IJAAR PUBLISHING® DOI: 10.46654

International Journal of Advanced Academic Research (Sciences, Technology and Engineering) | ISSN: 2488-9849
Vol. 6, Issue 10 (October, 2020) | www.ijaar.org
Journal DOI: **10.46654/ij.24889849**
Article DOI: **10.46654/ij.24889849.e6107**

Efficacy of Crude Leaf Extracts of *Ficus exasperata* (Vahl) in the Control of Powdery Mildew on *Vernonia amygdalina* (Del)

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Abstract

Over the years, there has been pressure to reduce the use of field inventories (synthetic chemicals) in agriculture, owing to their side effects in form of carcinogenicity, and detrimental effect of residual toxicities. Based on the fore-goings, alternative choice therefore has been the use of plant bioactive chemicals as biocides which are found to be non-toxic, biodegradable and eco-friendly. This study used the crude leaf extracts of *Ficus exasparata* (sand paper plant) to control the impact of powdery mildew on *Vernonia amydalina* (bitter leaf plant). The phytoconstituents of *F. exasparata* were identified. This gave valuable information about the activity of the plant. Screening of the test plant was carried out to determine their effect on the colony diameter and sporulation of *Podosphaera xanthii* using standard methods. The crude extract was used as topical biofungicidal spray on the powdery mildew infected *Vernonia amydalina* plant stands twice daily at three concentrations (25%, 50% and 100%), twice daily for 18 days. From the radial growth results, it revealed that the crude extract at 100 % concentration recorded for the lowest radial growth rate of the fungal pathogen. The result implied that the plant bioactive chemicals in the crude leaf extracts had acted on the pathogen in a novel mechanism to protect the bitter leaf Plant which is an important soup decoctant in Nigeria, ladden with antifungal and ethno medicinal potencies. It was concluded that the plant extracts used at different concentrations showed promising prospects for control of *Podosphaera xanthii* growth of the plant in the field effectively. Subsequently, this is recommended for vegetable farmers especially those that cultivate bitter leaf plant and in the attack of powdery mildew or any other plant pathogenic fungi.

Keywords: Biocide, *Ficus exasperata*, Pathogenic fungi, Powdery mildew, *Vernonia amygdalina*.

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Introduction

Fungi are ubiquitous and cosmopolitan in distribution, being found wherever life exists. Fungal parasitism account for about 85% of plant diseases (Akinbode and Ikotun, 2008; Agrois, 2004; Lopes and Martins, 2008). Plant fungal diseases cause significant economic loses of crops, ranging from poor nutritional value, organoleptic characteristics to reduction in shelf life of post harvest crops.

Chemical fungicides have often been used to manage and control these diseases but this conduct is associated to negative impact on consumers and the environment. Moreso, the effectiveness of agro-chemicals has been reduced by the frequent development of resistance by the pathogens; negative impacts on the environments and the consequent potential hazard of the chemical residues impact on the health of the consumers (Balakumar et al., 2012; Kumar et al. 2016; Liu et al., 2016).

Currently, the search for bioactive products with novel uses and directed towards plants diseases management and control is very active. Varieties of medicinal and aromatic plants have attracted great interest in the field of plant disease control. These are plants which extracts contain antimicrobial potencies (Gahukar, 2012; Gillitzer, 2012).

Plant extracts is a group of substances extracted from different parts of the plants (leaf, roots, buds, flowers, bud or seeds) which contain compounds with anti-microbial properties. Plants extracts, essential oils, gums, resins etc have been shown to exert biological actions on plant disease pathogens (Fawazi et al., 2009; Jalili et al., 2010). These compounds are natural in origin, have minimal or no adverse effects on the physiological processes of plants; being easily convertible into organic eco-friendly molecules (Gnanamickan, 2002).

