence of cotyledons, b) in case of absence of cotyledons.

As shown from Figure (2a) fungal hyphae were collected together and grown vigorously toward the host comparing with control plate were the fungus poorly grows regularly toward the moist filter paper. When it reaches its host it begins to produce its infection cushions (Figure 3) in order to penetrate host tissues.

This phenomenon was deeply studied using SEM. Germinated faba bean cotyledons were fixed vertically away from the fungus and a piece of the fungus laid beneath cotyledons was taken for SEM after different intervals that is, 24, 48 and 72 h. Figures (4a, b, c, d and f) illustrate the response of the fungus when it feels its host. As Figures indicate, after 24 h the fungal hyphae swelled to very great extent comparing to fungal hyphae very far from the host, the deviation from the normal case (control) include hyphal anastomosis, strong hyphal branching, hyphal likelihood strands and swelling. After 48 h, in addition to the previously mentioned observations, fungal hyphae started to differentiate forming strands consist of at least two thick hyphae and started to grow vertically. After 72 h these strands grow vigorously vertically toward the host. Swellings of hyphae began to decline indicating that nutrients were translocated from non invading hyphae to invading ones.

Effect of starvation of *R. solani* on its aggressiveness

The fungus was starved by successive cultivation from PSA medium to water agar. The transfer on water agar

Figure 3. Infection cushions on invaded by *Rhizoctonia solani* faba bean cotyledons (X 100).

was carried out twisly. Germinated faba bean cotyledons were put away (6 mm) from fugal growth in order to rule out the effect of fungal mass on disease incidence. This experiment was repeated three times, and data are presented in Table 1, Figures 5 and 6. It is evident that starvation of *R. solani* resulted in significant increase of disease incidence. More starvation led to the higher aggressiveness. The higher five cotyledons severely injured from the three experiments were chosen for photographing (Figure 5). As shown in the figure, starvation of the fungus led to severe disease incidence.

In order to make sure that starvation of the fungus led to increase in the aggressiveness of the pathogen, PPO activity was determined in severely infected five cotyledons subjected to the pathogen. Data are illustrated in Figure 7. PPO activity significantly increased in cotyledons subjected to the more starved growth compared to cotyledons subjected to feed growth.

DISCUSSION

What is going on when the fungus *R. solani* was away from its host? Does it feel it and what are the events that will be followed when the pathogen sense the host? These questions were the subject of the present study. It was noticed that the pathogen *R. solani* sense its host even when it was away from it. This phenomenon was noticed either when the host was found vertically or horizontally for the pathogen. What are the stimuli which moved to recognize the pathogen host? In this regard, many authors - as mentioned before - postulated that infection cushion is induced by host exudates. On the other hand, Weinhold and Bowman (1974) found that glucose and 3-O-methyl glucose repressed virulence of *R. solani* on rice seedling. It could explain these results on the base that these compounds may down regulate pathogenicity genes of *R. solani* (Lopez-Berges et al., 2010). All the previous studies were carried on models where *R. solani* was in close proximity with its host. The obtained results revealed that the fungus sense its host either by moisture or heat emitted from the host or by evaporate substances or by all of them. This means that these stimuli interact with the pathogen through receptor sites located in cytoplasmic membrane of *R. solani*. The results of this interaction may lead to provoke the fungus to react with it resulting in great effects on its morphology. The interaction between stimuli and receptors during pathogenesis still obscure and need further research. However the interaction between host and pathogen during elicitation of immune system in plants was deeply studied (Chishelm et al., 2006; Spoel and Dong, 2008).

