E-ISSN: 2789-3073
P-ISSN: 2789-3065
IJPPM 2021; 1(1): 23-26
Received: 16-01-2021
Accepted: 19-03-2021

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Mycological quality of noni (*Morinda citrifolia*) foliar infection and susceptibility to a novel agent

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Abstract
Foliar infection of Noni poses a great challenge to farmers, mycobiologists and phytopathologists despite the broad spectrum of bioactive and therapeutic potentials of the plant. This study investigates the mycological quality of Noni foliar infection and susceptibility to a novel agent (Alum). The mycological quality was assessed by culture-dependent technique using standard mycological procedures and susceptibility of isolates determined by disc and agar well diffusion methods and inhibition zones (IZs) were measured in millimetre. Fungi identified include *Aspergillus flavus*, *A. fumigatus, A. niger* and *Penicillium* species. Relative abundance (%) of fungal species in foliar infection were *A. fumigatus* (75%) being the most abundant followed by *A. niger* and *A. flavus* (50%) respectively and *Penicillium* (25%) species. Susceptibility assay of these species indicated that Alum compared appreciably with Ketoconazole (control) on dose-dependent fashion by both techniques. Data revealed that *A. flavus* (36.0mm), *A. fumigatus* (32.5mm) and *Penicillium* (30.2mm) were the most susceptible and the least was *A. niger* (30.0mm) with the novel agent. This research concludes that Alum could be used as an alternative cost-effective and ecofriendly novel or natural treatment agent to foliar related diseases or infections.

Keywords: noni, mycoflora, alum, foliar infection, ketoconazole

Introduction
Morinda citrifolia (Noni) is an important and most popularly cultivated plant of the family (Rubiaceae), genus *Morinda*, a native of Southeast Asia and now pan-tropically distributed [1, 2]. Traditionally, Noni was used as food and for treatment of a variety of diseases [3-6]. Recently, several parts of the plant such as leaves, roots, fruits, stem bark are extensively studied due to wide spectrum bioactivity and therapeutic significance.
A variety of diseases have been associated with the shoot of Noni plant ranging from leaf blight, shot hole, black leaf spot, anthracnose, sooty mould, stem cancer and blight, fruit blight, cracking and rot, black flag to plant death as well as phytoplasma disease [7, 8, 9, 10]. The plant leaves with phytoplasma (foliar) disease presents profound reduction in growth, stunted or dwarfed with puckers upwards and inwards as well as lamina shrinkages from petiole to the tip [9]. However, study on the mycoflora of Noni shoot is limited especially those associated with foliar diseases, prevention or management. Many genera of fungi have been implicated as aetiogic agents of Noni shoot diseases such as *Phytophthora*, *Sclerotiumrol, Colletotrichum* and *Rhizopus* [7, 11].
Mycoflora from foliar infection was treated with novel or natural compounds such as potash alum (potassium aluminium sulphate) because of its nontoxicity, affordability and eco-friendliness. Alum finds application in a wide variety of anthropogenic activities such as drug, food processing and preservation, domestic and industrial water treatments, antimicrobial agent, cosmetics and pharmaceutical industries as well as for treatment of 'Ich and white eye' diseases in fishes [12-16]. The study attempts to identify the mycoflora associated with foliar infection and their susceptibility to a novel agent, 'Alum'.

Materials and Methods
Sample source and Collection
Noni with foliar disease were plucked with sterile hand gloves into sterile Polyethylene bag from Dilomat farm and services, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt.
Fresh Noni with foliar disease (infected foliage with wrinkles or shrinking upward and inward) Plate 1 were collected/harvested from Dilomat farms and conveyed to the Department of Microbiology, Rivers State University, Port Harcourt for analyses.

Plate 1: Foliar infection of Noni (shrinking upward and inward) as observed at Dilomat farm.

Preparation of Alum and discs

Potassium aluminium sulphate (Vickers Laboratories, Ltd, England) was prepared by reconstituting appropriate quantity in sterile distilled water (w/v). Different concentrations of Alum were formulated by reconstituting appropriate quantities such as 2.0,3.0,4.0 and 5.0g in 100ml (w/v) to obtain 2- 5% respectively. Sterile discs (6mm diameter) were made of Whatman filter paper using an office perforating device.

Preparation of samples for mycological analysis

A 25gm portion of foliar infected sample was washed, cut in pieces (with sterile knife) and blended with a mechanical grinder in 225ml of sterile normal saline to obtain the homogenate. Further decimal dilutions were carried out before inoculation of 0.1ml aliquot onto surface-dried pre-sterilised Sabouraud's dextrose agar (SDA; Titan Biotech Ltd, India). Followed by incubation at 25±2℃ for 2 or more days.

Macroscopic and microscopic fungal cultures

Discrete fungal colonies were isolated, characterised and identified according to their shape, colour, texture and contour, and microscopically under x10 and x40 magnification by infiltration with lactophenol cotton blue [17, 18]. Cultures of each fungus was further grown for viability, confirmed and maintained on SDA and slants at 5ºC for further assay.

Antibiogram/susceptibility testing procedure

Antimycotic susceptibility test was carried out by agar well diffusion (AWD) and disc diffusion (DD) methods [19]. After adjustment of fungal cultures of 2-5days on SDA to a 0.5 McFarland turbidity standard, they were asceptically plated with the various Alum concentrations and equidistantly spaced from one another and incubated. An antimycotic drug, Ketoconazole (2.0mg/ml; Janseen, Pharmaceuticals, New Jersey, USA) was used as control both for AWD and DD methods respectively. Colony diameters were measured and the mean and standard

Statistical Analysis

Means of duplicate measurements were determined for each sample using Microsoft Excel® 2016.

