An investigation of the effectiveness of plant extracts from *Azadirachta indica* (neem) and *Persea americana* (pear) against rice sheath blight disease induced by *Rhizoctonia solani*

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Abstract

Biological controls of diseases have an important role in the effort to reduce the use of synthetic chemicals in controlling and preventing destructive rice diseases in Guyana. Leaves from *Azadirachta indica* (neem) and *Persea americana* (pear) plants were extracted selectively with solvents of increasing polarity: hexane, ethanol and water. These extracts were later diluted in concentrations of 0.10%, 0.25% and 0.50% and tested against the fungus, *Rhizoctonia solani* , isolated from infected plants. The antifungal effect of these extracts against the pathogen, *R. solani*, was then evaluated by the disc diffusion assay. Both plant extracts showed varying degrees of antifungal effect at different concentrations against the pathogen. Results indicated that pear leaf ethanol extract at 0.50% concentration with induced AZOI = 251.24 mm^2 was the most effective against *R. solani*, followed by neem leaf hexane extract at 0.50% concentration (AZOI = 195.5 mm^2). Interestingly, the aqueous extract showed the same AZOI at all concentration for both neem (AZOI = 75.1 mm^2) and pear leaf (AZOI = 57.5 mm^2) extracts. Overall, neem and pear leaf extracts were determined to be effective biological control against the sheath blight of rice and their future application in the rice industry is pending.

Keywords: Biological control, synthetic chemicals, plant extracts, rice sheath blight disease, *Azadirachta indica* (neem), Persea Americana (pear), *Rhizoctonia solani*

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1.0 INTRODUCTION

Rice is one of the most essential food crops in the developing world and provides the daily supply of staple food for more than half of the world's people[1.] It is also one of Guyana's major crops produced, and one of the most important crops in the agricultural sector in the economy. Rice contributes in excess of US$80M annually, 12.5% of export earnings and contributes 20% of the agricultural GDP[2]. As such, it is essential to maintain and/or improve production levels, given the demand for this staple commodity. This can be addressed by curbing or controlling the numbers of pests and diseases that threaten any of the stages of cultivation.

Rice has been under cultivation in different climatic conditions since ancient times and is widely affected by many diseases caused by fungi, bacteria and viruses resulting in significant yield losses. It's pests and diseases are a cause for concern and, control of these is of primary importance. A fungal rice disease of noteworthy is the sheath blight, induced by *Rhizoctonia solani*. It's one of the most serious diseases of rice worldwide, resulting in considerable yield losses [3]. Annual yield losses up to 40% were reported under optimum conditions of disease development [4]. Sheath blight of rice is one of the most serious rice diseases worldwide; this is second to rice blast. It's one of the major problems in intensive rice production of the world, causing huge economic losses, especially under warm and humid conditions. Initial symptoms are noticed on leaf sheaths near water level. On the leaf sheath, oval or elliptical or irregular greenish-gray spots are formed. As the spots enlarge, the center becomes grayish-white with an irregular blackish-brown or purple-brown border.

Sheath blight occurs in areas with high temperatures (28−32 °C), high levels of nitrogen fertilizers, and relative humidity of crop canopy from 85−100%. Plants are more vulnerable to sheath blight during the rainy season. High seeding rate or close plant spacing, dense canopy, disease in the soil, sclerotia or infection bodies floating on the water, and growing of high yield improved varieties also favor disease development [5]. Sheath blight symptoms may sometimes

appear on seedling rice, but symptoms are more likely to develop after jointing begins. The first symptom is an oblong, water-soaked lesion on leaf sheaths at or near the water line. In 2 or 3 days, the lesion will have a grayish-white center surrounded by a dark purplish- or reddish-brown margin and may be up to 1 inch long. This lesion interrupts the flow of water and nutrients to the leaf tip and the tip may die. Tissue below the lesion may remain green [6]. As the plant grows and the canopy closes, the humidity inside the canopy increases. In this humid environment, the fungus grows upward inside the plant and on the plant's surface, causing new lesions. The fungus can also spread to nearby plants. Severely damaged plants may lodge. This disease is described by IRRI [7] as the second most important rice disease and as one most expensive diseases for rice farmers.

