Original Research Article

In vitro Effect of Alum on Microbes Associated with Foliar Phytoplasma Disease of Noni

ABSTRACT

Foliar phytoplasma disease of Noni (*Morinda citrifolia)* has a devastating socioeconomic impact on host crop species and a huge problem to farmers and scientists. Therefore, this study investigates the *in vitro* effect of alum on microorganisms associated with foliar phytoplasma disease (FPD) of Noni. Bacteria and fungi were isolated and identified by culture-dependent technique using standard microbiological procedures and susceptibility of species were evaluated by disc and agar well diffusion techniques and inhibition zones (IZs) were measured in millimeter (mm). The microbes identified were *Bacillus subtilis*, *B. tequilensis*, *Brevundimonas vesicularis*, *B. cereus*, *Staphylococcus aureus* and *Serratia* species as well as *Aspergillus flavus*, *A. fumigatus, A. niger* and *Penicillium* species. Relative abundance (%) of bacterial species occurred in the order; *S. aureus* > *B. tequilensis* > *B. cereus* and least being *Br. vesicularis* whereas fungi were *A. fumigatu*s (75%), *A. niger* and *A. flavus* (50%) respectively and *Penicillium* (25%) species. Susceptibility test efficacy of Alum was concentration dependent with *Serratia* sp. as (6.0mm) and *S. aureus* (4.0mm) whilst *A. flavus* (36.0mm), *A. fumigatus* (32.5mm), *Penicillium* (30.2mm) and *A. niger* (30.0mm) at 5.0% Alum concentrations as the largest IZs respectively. The minimum inhibitory concentration (MIC) of Alum against all the microflora were < 2.0gm/mL except for *S. aureus* which portends a good future prospects in agronomy. However, the higher concentrations of Alum compared favourably with Ketoconazole (control) but showed wide disparity with Ofloxacin (control). This study, however, may proffer solutions for the prevention and management of FPD or foliar related diseases by periodic spraying or fumigation with Alum.

UNDER PEER REVIEW

Keywords: Foliar phytoplasma disease, alum, noni, fumigation, ofloxacin, ketoconazole.

1. INTRODUCTION

Noni (*M. citrifolia*) is a plant with tremendous socioeconomic significance and most popularly cultivated plant of the genus *Morinda* and belongs to the family (Rubiaceae). Originally a native of Southeast Asia but now grown in a variety of tropical and subtropical environments [1,2,3]. Noni became popular following its usage as food as well as for therapeutic and pharmaceutical purposes [4-7]. As a result, several parts of the plant such as

leaves, roots, fruits, stem bark are being studied due to wide spectrum bioactivity and therapeutic importance [8,9].

A plethora of diseases have been associated with Noni shoot such as leaf blight, shot hole, black leaf spot, anthracnose, sooty mould, cracking and rot, stem cancer and blight, black flag, foliar phytoplasma disease, eventually culminating in plant death ([10-13]. FPD of Noni present profound reduction in growth, stunted or dwarfed with puckers upwards and inwards as well as lamina shrinkages from petiole to the tip [12]. However, this pathology features the connection with microorganisms, plant and climate as demonstrated by the "disease triangle concept" [14,15]. Disease development requires an adaptive plant, a harmful microorganism and appropriate natural conditions, absence of ideal conditions for any of these three elements may be negatively impacted. There is paucity of literatures on the microbiology of Noni shoot especially those associated with FPD as well as its prevention, control or management. However, earlier reports have shown that microbial community structures of Noni shoot ~~of~~ consists of *Enterobacter*, *Bacillus*, *Serratia*, *Pelomonas* and *Ochrobactrum* as well as *Phytophthora*, *Sclerotiumrol*, *Colletotrichum* and *Rhizopus* [10,16,17].

Potash alum, a natural substance have been known to possess antimicrobial properties and would be used as a treatment agent against foliar phytoplasma disease. It is nontoxic, affordable and eco-friendly. It has found application on/in several items and processes beneficial to humans such as drug, food processing and preservation, domestic and industrial water treatments, adjuvant to potentiate immune response in humans, anticariogenic agent, cosmetics and pharmaceutical industries as well as for treatment of 'Ich and white eye' diseases in fishes [18-22]. Therefore, this study was designed to investigate the *in vitro* effect of Alum on microbes associated with foliar phytoplasma disease of Noni.

