Effect of Five Plant Extracts on Damping off Disease Control of *Causuarina Equisetifolia* L. in the Nursery

Omokhua, Godwin E. and Kalagbor, S.

Department of Forestry and Wildlife Management, University of Port Harcourt, Port Harcourt, Nigeria
profomokhua@yahoo.com

Abstract: Damping off of forest seedlings is a common fungal disease causing severe mortality in the nursery in Nigeria. The effect of five plant extracts, i.e. leaf of *Azadirachta Indica,* seed of *A. indica,* bulb of *Allium sativum,* root of *Zingiber officinale* and leaf of *Vernonia amygdalina* was tested as organic fungicides on seedlings of *Casuarina equisetifolia* in the green house. Results showed *A indica, Z, officinale*, *A. sativum* and *V. amygdalina* significantly reduced damping off disease incidence of *C. equisetifolia*. The plant extracts significantly reduced the incidence of damping off disease where the percentage incidences were in the range of 21.11-24% compared to 77.50% in control seedlings. The treatments also gave the highest shoot growth in the range of 41.67 – 47.00cm compared to the 32cm-33cm in control seedlings. This result has revealed that the five organic fungicides used in the study gave the highest survival percentage and improved growth of *C. equisetifolia.* Therefore, there is need to focus a great interest on *biofugicides* that are relatively cost effective and have minimal toxicity to mammal and the environment.

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1. Introduction

Protection of forest species in both field and nursery from diseases is an important aspect of forest pathology. In Nigeria, losses of forest species due to degradation and deterioration by bio-deteriorating agents have not been duly assessed and limited available data are often unreliable with little or no scientific validity. Where some assessments have been made, losses from actions of the biodegrading agents indicate serious wastage of forest seedlings in the nursery.

Damping off is a common disease that affects seeds, germinants and young seedlings of many tropical forest species including *Casuarina equisetifolia*. Its scope covers many plant species involving water, soil and seed borne diseases of forest plants (Omokhua *et. al*, 2009). Damping off occurs very often in container nurseries and is caused by some group of fungi.

These fungal pathogens are considered among the most devastating disease of tree seedlings in forestry. The main causal agents of damping off in some parts of the world are *Fusarium, Rhizoctonia, Solani* and *Sclerotinia Sclerotiorum, Pythium* and *Phytophthora* species. In Port Harcourt the incidence of damping off in the nursery is alarming in tree seedlings (Omokhua *et. al,* 2011). Where there are no seedlings to plant, afforestation and reforestation programmes are usually affected. Damping off results in a poor and uneven stand of seedlings which can be confused with low seed viability in a forest nursery. The seed may germinate but later wither and die or may suddenly fall over as in post emergence damping-off, lesions may appear on infected seedlings just at or below the ground level and could become discolored, collapsed and die suddenly (Omokhua *et, al*, 2009).

In plant protection, the use of industrial fungicides is fast becoming an environmental hazard. There is growing concern as to what can be done to prevent environmental problems often associated with fungicide application. This is because toxicity of fungicide residues can lead to environmental pollution and human health hazard.

As result of the problems enumerated above, there is need to look at environmental friendly, less hazardous and inexpensive practices in forest protection. Hence the growing trend to develop *biofungicides* as alternative to synthetic or industrial fungicides. This creates the need for this study. Therefore, the objectives of this study is to investigate the impact of extracts from A*zadirachta* indica, *Allium sativum, Zingiber officinale and Vernonia amygdalina* as biofungicides on damping off *incidemce* and early seedling growth of *C equisetifolia* in the nursery.

2. Research Methods

The study was carried out at the forest nursery of the Department of Forestry and Wildlife Management, Faculty of Agriculture, University of Port Harcourt, Choba, Port Harcourt (Lat04^0 53^I 38.3^{II} N and Long 0.06^0 54^I 38.0^{II} E)

Fruits of *Casuarina equisetifolia* used for this study were collected from three (3) mother trees at the University of Port Harcourt, Delta campus, Port Harcourt. The fruits were processed by sun-drying. All processed seeds were kept in a refrigerator to avoid depreciation. The floatation method was used to test for viable seeds before sowing.

The study involves the use of five (5) extracts as treatments. These include: extracts from leaf of *Azadirachta indica* (A); seed of *Azadirachta indica* (B), bulb of *Allium sativum* (C), root of *Zingiber officinale* (D) and leaf of *Vernonia amygdalina* (E).

The plant parts listed above were collected, washed thoroughly with deionized water, sterilized with 125mg *Streptopenicillin* and rinsed several times, and oven dried at 50^0C for 24hours. A sterilized grinder was used to crush the plant parts and sieved with 80 mesh sieve to obtain a 100g weight of each of them. Ethanol extracts of the five treatments were obtained by adding 100ml of 50% ethanol (wt/v) to each of the 100g plant parts. The extract was then filtered using whatmann filter paper No. 1 (Phasuda and Varipat, 2004). The filterate was stored in the refrigerator at-20^0C before use.