Sheath blight can cause partial infection of the lower leaves with little effect on grain development, to premature plant death, Fig. 1.0. Both yield and grain quality is reduced when the infection prevents the flow of water and nutrients to the grain. Grain may develop only partially or not at all. Poorly developed grain usually breaks up during milling, thus reducing quality. In Japan, the disease has caused a yield loss of as high as 20% and affected about 120,000−190,000 hectares. A yield loss of 25% was reported, if the flag leaves are infected. In the United States, a yield loss of 50% was reported when susceptible cultivars were planted. Sheath blight has also caused a yield loss of 6% in tropical Asia [7].

Fig. 1.0 Rice infected with Sheath Blight disesase.

The use of synthetic chemicals is widely practiced as a solution to these issues. However, these compounds are often harmful to organisms in the environment as well as the rice plant itself, making it unsafe for consumption. There is need for other alternatives of controlling pests and diseases, not only in rice ecosystems, but generally in all types of cultivation, where the use of synthetic chemicals is a common practice. Because of the continuous use of synthetic chemicals as fungicides, fungal pathogens are becoming more and more resistant to the chemicals. Alternatively, biological controls offer eco-friendly and cheaper methods of control. A number of plants worldwide have been tested for their antifungal properties. This promotes their use as biological controls to fungal diseases in rice. The Guyana Rice Development Board, GRDB has succeeded to incorporate sheath blight resistance in new varieties. This has been a continuing program, since the fungus continues to develop new strains that overcome the resistance in the new varieties. The new varieties do not always resist this disease and, in some cases, the crop becomes infected, thus resulting in an overall loss in produce [8].

Biological control measures, such as medicinal and or antifungal plant extract assist plants to combat diseases and pests. This measure is safe, cheap and is not harmful to the environment. Medicinal and antifungal plants are grown in Guyana on a medium size scale and are usually available in farming areas, as such farmers will find it very convenient to prepare and apply this to infected crops.

This paper reports the effectiveness of the ethanolic and aqueous plant extracts of neem, *Azadirachta indica* and pear leaf, *Persea Americana* at different concentrations as biological control (antifungal agents against the prevalent fungal sheath blight disease which attacks rice and is induced by the fungus *Rhizoctonia solani*. The significance of this study is to positively impact the rice producers of Guyana in reducing the high level of chemical application to rice crops, and to adapt a biological control to combat diseases. This method of control is economical and environmentally–friendly.

Sukanya *et al.* [9] reported that management of sheath blight disease is best when using an integrated approach which entails use of fungicides, growing resistant varieties, application of organic amendments, balanced nutrition, biological agents and resistance inducing chemicals. However, the excess use of chemicals results in environmental pollution and is harmful to biotic community as a whole. Therefore, biological controls are often encouraged over the use of chemical controls.

Neem and pear leaves contain vital phytochemicals that are responsible for their antifungal and antimicrobial ability. Studies [10],[11] have also proven that antimicrobial and antifungal activity is solvent-dependent.

Earlier studies proved ethanol as the most efficient solvent for extracting a broad spectrum of antimicrobial compounds from plants. Srinivasan *et al*.[9] also observed the antifungal and antibacterial activity of *A. indica* due to its phytochemical components.

Flavonoids have been found in the hexane leaf extracts of *A. indica. which* has low antifungal activity [11,12,13,14,15]. Ethanol extracts showed a large number of secondary metabolites such as carbohydrates, alkaloids, phenols, flavonoids and saponins. These findings corroborate those of this experiment, since ethanol extracts of pear had a greater inhibitory effect against *R. solani* than hexane or water extracts.

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Zaki *et al*.[17] readily extracted and detected the antifungal compound "borbonol" in leaves, stems and roots of *Persea* species. When applied to cultured plates of *R. solani* and other plant pathogens, methanol extracts of the leaves exhibited the greatest fungitoxicity. It was also noted that on average, post-harvest pathogens were inhibited to a considerably higher degree by extracts from leaves than were root pathogens. The leaves, fruits and seed of *Persea Americana* have been shown to be rich in phenols, saponins, and flavonoids in appreciable quantities[18].

2.0 Procedure

2.1 Sample Collection

Samples of rice plants infected with sheath blight disease were collected from the East Coast Demerara. Latex gloves were worn during the collection to prevent unnecessary contamination of samples. Samples, were then washed with running tap waters. The freshly picked leaves from *Azadirachta indica* and *Persea americana* trees were obtained from farm lands in the Demerara region of Guyana and were dried prior to extraction, Fig. 2.0.