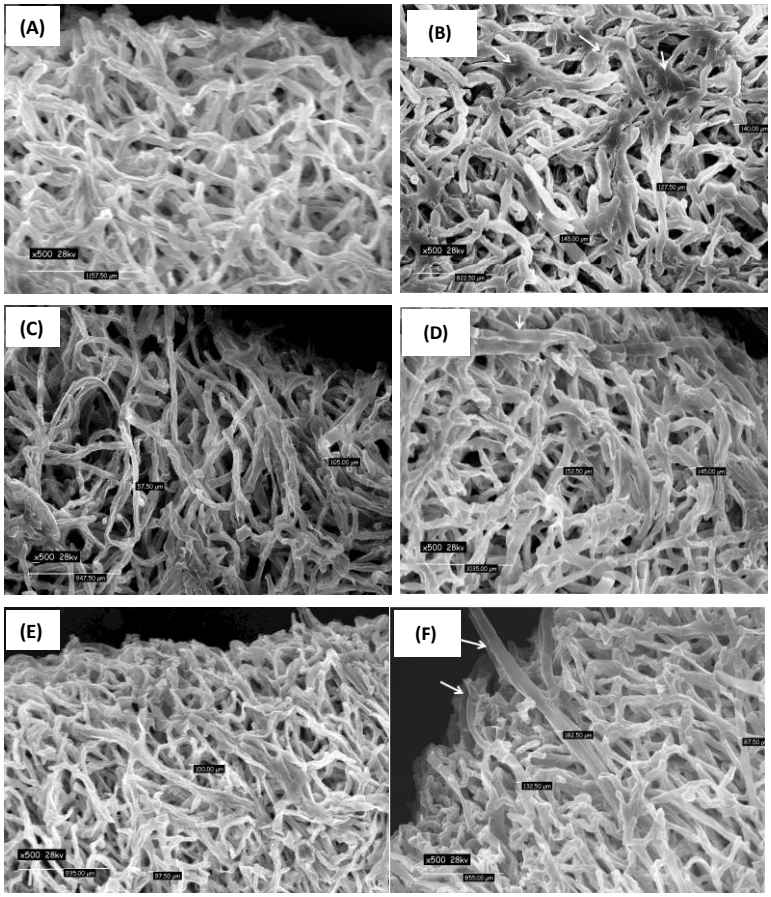

Figure 4. Behavior of *Rhizoctonia solani* hyphae which laid beneath to cotyledons . Where A, C, and D :Hyphae very far from cotyledons, after 24, 48 and 72 h respectively. B, D and F: Hyphae laid beneath cotyledons, after 24, 48 and 72 h respectively. In B stars indicate abnormal hyphal thickness, arrows indicate anastmosis and circle indicates abnormal hyphae. In D arrow indicates mycelium likelihood strand. In F arrow indicates newly differentiated hyphae, growing toward cotyledons.

The most noticed morphological deviations from the normal state - in this study are swelling the hyphae beneath the host which was observed after 24 h from the diffusion of this stimulus, this phenomenon was followed by differentiation of fungal hyphae, and strengthen of hyphae by anastmosis and by formation of something likelihood mycelial threads which grow toward the host forming infection cushions. This result revealed that not all hyphae able to infect the host but only some newly differentiated ones had the ability to infect the host. Other non invading hyphae lost their thickness after the differentiation of invading hyphae had been occurred, this action may due to the migration of biomass from non invasive hyphae toward invading ones and it is very important to investigate the type of stimuli that provoke fungal differentiation.

In this study, the role of nutritional status of the fungus on its aggressiveness was investigated. In order to reach this goal, the fungus was grown on PSA medium. After the fungal growth has reached the edge of the plate, a piece of fungal growth at the edge was taken and transferred to another dish contained water agar and this technique was repeated again on WA.

Germinated sterilized cotyledons were put on the growth leaving 6 mm distance between fungal growth and cotyledon surface. Three days later at 25°C disease severity was assayed either visually or by determination of PPO activity in the most five injuries cotyledons since PPO activity is increased in *Rhizoctonia* diseased hosts (Stackwell and Hanchey, 1987; Seo et al., 2012). Data obtained indicated that starvation of *R. solani* led to great increase of its aggressiveness, and this increasing of aggressiveness was increased more than an increase of starvation.

How the starvation does increase the aggressiveness of *R. solani*? In this respect Lopez-Berges et al. (2010)

Table 1. Disease severity on faba bean cotyledons infected by *Rhizoctonia solani* grown either on potato sucrose medium (PSA), water agar (1st transfer) or another water agar (2ed transfer). Three distinct experiments were carried out.