Plant extracts of *Azadirachta indica , Brassica campestris, Piper nigrum, Ageratum conyzoides, Aegle marmelos, Tagetes patula, Vitax ngundu, Pongnia pinata, Eupharbia tirucalli, Zinghiphus jujube, Ficus exasperata* and many more have been shown to contain some plant product effective as antimicrobials. These products include: Alkaloids, Saponins, Phenols, Steroids, glycosides, anthraquinones, flavonoids, resins, terpens, gum, tannins, monoterpine citrals, and many others (Bowers and Locke, 2004; Satish et al., 2007; Keller and Kumu, 2011; Hadian, 2012).

These plant products are bioactive compounds and are of low molecular weight, often referred to as plant secondary product which are not essential in sustaining plant life but are crucial for the survival of the producing organism (Hadacek, 2002). Frequently encountered plant fungal diseases include: Anthracnose, leaf and stem rusts, angular leaf spots, white moulds, downy and powdery mildews.

Powdery mildew is fungal pathogens that infect majorly, the aerial parts of plant. They cause the disease of wild and vegetable crops and depress their yield as low as 30% (Braun et al., 2002).

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Powdery mildew fungi are the Erysiphales; *Sphaerotheca fuliginea* is the most commonly encountered (Hulkelhoven, 2005).

They are biotrophic ascomycetes that invade only the epidermal cells of plants. After the comdia of the powdery mildew fungi land on the surface of the host plant; they attach to the surface of the host and penetrate the host cuticle and the cell walls, build up germ tube, depending on the genera attacking. The appressorial germ tube swells at the tip to build up an appressorium; the penetrating organ. The fungi could also penetrate the host directly through the cell wall by enzymatic degradation and mechanical means (Green et al., 2002).

Plant diseases leave our agricultural forests, grasslands, and urban landscapes diminished in economic values and beauty as they cause damages. In order to curb these damages on crops, recent developments of use of plant bioactive compounds are sought to manage the diseased plants as alternative to agrochemicals with negative impacts (Afolabi and oke, 1981; Riad et al., 2014).

Interestingly, growers have developed interest in the strategy of using phenolic-rich plant extracts as biofungicides against phytopathogenic fungi. An affordable, effective, and readily available packed with phenolic compounds is *F. exasperata*. *Ficus exasperata* belongs to the family Moraceae. It is commonly called sand paper plant. It is wide spread in all eco-region in Nigeria, in a secondary forest. *F. exasperata* is an anti-microbial agent and ethnobotanically reported to have diverse medicinal uses (Buniyamin et al., 2007; Borokuni et al., 2012).

Anti-fungal activities of *F.exasperata* have variously and extensively been investigated and reported (Odunbaka et al. 2008). It is reported to contain biochemicals such as tannins, saponins, terpenoids, phenols, alkaloids, glycosides with underlying modes of action. These biochemicals are reported to deplete fungal ions, disrupt their DNA, cause the leakage of cell contents and granulation and destruction of the cell membranes, dysfunction of mitochondrion, inhibition of spore formation, cutinase and laccases pathway (Goetz, 1999; Djipa et al., 2000; Saleem et al., 2017).

The fungal disease, powdery mildew is a very serious menace on various vegetable crops such as *V. amydalina*, a member of the daisy family, Asteraceae. Bitter leaf plant is a very important vegetable in Nigeria due to its bitter taste implicated for health benefits (Hamowia and Safran, 1994). With these knowledge on the importance of the leaves of *V. amygdalina*, the knowledge of the menace of powdery mildew on vegetable crops including the bitter leaf plant; coupled with the knowledge of the anti-fungal properties of *F. exasperate*, this study is conducted to control the impact/effect of powdery mildew on *V. amygdalina* using the crude leaf extracts of *F. exasperata.*

Materials and methods

Experimental Sites and Experimental Design

This experiment was carried out in the field of Plant Science and Biotechnology Department, Rivers State University, Port Harcourt, Rivers State, Nigeria. The experiment was done in a

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Completely Randomized Design (CRD) using *F. exasperata* at concentrations (25%, 50%, and 100%) and a control replicated three times, bringing the total number of treatments to (4) four which were assigned completely at random to each replicate.