Fig. 2.0: Neem leaves.

Fig. 3.0: Pear leaves.

2.2 Preparation of Plant Extracts

Leaves of *Azadirachta indica* (neem, 370.7g) and *Persea Americana* (pear, 136.5g) were collected, thoroughly washed with distilled water, dried and were subsequently weighed using a metric scale. They were selectively extracted thrice using solvents of increasing polarity: hexane (3000ml), ethanol (3000ml) and water (2500ml) over a period of six weeks. When extract was ready to be used, it was filtered. The filtrate was then poured into a clean glass jar and dried over anhydrous sodium sulphate, Na_2SO_4. The extracts were then concentrated using a rotary evaporator, where solvents were removed in *vacuo*. The hexane extract of Neem and Pear leaf constituted a weight of (88.67g, 23.92%) and (47.55g, 34.84%) respectively, whereas the ethanol extract of neem and pear leaf constituted a weight of (25.30g, 6.82%) and (26.55g, 19.5%) respectively. The extracts were then made up to the requisite concentrations of 0.1%, 0.25% and 0.5% via dilution with the respective solvents.

2.3 Antimicrobial studies[19-20]

500 mL of distilled water was added to a 1L beaker along with 200g of potatoes. The mixture was then boiled until the potatoes were tender. The contents of the beaker were filtered through gauze. 20g each of dissolved dextrose and agar was added to the filtrate obtained. The volume of this mixture was made up to 250mL (to prepare 7 agar plates) and was dispensed into a 250mL conical flask. The flask was then plugged with non-absorbent cotton and sterilized at 121°C for two hours in an autoclave. Approximately 33mL of media was poured, under aseptic conditions, into each Petri dish. The media was allowed to solidify to produce the agar plates.

2.4 Culturing and Sub-culturing of *R. solani* [19-20]

The infected leaf tissue was selected from the advancing margin of a lesion. The tissue was cut into small pieces (2-5mm) and contain both the diseased and healthy tissue. These pieces were kept in a sterile Petri dish. The pieces were placed into 1% sodium hypochlorite solution for one minute. They were then washed twice in sterile distilled water. The inoculation area was sterilized (swabbed) with 70% ethanol. The lid of the Petri dish was removed, near the flame of a lit burner, and four sterilized pieces of tissue was placed at different distances in a single PDA plate. The Petri dishes was inverted and incubated at 25°C for 3-5 days. Mycelia from the margin of colonies on PDA plates were then aseptically transferred (sub-cultured) to fresh PDA plates and pure cultures were obtained. From this pure culture replications were made.

2.5 Application of Treatment

The antifungal activity of the leaves extracts was determined by using the disc diffusion assay. The filter paper discs of about 6 mm in diameter were separately saturated with 10mL of extract at a particular concentration and placed on the agar which was previously inoculated with *R. solani*. The plates were then incubated for 48 hours at 37^{o}C.

Pure solvents were used as the control and this was also investigated via the disc diffusion method. Nystatin was also used as the reference. Each treatment was done in

triplicates. Antifungal activity was then assessed by measuring the diameter of the growth of Inhibition Zone in millimetres for the test fungus.

3.0. Results

Table 1.0: Shows the mean ± standard deviation of zones of inhibition observed and LSD (Least Significance Diffrence) all pairwise comparison

Extract	Solvent	0.1%	AZOI (mm^2)	0.25%	AZOI $(mm)^2$	0.5%	AZOI $(mm)^2$
Neem Leaf	Hexane	9.56 ± 0.27 C, B	65.87	12.67 ± 0.69 BC, AB	126.02	15.78 ± 1.51 BC, A	195.47
	Ethanol	10.11 ± 2.18 BC, B	80.24	11.67 ± 0.47 BCD, AB	106.91	12.78 ± 0.50 CD, A	128.21
	Water	9.78 ± 0.33 C	75.08	9.78 ± 0.33 C	75.08	9.78 ± 0.33 C	75.08
Pear Leaf	Hexane	9.56 ± 0.33 C	71.74	7.33 ± 3.03 D	42.18	11.22 ± 1.02 D	98.82
	Ethanol	11.89 ± 0.24 B	110.98	12.56 ± 0.73 BC	123.84	17.89 ± 0.32 AB	251.24
	Water	8.56 ±1.03 BC	57.52	8.56 ±1.03 BC	57.52	0 ± 0 E	0.0
Control	-	0 ± 0 E		0 ± 0 E		0 ± 0 E	
Reference	-	21.67± 0 A	368.63	21.67+ 0 A	368.63	21.67+ 0 A	368.63

* Means with similar letters indicate no statistical difference.