Three hundred viable seeds of *C. equisetifolia* were propagated per treatment involving three replicates in a completely randomized design (CRD). A total of one thousand eight hundred (1800) seeds were used plus the control. The seeds were broadcast in germination trays (60x60cm) filled with top forest soil. The germination trays were watered daily and kept in a propagator. Inception of germination was observed two weeks after sowing and recorded.

Final germination count was recorded at one month after inception. The five treatments as *biofungicides* were applied immediately after the final germination count. Disease incidence was calculated at two (2) months after the application of treatments and final seedlings height were measured using a meter rule at the end of six (6) months after inception of germination as shown below:

$$D1\ (\%) = \frac{x}{\gamma} X \frac{100}{1}$$

D1=disease incidence

x = no of seedlings infected

y = no of seedlings germinated

Disease incidence and final height of *C. equisetifolia* were statistically analyzed using analysis of variance (ANOVA) in a completely randomized design. The least significance difference was used to separate the means.

3. Analysis of Results and Discussion

The highest mean disease incidence was recorded in the control which as not treated and had 77.5 ± 1.44%. The other treatments: leaf of *Azadirachta indica*; seed of *Azadirachta indica*; bulb of *Allium sativum*; roots of *Zingiber officinale* and leaf of *Vernonia amygdalina* had 14.0 ± 0.7%, 23.3 ± 0.83%, 22.5 ± 1.44%, 21.8 ± 0.66% and 21.1 ± 44% respectively (Table 1). The five treatments were significantly different from the control at 5% level of probability (Table 2).

Final seedling growth of *Casuarina equisetifolia* using five (5) plant extracts as *biofungicide* treatment is shown in Table 3. The result showed that the highest mean height was recorded in *A. indica* leaf extract treatment which had 57.00 ± 1.52cm, while extract from seed of *Azadirachta indica*, bulb of *Allium sativum*, root of *Zingiber officinale* and leaf of *Vernonia amygdalina* had 55. ± 2.51cm, 43.7 ± 0.88cm, 43.7 ± 1.45cm, 41.7 ± 1.20cm respectively. The control had the least height of 32.3 ± 1.45cm. The analysis of variance showed that there was no significant difference between the five treatments including control.

This result revealed that seeds of *Azadirachta indica*, leaf of *Azadirachta indica*, bulb of *Allium sativum,* root of *Zingiber officinale* and leaf of *Vernonia amygdalina* had antagonistic effects on damping off disease pathogens of *Casuarina equisetifolia*. Apart from the control all the treatments reduced the mortality of *Casuarina equisetifolia* seedlings in the nursery. The implication of this is that bio-control methods used in controlling damping off disease in this study has an advantage over chemical methods, since chemicals are not environmentally friendly because they are pollutants and are very expensive to buy. The results on final height growth of seedling of *Casuarina equisetifolia* in the nursery showed that the highest height was recorded in leaf of *Azadirachta indica* which had 57.0 ± 1.52cm, while the seed of *Azadirachta indica*, bulb of *Allium sativum*, root of *Zingiber officinale* and leaf of *Vernonia amygdalina* had 55.0 ± 2. 51cm, 43.7 ± 0.88cm, 42. 7 ± 1.45cm, 41, 7 ± 1.20cm respectively. This trend discussed above is similar to the observation on disease incidence by many scientists (AI-Dalmasini *et. al,* 2003; Ben and Nelson, 1999; Bennard and Clement, 1983; Benhamous *et al,* 1997; Bennard and Pesando, 1989; Boehm and Hoitink, 1992; 1997; Bonsi *et. al,* 1995*;* Cariellon and Zanetti, 1979; Cotxarrera *et. al,* 2002; Craft and Nelson, 1996; Delaney *et. al,* 2001; Elad and Shteinberg, 1994; Erthart and Burian, 1997; Folman *et. al,* 2004; Fuchs and Larbi, 2004; Hoitink *et. al,* 1997; Kao and Ko, 1986; Verma and Kharmar, 2006). Also, several studies have been conducted to investigate the peculiar effects of plant extracts as

fungicides, pesticides and as antibacterial agents on plant growth worldwide. The antifungal activity of *A. sativum* was reported by Grewal (2006). The author, observed that essential oil of *A. sativum* on agar plate was active on *Lenzites trabea*, *Lentinus lepideus* and *Polyporus versicolor*. Similarly, the species antibacterial activity of ethanol extract was active on *Escherichia coli, Salmonella typhosa, Shigella sonnei and Staphylococcus aureus*. The author observed that water extract of the species was active on *Bacillusi klebsiella*, *Proteus vulgaris,* S. *styphosa*, S. *sonnei* and S. *aureus*. Moslem and Kholie (2009) also reported the antifungal effects of neem leaf and seed extracts obtained by ethanol, on *Fusarium oxysporum, Rhizoctionia solani, Alternaria solani* and *Sclerotiorum solani.* The authors observed growth inhabitation of the tested fungi.