Collection of Plant Materials

The samples of *F. exasperata* were obtained from a secondary forest of home garden, in Akwaka Rumuodumaya in Obio/Akpor Local Government Area of Rivers State. The samples were taken to the Department of Plant Science and Biotechnology of Rivers State University, Port Harcourt, Rivers State, Nigeria. The plant sample was authenticated by Dr. Ajuru, M.G. a Plant taxonomist.

Preparation of crude leaf extracts

The samples of *F. exasperata* were rinsed and washed with10 % sodium hypochlorite (NaOCl) which was prepared by adding 1 part bleach to 9 part water, air-dried under a shade and later oven dried at 70^{o}C for 20 minutes, according to Akinbode and Ikotun (2008). The plants were grinded using pestle and mortar, sieved through a 40 mm mesh and 300g of the plant material was weighed out and soaked in 600ml of distilled water in a 1000ml flat bottom flask for 24 hours. The content was filtered using a muslin cloth and kept in glass bottles until needed. The extracts at different concentrations were prepared by mixing 25 ml, 50 ml and 100 ml of the stock with 100 ml of distilled water to give final concentrations of extract at 25 %, 50 % and 100 % respectively. The extracts were dispensed into air-tight spray bottles (Mumtaz et al. 2014).

Isolation and Identification of *Podosphaera xanthii*

Diseased leaves of *V. amydalina* with the symptoms of *P. xanthii* infection were collected from a secondary forest of home garden in Rivers State. A small piece of an advancing margin of a lesion was cut with a sterile pair of scissors after sterilizing with 10 % sodium hypochlorite according to Larone, (1995). The tissues were then washed thoroughly several times with sterile distilled water and placed aseptically into 9 cm diameter Petri dishes containing 15 ml of molten Potato Dextrose Agar (PDA). The medium was impregnated with streptomycin to prevent bacterial contamination, and cultured for 7 days at room temperature (28-30^{o}C) in a sterile fume cupboard. Distinct colonies present on the plates were selected, purified by repeated culturing and maintained on PDA slants. The identification of the fungal isolate was carried out using the macroscopic and microscopic identification guide according to Larone (1995). The isolate (*P. xanthii*) was stored in an agar slant for other experiments.

Fungal culture

The plant extract was tested against *P. xanthii*. The fungal pathogen was identified and, after verification, was maintained in the laboratory in potato dextrose agar (PDA) slants at 4°C.

Effect of plant extracts on spore germination

Plant extract was placed at two spots 3-cm apart on each microscopic slide taken in duplicate. Spore suspension was prepared from 12-day-old cultures of the fungus in sterile distilled water and added (30 µl) to the plant extracts. Control slides contained appropriate solvents in place of plant extracts. The slides were incubated at 30°C in a humid chamber. After 24 h, they were stained with cotton blue/lactophenol mixture and observed (500 spores) under the microscope. Germination, if any, was noted, and the percentage inhibition was calculated.

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Mode of treatment

The crude leaf extract was prepared at three different concentrations (25%, 50% and 100%) and applied as a topical biofungicidal spray on the leaves, using a hand sprayer until wetness of plants twice daily (8am and 6:30 pm) respectively for 18 days. The plants were sprayed at onset of plant growth, prior to the artificial inoculation of plants, by *P. xanthii* isolates. Dusting was carried out at sunset before dew onset. The non-treated control plots were sprayed with distilled water.

Bioactive compounds screening:
Volumes of literatures indicate that *F. exasperata* contain bioactive chemicals such as flavonoids, alkaloids anthracquinones, steroids, tannins and terpenoids (Lawal et al., 2012; kazeen et al, 2013). The confirmatory test for bioactive compounds was a qualitative study.