4.0. Graphs

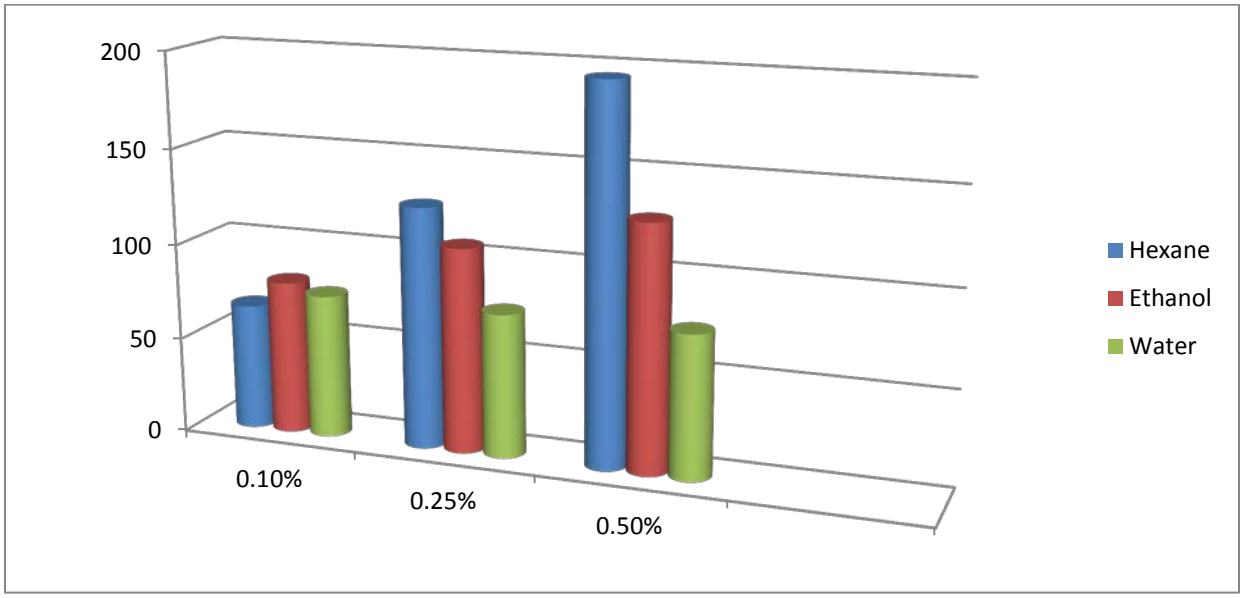

Fig. 4.0. A plot of the Area of Zone of Inhibition, AZOI of the Neam leaf extract versus increasing concentration of the plant extract.

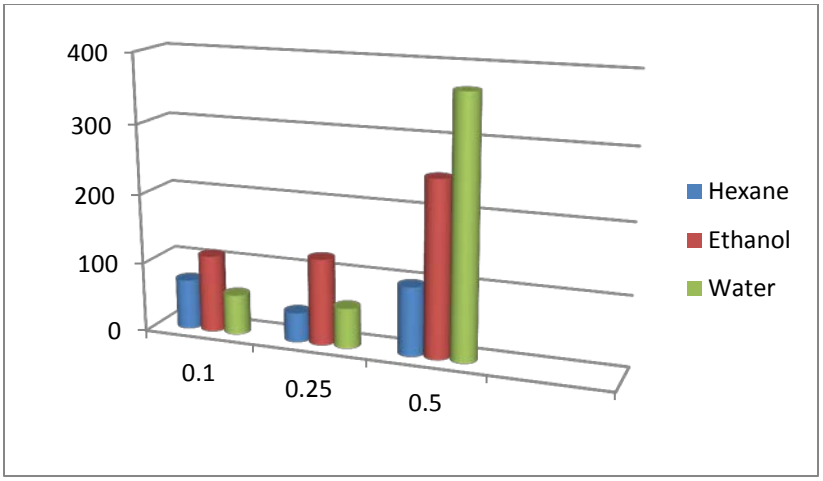

Fig. 5.0. A plot of the Area of Zone of Inhibition, AZOI of the Pear leaf extract versus type and increasing concentration of the plant extract.