UNDER PEER REVIEW

2. MATERIALS AND METHODS

2.1 Collection of samples

Typical features of the affected shoot in the field with FPD are stunted growth, reduced leaf size with puckers (upward and inward) and lamina shrinkages from petiole to the tip (**Plate 1**). Such samples were collected from Dilomat farm and conveyed to the Department of Microbiology, Rivers State University, Port Harcourt for analyses.

Plate 1. Foliar phytoplasma disease of Noni, sandwiched with the uninfected. Infected shoot are shorter with reduced leaves shrinking upward and inward.

2.2 Preparation of Alum and discs

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

2.3 Preparation of samples for microbiological analysis

A 25gm portion of FPD samples were washed, dried 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 nutrient agar (NA) for bacteria and Sabouraud's dextrose agar (SDA) for fungi. Followed by incubation at 37°C for 24h and 25±2°C for 2 or more days for bacteria and fungi respectively.

2.4 Macroscopy and microscopy of microbial cultures

Discrete microbial colonies were phenotypically identified according to their shape, colour, texture and contour and microscopically under oil-immersion objective, followed by biochemical tests for bacteria. Additionally, x10 and x40 magnification by infiltration with lactophenol cotton blue was carried out for fungi [23,24]. Cultures of bacteria were purified and preserved on NA at 5°C and each fungus was further grown for viability, confirmed and maintained on SDA slants at 5ºC for further assay.

2.5 Antibiogram procedure

Antimicrobial susceptibility tests were carried out by agar well diffusion (AWD) and disc diffusion (DD) methods. After the growth of bacterial and fungal cultures for 18h (~ x 10^8 overnight cultures) and 2-5days respectively on respective media, they were adjusted to 0.5 McFarland turbidity standard. Cultures were asceptically plated with various Alum concentrations and equidistantly spaced from one another and incubated at the different temperatures (for bacteria and fungi). Antibacterial and antifungal drugs; Ofloxacin (2,0mg) and Ketoconazole were used as controls respectively. Colony diameters were measured. The minimum inhibitory concentration (MIC), i.e., the lowest concentration able to inhibit any visible bacterial growth, was determined by measuring cell growth after 18-24h incubation at 37ºC with the various concentrations used [25].

2.6 Statistical Analysis

UNDER PEER REVIEW

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

3. RESULTS

Bacterial profile associated with foliar phytoplasma disease (FPD) and their susceptibility to Alum concentrations are presented in **Table 1**. The Inhibitory effect of Alum on bacterial isolates was concentration dependent using Agar well diffusion (AWD (values in bold) and disc diffusion (DD) techniques. The largest inhibitory zones occurred at 5.0% Alum concentrations. *Serratia* species had the larger IZs than those of other species but the inhibitory effect was much more pronounced with the control. However, IZs by AWD technique were much larger than those of DD whilst the control showed exceptionally the largest IZs.

Table 1. Inhibitory effect of Alum concentrations on bacteria associated with foliar phytoplasma disease of Noni using to AWD and DD techniques

Bacteria	**Concentration of Alum (%) and Mean IZ (mm)**									
	AWDT				DDT				Control	
	2.0%	**3.0%**	**4.0%**	**5.0%**	2.0%	3.0%	4.0%	5.0%	2.0mg/mL	
Bacillus subtilis	**1.0**	**3.0**	**3.0**	**4.0**	0.0	1.5	1.8	2.0	**49.5**	32.0
B. tequilensis	**1.0**	**2.0**	**3.0**	**4.0**	0.0	2.0	2.5	3.0	**45.6**	25.5
St. aureus	**0.0**	**1.5**	**2.5**	**3.5**	0.0	2.5	3.5	4.0	**41.0**	22.5
Serratia sp.	**2.0**	**3.0**	**4.0**	**6.0**	0.0	2.5	3.0	4.0	**51.0**	22.0
Br. vesicularis	**1.0**	**2.0**	**3.0**	**4.0**	0.0	1.6	1.8	2.0	**43.0**	30.5
B. cereus	**1.0**	**1.5**	**2.0**	**4.0**	0.0	1.8	2.0	2.5	**42.0**	33.8

Legend: AWDT = Agar well diffusion technique (in **Bold**); DDT = Disc diffusion technique = IZ = Inhibition zone (measured in millimeter (mm). Control = Ofloxacin.