It is important to note that bio-control agents induces the accumulation of enzymes such as *Chitinnase, Peroxidase* and *Polyphenol oxidase* which plays an important role in plant defense mechanisms against pathogens (Abd-EL-Khain *et.al,* 2003). Raupach *et. al,* (1996) reported on induced systematic resistance in cucumber and tomato against mosaic *Cucumovirus* using plant growth-promoting *Rhizobacteria* (PGPR): *Pseudomonas fluorescens.*

This study indicates that the application of the plant extracts increased the growth and reduced disease incidence in C. *equisetifolia.* The increase in plant growth might be associated with secretion of *auxin, gibberellins* and *cytokinnins* and suppression of deleterious microorganism causing damping off (Gamliel & Katan, 1993). In addition to the direct antagonism and plant growth promotion, plant extracts from leaf of *Azadirachta indica,* seed of *Azadirachta indica,* bulb of *Allium sativum,* root of *Zingiber officinale* and leaf of *Vernonia amygdalina* increased the activities of various defense related enzymes and chemicals in response to infection by the pathogen. Many plants are endowed with defense mechanisms which can protect them against fungi (Dutta, 2003).

Also, it has been reported that application of plant extracts triggers plants' latent defense mechanism in responses to infection by pathogen. Inducing a plants' own defense mechanism by prior application of a biological agent is a novel strategy in plant disease management. Aqueous plant extract is rich with nutrient and microorganism. It can stimulate growth, protect plants from disease and help suppress soil borne pathogen (Quarles, 2011).

In this study, it could be stated that seedlings treated with the (5) five plants extracts: leaf of *Azardirachta indica,* seed of *Azadirachta indica,* bulb of *Allium sativum,* root of *Zingiber officinale* and leaf of *Vernonia amygdalina* increased the activities of various defense-related enzymes which led to the synthesis of defense chemical in *Casuarina equisetifolia.* The result implies that the use of five plant extracts inhibited the growth and development of damping off causing fungi.

Therefore, new forest protection options involving the use of bio- fungicides need to be explored in order to ensure the success of any defforestation and reforestation programmes. A great interest should be focused on biofungicides that are relatively cost effective and have minimal toxicity to both mammals and the environment. Unlike inorganic fungicides, the use of organic fungicides can cause no disruption to any stable food chain.

Table 1: Mean disease incidence (%) of damping off using 5 treatments at 4 weeks after sowing (WAS)

Treatment	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
A	3	14. 1000	1.32288	.76.376	12.50	15.00
B	3	23.3333	1.41338	.83333	22.50	25.00
C	3	22.5000	2.50000	1.44338	20.00	25.00
D	3	21.8333	1.15470	.66667	20.50	22.50
E	3	21.1667	1.25831	.72648	20.00	22.50
Control	3	77.5000	2.50000	144338	75.00	80.00
	18	31.7222	21.14114	4.98301	20.00	80.00

Table 2: Analysis of variance (ANOVA) for mean disease incidence of damping off using 5 treatments at 4 weeks after sowing (WAS)

	Sum of Squares	df	Mean Square	F cal	F tab
Treatment	7559.611	5	1511.922	471.248 *	3.11
Error	38.500	12	3.208		
Total	7598.111	17			

* Significant at 5% level of probability

Table 3: Mean shoot height (cm) of *Casuarina equisetifolia* seedlings using 5 treatments at 6 months after planting (MAP)

Treatment	N	Mean	Std. Dev	Std. Error	Minimum	Maximum
A	3	57.000	2.64575	1.52753	45.00	60.00
B	3	55.0000	4.35890	2.51661	40.00	58.00
C	3	43.6667	1.52753	.88192	42.00	45.00
D	3	43.6667	2.51661	1.45297	40.00	45.00
E	3	41.6667	2.08167	1.20185	40.00	44.00
Control	3	32.3333	2.51661	1.45297	30.00	35.00
	18	42.0556	5.32996	1.25628	30.00	50.00

Table 4: Analysis of variance (ANOVA) for final height of *Casuarina* equisetifolia using 5 treatments at 4 weeks after sowing (WAS)

	Sum of Squares	df	Mean Square	F cal	F tab
Treatment	392.278	5	78.456	10.384 *	3.11
Error	90.667	12	7.556		
Total	482.944	17			

* Significant at 5% level of probability

5. Conclusion and Recommendation

The findings from this study has revealed that some plant extracts can be used as organic fungicides in place of chemical substances currently being used in horticultural and forest nurseries all over the world. The plant extracts used in this study (*A indica, V. amygdalina, A sativum, Z. officinale*) are cheap, readily available and environmentally friendly. Further investigations on use of plant extracts as organic fungicides are recommended not only on the species reported in this study, but other plant species that have the potentials as organic fungicides.

Corresponding Author:
Dr. Godwin E Omokhua,
Department of Forestry and Wildlife Management,
University of Port Harcourt, Port Harcourt, Nigeria.
E-mail: profomokhua@yahoo.com

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