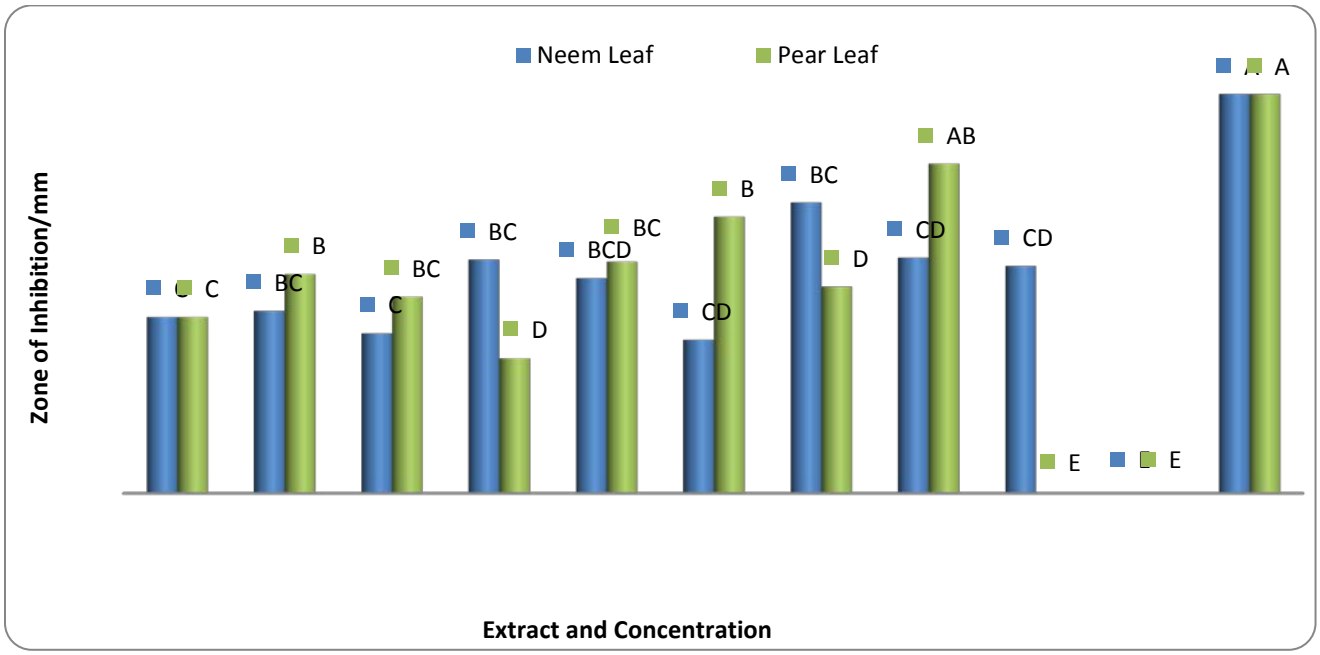

Figure 6.0: Comparing the zones of inhibition observed when different extracts are applied to fungal culture.

Table 2: Showing comparison of zones of inhibition when different extracts are applied to fungal cultures using ANOVA Two Factor with Replication ($p < 0.05$ = significance)

Comparison	***f* value**	***f* crit**	***p* value**	**Significance**
Between extracts	71.26	2.21	0.14	Not significant
Between concentrations	2.08	3.19	3.56×10^{-23}	Significant

5.0 Discussion

The hexane, ethanol and aqueous extract of both neem, *Azadirachta indica* and pear leaf, *Persea americana* were prepared and used to investigate antifungal activity against *R. solani.* These extracts were obtained according to the procedure and were made up to a concentration of 0.1, 0.25 and 0.50 % respectively. Antifungal assay was done using the Disc Diffusion method. Here the diameter of zone of inhibition, DZOI against the mycelial growth and resulting calculated AZOI were used as indicators of the plant extract's antifungal activity. Results are presented in Table 1.0, which shows the diameter of zone of inhibition, DZOI expressed as mean with standard deviation and the computed, area of zone of inhibition, AZOI .