Each value represents mean of 2 experiments.

The inhibitory effect of Alum against fungal isolates associated with foliar phytoplasma disease of Noni are presented in **Table 2**. Susceptibility of fungal isolates to Alum exhibited

UNDER PEER REVIEW

similar trend of dose dependence, with 5% concentration having the larger IZs. The inhibitory pattern of different concentrations of Alum revealed more profound effects on the isolates by AWD than with DD (**Table 2**). Of the two genera of fungi; *Aspergillus* and *Penicillium* the most sensitive to Alum at various concentrations was *Aspergillus flavus,* followed by *A. fumigatus* and *Penicillium* sp with *A. niger* as the least. However, higher concentrations of Alum also depicted comparable results with those of control.

Table 2. Inhibitory effect of Alum concentrations on fungi associated with foliar phytoplasma disease of Noni using AWD and DD techniques

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

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 minimum inhibitory concentration (MIC) of Alum against the microflora associated with foliar phytoplasma disease (FPD) are depicted in **Table 3**. All the species had MIC of < 2.0gm/mL except *Staphylococcus aureus*.

Table 3. MIC of Alum against bacterial and fungal species associated with FPD of Noni

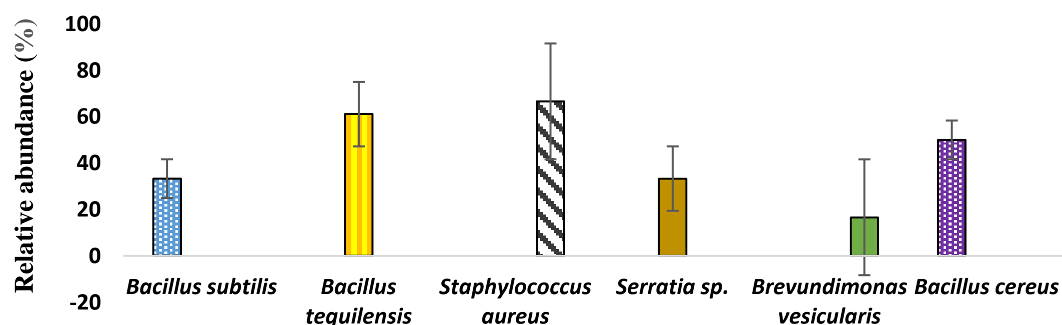
	Alum		
Bacteria	**gm/mL**	**Fungi**	**gm/mL**

UNDER PEER REVIEW

B. subtilis	< 2.0	*A. flavus*	< 2.0
B. tequilensis	< 2.0	*A. fumigatus*	< 2.0
S. aureus	> 2.0	*A. niger*	< 2.0
Serratia sp	< 2.0	*Penicillium* sp	< 2.0
Br. vesicularis	< 2.0		
B. cereus	< 2.0		

Relative abundance (%) of bacteria associated with foliar phytoplasma disease of Noni are presented in **Figure 1**. Basically, four genera of bacteria were identified, with the predominant genera as *Bacillus* followed by *Staphylococcus* and *Serratia* with *Brevundimonas* as the least. The most abundant single species was *Staphylococcus aureus* followed by *Bacillus tequilensis* and *B. cereus,* the least being *Brevundimonas vesicularis*.