Nutritional status	1st experiment	2nd experiment	3rd experiment
PSA medium	2.0 ± 0.0	2.17 ± 0.40	2.57 ± 0.42
1st transfer	2.67 ± 1.21	2.86 ± 0.59	3.0 ± 0.0
2nd transfer	4.0 ± 0.93	3.13 ± 0.60	3.25 ± 0.80

Disease severity was determined according to the following scale: 1: apparently healthy cotyledons; 2: very weak infection; 3: moderate infection; 4: very severe infection; 5: cotyledons completely covered by fungal mats and collapsed.

Figure 5. Disease symptoms due to *Rhizoctonia solani* invasion of faba bean cotyledons. PSA: the fungus was grown on potato sucrose medium; 1st , the first transfer from PSA to water agar, 2ed second transfer from water agar to water agar.

have found that nitrogen limitation act as a signal to trigger the *in planta* expression of virulence genes, They postulated that the mitiogen-activated protein kinase Fmk1 is required for plant infection, but the results obtained in the present study revealed that expression of genes of pathogenecity occurring prior to infection and before it reaches the host pathogen.

The enhancing effects of starvation on fungal aggressiveness may explain how organic amendments, such as animal and green manure, organic wastes, composts and peats, have proposed to control diseases caused by soil borne pathogens (Baker and Cook, 1974; Hoitink and Fahy, 1986). There are many examples of soil borne pathogen controlled effectively by the application of organic amendments among these *R. solani* (Papavizas and Davey, 1960). The explanation of this phenomenon was not clear until the discovery of the role played starvation in elicitation of pathogenecity genes during infection.

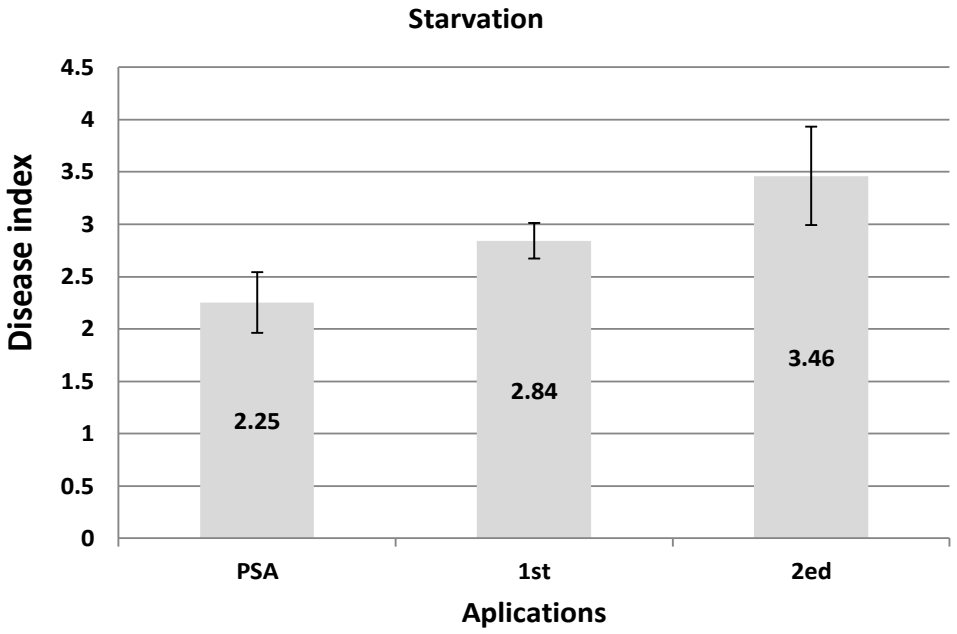

Figure 6. Effect of starvation of *Rhizoctonia solani* on its aggressiveness on faba bean cotyledons. Results are average of three distinct experiments.