All extracts showed inhibitory effect against the tested fungus, some greater than others. According to the results, there was a significant increase in the area of zone of inhibition, AZOI and antifungal activity for the neem hexane and ethanol extract for the concentration range 0.1% through 0.25% to 0.50% against the fungal pathogen. However, for the aqueous extract, the antimicrobial potency remains constant at 75.08 mm^2 and 57.5 mm^2 for the neem and pear leaf extracts respectively at all concentrations. For example, for the ethanol neem extract, AZOI of 80.24 mm^2, 106.91 mm^2 and 128.21 mm^2 were registered at 0.1, 0.25 and 0.50% of the extract against *R. solani*.

For the pear leaf extract, there was a general increase in antimicrobial potency as the concentration of the extract increases from 0.1 to 0.5%. For example, the antimicrobial potency of the hexane extract at 0.1 and 0.5% was observed to be 71.74 mm^2 and 98.32 mm^2 respectively. A decrease in AZOI of 42.18 mm^2 was noted at the 0.25% concentration. The lowest AZOI of 42.18 mm^2 was exhibited by the hexane extract of the pear leaf at 0.25% concentration. The highest AZOI of 251.2 mm^2 was induced by the ethanol pear leaf extract at 0.50% against *R. solani.* This is 68.2% of that of the antifungal agent, Nystatin.

For all the extracts analysed, there seems to be an increase in antifungal activity as the concentration is increased from 0.1% to 0.5%. Exception to this, being the hexane extract of pear leaves which showed a decrease in AZOI at 0.25% and the aqueous extract of pear leaf which showed a decrease in antifungal activity from 0.1 to 0 0.5% with registered value of 57.5 mm^2, 57.5 mm^2 , 0.0 mm^2 respectively.

Antifungal activity seems to follow the trend for the neem leaf extract at 0.1%. Ethanol > H_2O > Hexane. At 0.25 and 0.5%, the order is: Hexane > Ethanol > H_2O. At 0.5%, the order is Hexane > Ethanol > H_2O. For the peaf leaf extract, the antifungal activity at 0.1% follow the trend: Ethanol > Hexane > H_2O. At 0.25%, the antifungal activity follow the trend: Ethanol > H_2O > Hexane. At 0.5%, the antifungal activity follow the trend: Ethanol > Hexane > H_2O.

Antifungal activities are probably due to the presence of antifungal natural products or phytochemicals present in the plant hexane, ethanol and aqueous extract. Phytochemicals are non-nutritive plant chemicals that have protective or disease prevention properties [21-26]

Most report show that the antifungal effect increases as the polarity of the plant extract increases i.e the ethanol extract is more effective than the hexane extract [21-25]. However, this is not always the case as our research findings reveal. Some compounds might be more active in hexane extract than they were present in ethanol extracts [26].

Pear leaf ethanol extracts exhibited higher zones of inhibition at 0.5% when compared to neem leaf ethanol extracts. For example, AZOI of 251.24 mm^2 was noted for the ethanol extract at 0.5% (pear leaf). For the neem leaf at 0.5%, AZOI of 128.21 mm^2 was noted. The neem leaf ethanol extracts at 0.25 and 0.5% exhibit AZOI of 106.91 and 128.21 mm^2 respectively. The pear leaf hexane extract exhibit AZOI of 42.14 mm^2 and 98.82 mm^2 at 0.25 and 0.50% concentration respectively.

The opposite was seen for hexane extracts of neem leaf, exhibiting greater zones of inhibition. Differences were significant at 0.25% and 0.5%, but not at 0.1%. The hexane extract of neem leaf at 0.5% exhibited AZOI of 195.5 mm^2 compared with 98.8 mm^2 exhibited by the pear leaf hexane extract at 0.5%. In the case of water extracts, neem leaf extract exhibited higher zones of inhibition (AZOI = 75.08 mm^2) at all three concentrations: 0.1, 0.25 and 0.5%, compared to pear leaf (AZOI = 57.5 mm^2). Fig. 3.0. shows the Disc Diffusion assay of neem leaf extract, *Azadirachta indica* against *R. solani* at 0.5% concentration.

ANOVA Two Factor with Replication was used to determine significant differences between concentrations and between extract concentrations using the mean values of zones of inhibition when different extracts at different concentrations were applied to fungal cultures [27-28]. From Table 2, it is evident that differences between concentrations were significant throughout, since the calculated *p*-values are all less than 0.05 while between extracts did not

differ significantly in their ability to inhibit growth of the fungus, since ($P > 0.05$, $P = 0.14$). This was also done for the control and reference.