PEER REVIEW



Figures 1. Relatiive abundance (%) of bacterial isolates assocaited with foliar phyoplasma disease of Noni

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

PEER REVIEW

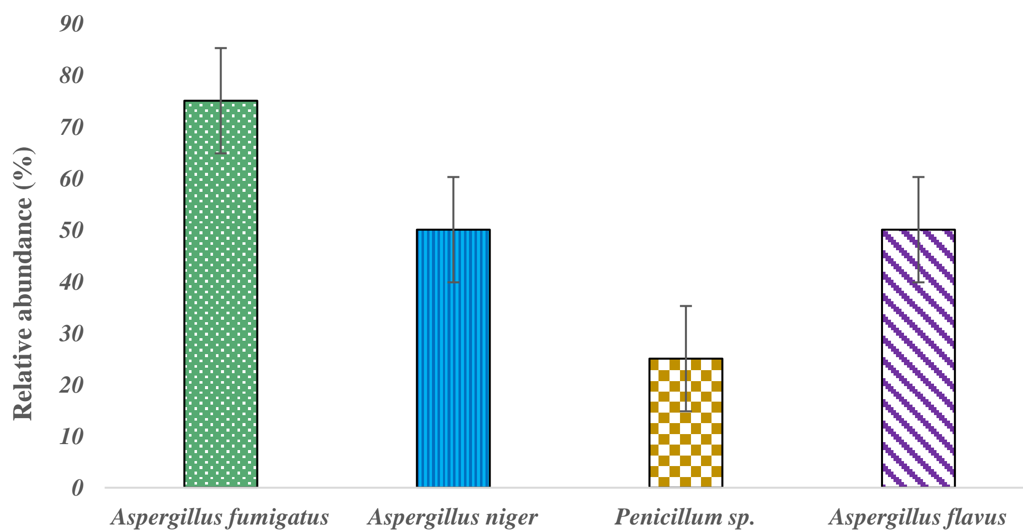


Figure 2. Relative abundance (%) of fungal isolates associated with foliar phytoplasma disease of Noni.

4. DISCUSSION

Foliar phytoplasma disease (FPD) has been a challenge to farmers and persistent occurrence of such exogenous pathology can have huge negative impact on income and well-being. The inhibition of microbes of which microorganism is one of the major components of the 'disease triangle' can proffer solution to the prevention and/or management of the foliar phytoplasma disease. The microbes associated with FPD belongs to six genera; *Bacillus*, *Staphylococcus*, *Serratia, Brevundimonas*, *Aspergillus* and *Penicillium* as revealed in this work.

Literature on foliar phyoplasma disease of Noni has been earlier reported [12] but devoid of the aetiologic agents or microbes associated with the disease. However, two major microbial genera from this study; *Bacillus* (*B. tequilensis*) and *Aspergillus* (*A. niger*) have been reported to produce enzymes (e.g., pectinases; polygalacturonase, pectin lyase and pectinesterase) capable of degrading starches, hemicelluloses, celluloses and other cell wall components [26,27,28,29]. Beyond the role of degradation, *Aspergillus* is also a known phytopathogen [30]. These microbial attributes can negatively impact the tender morphological architecture of Noni foliage to present the typical condition of foliar phytoplasma disease (**Plate 1**).

The relative abundance (%) of foliar microbes may be attributed to soil, weather and other environmental conditions which in turn impacts the microbial community structure where the plant is grown [16,30,31,32]. The antimicrobial potentials of Alum is well documented in

UNDER PEER REVIEW

previous studies [22,33,34] and validates the present study. The low MIC of Alum of < 2.0 gm/mL suggests future prospects for its agronomic applications. As an eco-friendly natural agent, it can be explored and adopted to ameliorate or combat foliar phytoplasma disease as well as other foliar pathogens. Although, there was wide disparity in relation to Alum's effect compared to the antibiotic (control), its relative comparison ~~at higher doses~~ with Ketoconazole underscores antifungal efficacy.

5. CONCLUSIONS

The research revealed the identification of six microbial genera; four of bacteria (*Bacillus, Staphylococcus*, *Serratia* and *Brevundimonas*) and two of fungi (*Aspergillus* and *Penicillium)* associated with foliar phytoplasma disease of Noni. Alum treatment against these microbes exhibited appreciable MIC and antimicrobial efficacy. This presupposes that Alum can be explored and used to ameliorate future challenges in agronomy especially foliar related diseases.

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DISCLAIMER

The samples used for this research are commonly used in our area of research and country. There is absolutely no conflict of interest between the author and producers of Noni fruits because we do not intend to use these samples as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the author.

DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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UNDER PEER REVIEW

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UNDER PEER REVIEW

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