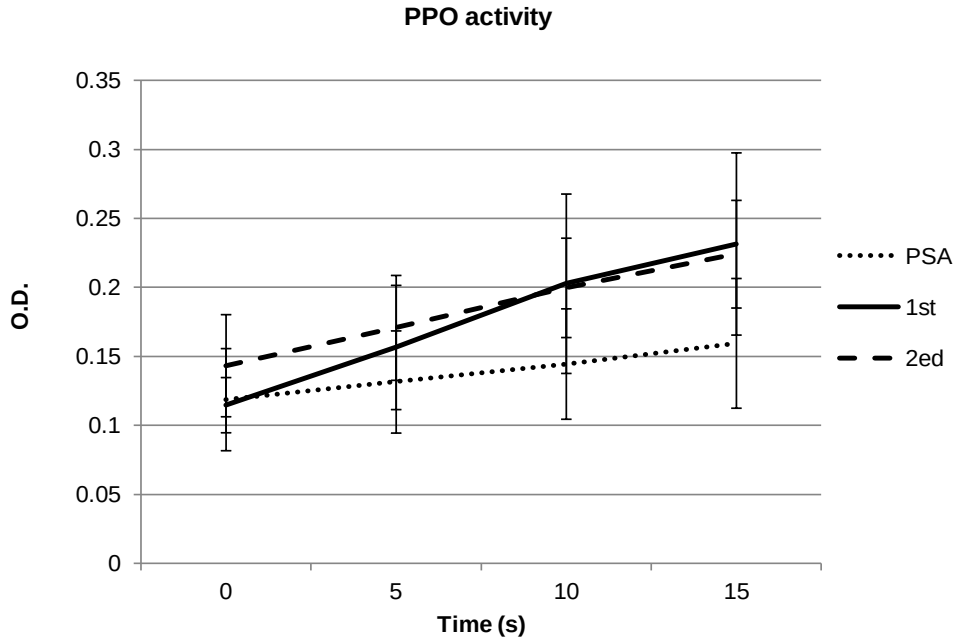

Figure 7. Polyphenol oxidase activity in cotyledons infected either by *Rhizoctonia solani* grown on PSA, 1st transfer to water ager or 2ed transfer from water agar to water agar.

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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 Euphorbiacae is gaining lot of importance for the production of biodiesel (Achten et al., 2007; Achten et al., 2008; Kumar and Sharma, 2008; http://www.worldauroforestrv.or /af/treedb/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

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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 (Isawunmi, 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 (Isawunmi, 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 subtropics 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).

Vernonia 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 (Okigbo 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:

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

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 aluminum 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

Table 1. Percentage occurrence of organism isolated.

Organism	Occurrence (%)
Fusanrium oxysporum	54
Aspergillus spp.	35
Septria apii	30
Rhizoctonia spp.	36
$LSD_{0.05}$	0.975

Table 2. Effect of bitter leaf extract and *P. guineensis* leaf extract on the inhibition of micro-organisms that attacks *J. curcas* in the field

Treatment	Treatment concentration (%)	Inhibition (%)
Bitter leaf extract	20	66.9
	30	74.4
	50	80.0
Uziza leaf extract	20	83.6
	30	89.8
	50	94.0
$LSD_{0.05}$	8.40	

Table 3. Effects of seed condition on the percentage germination of *J. curcas*.

***Jatropha* seed**	**Days after sowing**			
	5	**7**	**9**	**11**
Healthy *Jatropha* seeds	70	80	95	95
Unhealthy *Jatropha* seeds	30	40	55	55
$LSD_{0.05}$	10.146			

incubated for 7 days. At the 7th day, filter paper was used to harvest the growth. The filter paper was oven dried and weighed and recorded.

The inhibition of *V. amygdalina* and *P. guineensis* on the fungi was determined using the following formula:

$$\text{Growth inhibition (\%)} = \frac{\text{Average weight in control} - \text{Average weight in treatment}}{\text{Average growth weight in control}} \times \frac{100}{1}$$

Examination and incubation of the organism

A microscope was used to identify and observe the organism. A drop of phenolphthalein was dropped on a glass slide and the pure culture was placed on the slide and observed, drawn and identified using genera of imperfect fungi by Barnett and Hunter (1998).

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 Isawunmi (1984) who reported *Asperigillus* 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 fungi toxic on *F. oxysporum, Aspergillus* spp., *Rhizoctonia* spp. and *S. apii*. The highest inhibition was recorded by *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 Levingston and Zarnora (2006); as well as Amusan and Okorie (2002), who reported that *P. guineensis* leaf extract recorded the highest inhibition of *Penicillin 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 nutriment 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.

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