Results

Table 1, depicts the inhibitory effects of Alum to fungi isolated from foliar disease/infection of Noni. Inhibition zones or susceptibility of fungal isolates to Alum were observed with increasing concentrations of Alum treatment (dose dependent). The inhibitory pattern of different concentrations of Alum by AWD and DD techniques revealed more profound effects on the isolates by AWD than with DD (Table 1). The data revealed that two (2) genera of fungi were isolated; *Aspergillus* and *Penicillium*. The most sensitive to Alum at various concentrations was *A. flavus*, followed by *A. fumigatus* and *Penicillium* sp with *A. niger* as the least. Results showed relatively larger IZs as Alum concentrations increased which almost equals those of control.

Table 1. Susceptibility of fungi isolated from Foliar infected Noni to Alum by AWD and DD techniques

	Concentration of Alum (%) and Mean IZ (mm)									
	AWDT				DDT				Control	
Fungi	2.0%	3.0%	4.0%	5.0%	2.0%	3.0%	4.0%	5.0%	2.0	mg/mL
Aspergillus flavus	28.0	30.0	34.0	36.0	22.0	25.0	30.0	24.8	34.0	39.0
A. fumigatus	22.0	29.0	30.3	32.5	15.0	25.0	29.5	25.0	30.0	35.2
A, niger	15.0	19.4	21.0	30.0	10.0	18.2	20.4	20.2	22.0	25.3
Penicillium sp.	20.0	22.5	25.0	30.2	20.0	21.4	23.0	22.53	29.6	35.0

Legend: AWDT = Agar well diffusion technique (in Bold); DDT = Disc diffusion technique; IZ = Inhibition zone (measured in millimeter (mm). Control = Ketoconazole.
Each value represents mean of 2 experiments.

The relative abundance (%) of fungal isolates associated with Noni foliar disease/infection are shown in Figure 1. The data shows that there are two (2) genera of fungi; *Aspergillus* and *Penicillium*. The dominant genus, *Aspergillus*, has *A. fumigatus* (75%) as most abundant species, followed by *A. niger* and *A. flavus* (50%) respectively, the least being *Penicillium* species (25%).

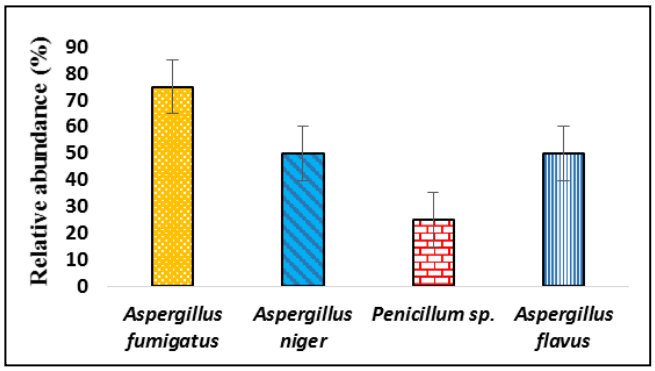

Fig 1: Relative abundance (%) of Fungal isolates associated with Noni foliar disease.

Discussion

Foliar infection or disease has over the years been a major challenge to farmers, mycobiologists and phytopathologists considering the huge economic potentials of Noni. The major genera of fungi identified were *Aspergillus* and *Penicillium* and may not be unconnected with foliar phyoplasma disease first reported by [9]. However, the report did not identify the microbes associated with the disease.
Aspergillus plays significant role phytopathogenetically as well as ecologically by production of enzymes, e.g,, polygalacturonase, capable of degrading starches, hemicelluloses, celluloses, etc [20, 21, 22]. However, It could be said that, these enzymes can negatively affect the tender structural architecture of Noni foliage to present the typical condition shown in Plate 1. The relative abundance (%) or

diversity of foliar mycoflora may be attributed to soil, climate/weather and other environmental conditions which in turn impacts the microbial community structure where the plant is grown [7, 20, 23, 24, 25].

The antimycotic potentials of Alum has been well documented in our previous studies (*in vitro* and in fields) against fungi such as *Aspergillus niger*, *A. flavus*, *A. terreus*, *Penicillium crystallium*, *Saccharomyces cerevisiae*, *Candida albicans*, *Geotrichum candidum* and *Fusarium* species [15, 26]. Its activities against fungi in the present study was in consonance with previous reports. As an eco-friendly natural agent it could be exploited and adopted to ameliorate or combat phytoplasma disease as well as other mycotic infections of plants. Its close comparison with Ketoconazole underscores antifungal efficacy.

Conclusion

The study revealed that two genera (*Aspergillus* and *Penicilluim*) of fungi were identified to be associated with phytoplasma disease of Noni. Alum treatment against these fungi exhibited appreciable mycotic activity comparable to the control which if explored could serve as a novel agent for the prevention, control and/or management of fungal infections.

Acknowledgements

We appreciate the magnanimity of Chief MM. Chinda, CEO/MD and his Technical team lead by Mr. Francis U. Abel all of Dilomat Farms and Services Ltd, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt for supplying the Noni plant samples used for this study.

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