Test for flavonoids:
Test was performed by using the method of Harborne, (2005). One grame (Ig) powdered sample was heated with 10ml ethyl acetate over a steam bath (40-50^{o}c) for 5 minutes. Filtrate was treated with 1ml dilute ammonia. A yellow colouration indicated positive result for flavonoids (Mumtaz et al. 2014).

Test for Anthracquinones:
Five (5ml) of chloroform was added to 0.5g of the plant material in coarse powder. The resulting mixture was shaken for 5 minutes and filtered thereafter. The filtrate was shaken with equal volume of 10% ammonium. The presence of a bright pink colour in the aqueous was an indication of the presence of anthracquinones. This was done and determined using the method of Sofowara (2006); Kazeem et al; (2013); Edeoga et al, (2005).

Test for steroids:
Test was also done according to the method of Treas and Evans and Sofowara, (2006). Onto 0.5g plant material was added 2ml acetic anhydride with 2ml concentrated sulphuric acid (H2so4). A colour change from violet to blue or green confirmed the presence of steroids in the plant sample material (Kazeem *et al*, 2013).

Test for tannins
This was carried out according to the method of Kumar *et al*, (2007). Alcoholic ferric chloride solution (10%) was added to 2-3ml crude extract of the plant material (l:l). The development of dark blue colour of the solution was an indication of the presence of tannins (Mumtaz et al. 2014).

Testing for Terpenoids:
Unto 5ml plant extract was added 2ml chloroform and 3ml $^{H}2^{SO}4$ carefully to form a layer. And a reddish brown colouration of interface indicated the presence terpenoids (Sofoworo, 2006; kazeem, 2013).

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Test for Alkaloids:
Using the method of Harborne (2005), lg of the coarse powdered plant extract was mixed with 5ml methanol and 5ml of 2n hydrochloric acid. Then filtrate was treated with Meyer and Wagner's reagents. On the basis of turbidity, the sample indicated the presence of alkaloids.

Test for Glycosides:
Kellar-kiliani test was performed using the method of Parekh and Chanda (2007). Two (2ml) of filtrate was added with 1ml of glacial acetic acid. Then 1ml of ferric chloride was added with 1ml concentrated H_2SO_4. Blue-green colouration of the solution indicated the presence of glycosides.

Test for Phenolic Compounds:
The method of Sofowora (2006) was used. 2ml of the plant extract was taken in a beaker. Then 2ml ferric chloride solution was added. A deep bluish-green colour of the solution was an indication of the presence of phenolic compounds (Mumtaz et al. 2014).

Results and Discussion

The results of the application of the biofungicide of the crude leaf extracts of *F. exasperata* for the control of powdery mildew on the infected stands of *V. amydalina* showed the effect of aqueous extracts of the test plant on spore germination of the fungal pathogen. The extract showed significant inhibition against the tested fungi. Spore germination of *P. xanthii* was totally inhibited (100% inhibition) by aqueous extracts from *F. exasperata*.

At the end of the 12^{th} day treatment, the recovery of the plant from the fungal attack became profuse. And at the end of the treatment on the 18^{th} day, there was a total expulsion of every symptoms and signs of the powdery mildew on the test plant. The controlled stands were recorded as dead by the 18^{th} day treatment.

Phytochemical analysis revealed the presence of glycosides, Steriods, tannins, alkaloids, Terpenoids, Phenolic Comps, Anthracquinones and flavonoids in the plant leaf extracts (Table1)

Table 1: Phytochemical components of leaf extract of *F. exasperata* plant against *P. xanthii*

Plant extracts	Alkaloids	Tannins	Glycosides	Phenolic Comps	Steriods	Terpenoids	Anthracquinones	Flavonoids
Leaves	+	+	+	+	+	+	+	+

Key: + = Present

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The results obtained from this study have shown that the use of crude leaf extracts of *F. exasperata* was effective in eliminating the fungi of the powdery mildew attack on *V.amydalina*. It is obvious that the biochemicals in the organic fungicide affected the phytopathogenic fungi via interference with the fungal molecular targets in the tissues and cells. The chemicals may have affected the fungal biomembranes, proteins and nucleic acid. This is clearly in line with the reports of Djipa et al., (2000); Ribera and Zuniga (2012). These researchers had reported on the efficacy of some bioactive chemicals in *F.exasperata* on plant microbial attacks.