LSD (Least Significance Difference), All-Pairwise Comparisons test was also used to compare concentrations within each extract using the mean value obtained. The LSD facilitates pairwise comparison of all extracts. Means with the same letters indicate no significant difference, whereas those with different letters are significantly different from each other, Fig. 3.0. The letters at the top of each bar were generated from this analysis. From the LSD analysis in Table 1.0 above, it is seen that the reference and control differed significantly from both neem leaf and pear leaf extracts at 0.1% and 0.25%. At 0.5%, the pear leaf water extract had no zone of inhibition hence, was statistically similar to the control. At 0.1%, the pear leaf ethanol extract differed significantly for the pear leaf hexane extract as well as the neem leaf hexane and water extracts. At 0.25%, the pear leaf hexane extract was different from all others except the neem leaf hexane extract. This was also observed at 0.5%. Additionally, the pear leaf ethanol extract differed significantly from all others.

Fig. 7.0. Disc Diffusion assay of neem leaf extract, *Azadirachta indica* against *R. solani* at 0.5% concentration.

6.0. Conclusion

The use of plant extracts as biological control should play an important role in the control and eradication of sheath blight of rice disease. The antifungal properties of pear and neem leaf have proven their ability to inhibit the growth of this pathogen. Results indicated that pear leaf ethanol extract at 0.50% concentration with the highest induced AZOI = 251.24 mm^2 was the most effective against *R. solani* followed by neem leaf hexane extract at 0.50% concentration (AZOI = 195.5 mm^2). Interestingly, the aqueous extract showed the same AZOI at all concentration for both neem (AZOI = 75.1 mm^2) and pear leaf (AZOI = 57.5 mm^2). The lowest AZOI of 0.0 mm2 was induced by the pear leaf aqueous extract at 0.5% concentration. This method of control is economical and eco-friendly and rice farmers and rice sectors are encouraged to promote the use of these biological controls to infected fields and reduce and eliminate the use to harsh synthetic chemicals. It is anticipated that the results obtained from this research project will be shared with the rice sector of Guyana, the GRDB, GRPA and other rice production related entities and their research departments. This information will also be shared with rice farmers countrywide and the general public.

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References

1.Zeigler RS, Leong SA, Teng PS. Rice blast disease. *In Rice Blast Disease*, 1994; 1-626. Cab International, Wallingford.

2. Rampertab R. The price of rice in Guyana, *Guyana under siege*. 2012; Retrieved from http://www.guyanaundersiege.com/Industrial/Price%20of%20Rice.htm , on November1, 2013.

3. Sudhakar R, Rao KC, Reddy CS. Studies on antagonism of *Trichoderma* and *Gliocladium* species against *Rhizoctonia solani* of rice. *Indian Journal of Plant Protection,* 2014; 26, 25–29.

4. Zhong T, Zhang W, Zeng QO, Cheng WL, Zhou GJ, Wang Z, Yin, L. Analyses of temporal development and yield losses due to sheath blight of rice, *Rhizoctonia solani* AG1. 1A). *Agricultural Sciences in China.* 2007; 6(9): 1074-1081.

5. Sateesh. K, Thambiayya M, Jayashree K, Balsamy T, Ramasamy S, Induction of Systemic resistance in rice by leaf extracts of against *Rhizoctonia solani*, Plant Signal Behav. 2011; 6(7):919–923.

6. Persaud, R. *Disease Management In Rice* ,Guyana Rice Development Board, Rice Research Station Burma, M/cony, E.C.D., 2010.

7. IRRI Rice Knowledge Bank (2014), *Sheath blight* http://www.knowledgebank.irri.org/training/factsheets/pestmanagement/diseases/item/sheath-blight?tmpl=component&print=1,on July 22, 2014.

8. Payman, SH, Bagheri I, Zareiforoush H. Milling. Characteristics of rice grains as affected by paddy mixture ratio and moisture content, *International Journal of Biosciences*. 2014; 4 (2): 87-97.

9. Sukanya S.L, Yamini D. and Fathima S. K. *Eco-friendly management of Pyricularia oryzae-The causal agent of blast of paddy, 2011;* Retrieved from http://currentbotany.org/index.php/currbotany/article/view/10090, on November 1, 2013.