Keuken, (1992) had also earlier reported that tannins in plant extracts attack fungi by metal depletion or direct action on their cell membranes. The result may also suggest that the efficacy of the phytochemicals such as steroids interfered with the fungal membrane integrity. This also was the report of Keuken, (1992) on the mode of actions of steroids on phytopathogenic fungi. It could also mean that the phenolic compounds acted in form of chlorogenic and caffeic acids contained in the extracts by inhibiting the formation of stilbene oxidase of the phytopathogenic fungi. This agrees with the report of Bostock et al., (1999) who reported on the inhibition of stilbene oxidase of *Monilia Fructicola* fungus.

Presence of steroids may have also affected the fungal membrane sterol with free hydroxyl groups and may have resulted in pore formation and subsequent loss of the fungal membrane integrity, in line with Ito *et al* (2005) who reported that steroid has the ability to complex with fungi free hydroxyl groups. Terpenoid may have also caused the phytopathogenic fungi to develop cytoplasmic granulation, disruptions of plasma membrane and leakage of cellular contents in line with the submission of Harris and Dennis (1976, 1977) who reported that terpenoid type causes zoospores of some fungal pathogens to granulate and disrupt the plasma membrane as well as leakage of cell contents. Little wonder why the organic fungicide was able to control the powdery mildew within 18 days. However, Akaloids effect on fungal pathogens work best at its low concentration. Alkaloids in form of allosecurinne in the extract of *Phyllanthus amarus* was reported to inhibit fungal spore germinations of Heterosporium species. This result seems to agree with this report by Adrain et al., (1997).

Fungi usually attack plant cutins by synthesizing cutinases but biochemecals in plant material such as the *F. exasperata* inhibit the production of cutinasses and lacasses pathway with which the pathogen uses to degrade the plant cell wall, so by inhibiting the pathway, the pathogen is destroyed as reported by Goetz et al (1999). This seem to be what happened in this study that made the biochemical in the crude leaf extract of *F. exasperata* eradicate the pathogen in a short time.

In the same vein, flavonoids, together with glycosylated derivatives or acylated with phenolic acids, anthocyanidins are termed condensed tannins. These would have caused membrane disruption, induction of mitochandria dysfunction, inhibited cell wall formation, cell division, RNA protein synthesis and the efflux mediated pumping system of the pathogen, thereby disorganising the action integrity of the pathogen as reported by Seleem et al., (2017); Abdelfattah and Moussaoui et al., (2019).

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With these mechanism of actions of the bioactive chemicals it is obvious that the biofungicidal action on the powdery mildew was efficacious and this is one of the indications that organic chemicals for agribusiness is very possible in the near future as an alternative to exclude synthetic chemicals from our farms and the environment.

Conclusion and Recommendation

The results obtained from this study showed that the crude leaf extract of *F.exasperata* exhibited effective antifungal potency against powdery mildew in a short time of less than one month. It is obvious that the biofungicide affected the pathogen via interference with the pathogen's molecular targets of the cells and tissues. The efficacy showed that the biocide affected the pathogens biomembranes, proteins and nucleic acid and interfered with the membrane integrity which lead to the bioactive chemical to also have complexed against the sterol of the pathogen to disorganise its virulence, which otherwise means the safety to the plant.

Based on these, more studies on biofungicide should be carried out which in combination could raise good volumes of biofungicides for agricultural sustainability and exclusion of the elaborate use of field inventories with their attendant toxicity. The study recommends biofungicides for extensive agricultural practice.

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