10. Vinoth B, Manivasagapermaul R, Rajravindran M. Phytochemical analysis and antibacterial activity of *Azadirachta* a juss. 2012; *International Journal of Research in Plant.*

11. Jeyasakthy S, Jeyadevan JP, Thavaranjit AC, Manoranjan T, Srikaran R, Krishnapillai N. Antifungal activity and qualitative phytochemical analysis of extracts obtained by sequential extraction of some medicinal plants in Jaffna peninsula. *Arch. Appl. Sci. Res.*, 2013; 5 (6): 214-221.

12. Tiwari SN, Nayak M. Activity of four plant leaf extracts against three fungal pathogens of rice. *Tropical Agriculture.* 1991;68: 373-375.

13. Al-Abed, AS, Qasem, JR, Abu-Blam, HA. Antifungal effects of some common wild plants species on certain plant pathogenic fungi. Dirasat (Pure and Applied Science), 1993; 20: 149-158.

14. Amadioha AC. Fungitoxic activity of extracts of *Azadirachta itidica* and *Xylopia aethiopica* on *Cottetotrichum lindemuthianum* in Cowpea. Journal of Herbs, Spices and Medicinal Plants, 1998; 6: 33-40.

15. Amadioha AC. Fungitoxic effects of extracts of *Azadirachta indica* against *Cochliobolus miyabeanus* causing Brown Spot Disease of Rice. Acta. Phytopath. Pflanz. (Taylor & Francis), 2003; 35: 37-42.

16. Zaki Al, Zentmyer GA, Pettus J, Sins JJ, Keen NT, Sing VO. Borbonol from Persea spp, chemical properties and antifungal activity against Phytophthora cinnamomi. Physiology Plant Path. 1980; 16, 205 – 212.

18. Arukwe U, Amadi, BA, Duru MK, Agomuo C, Adindu EN, Odika EA, Lele PC, Egejuru, KC, Anudike J. The chemical composition of *P. americana* leaf, fruit and seed. International Journal of Research & Reviews in Applied Sciences. 2012; 11 (2): 346.

19. Johnson M, Sekhar VC. Principles of plant pathology. Acharya N.G. Ranga Agricultural University. 2012; Retrieved from http://www.angrau.ac.in/media/11003/271finalpracticalmanual.pdf, on October 26, 2013.

20. Barry AL, Coyle MB, Thornsberry CE, Gerlach H, Hawkinson RW. Methods of Measuring Zones of Inhibition with the Bauer-Kirby Disk Susceptibility Test, *Journal of Clinical Microbiology.* 1979; 10 (6): 88.

21. Jagessar RC, Mohamed N. Antimicrobial activity of selected plants extracts from Guyana's flora, Journal of Pure and Applied Microbiology, 2010; 4(2): 533-540.

22. Jagessar RC, Allen R. Antimicrobial Potency of the Aqueous Extract of leaves of *Terminalia catappa,* Academic Research International, 2011; 362-371.

23. Jagessar RC, Mars A, Gomathigayam S. "Selective Antimicrobial properties of Leaf extract of *Samanea Saman* against *Candida albicans, Staphylococcus aureus* and *Escherichia coli using several microbial techniques"*, Journal of American Science. 2011; 7(3): 108-119.

24. Jagessar RC, Mars A, Gomes G. "Leaf extract of *Smilax schomburgkiana* exhibit selective antimicrobial properties against pathogenic microorganisms", Life Science Journal, 2009; 6(1): 76-83.

25. Jagessar RC, Mohammed A, Gomes G. An evaluation of the antibacterial and antifungal activity of leaf extracts of *Momordica Charantia* against *Candida albicans*, *Staphylococcus aureus* and *Eschericia Coli,* Nature and Science, 2008; 6(1): 1-14.

26. Asif M. Antimicrobial Potential of *Azadirachta indica* gainst a pathogenic bacteria and fungi. J. Pharmacognosy and Phytochemistry. 2012; 1: 78-83.

27. Skoog DA, West DM, Holler FJ. Fundamentals of Analytical Chemistry, 7th ed. Thomson Learning, Inc; USA, 1996.

28. Daniel HC. Quantitative Chemical Analysis, 6th ed. W.H. Freeman and Company, New York, 2003. pp 61-79.