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Multi-seasonal evaluation of selected aqueous plant extracts for antimicrobial activities against rice blast disease

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Abstract
Rice blast is one of the most destructive fungal disease that limit rice production. The objective of this study is to evaluate the antifungal potentials of Neem tree (*Azadirachta indica*), Aleo plant (*Aleo vera*), Tobacco (*Nicotiana tabacum*) and Bitter leaf (*Vernonia amygdalina*) aqueous leaf extracts against isolated and characterized *Magnaporthe oryzae, a pathogen* causing rice blast disease at varied concentration (25, 50, and 100%) for three cropping seasons *in vitro* and *in vivo*. The study was laid out in a split-split plot arrangement using a randomized complete block design with three replications. Data were collected for disease severity, disease incidence, and numbers of tillers per plant, number of filled grains, the weight of 100 grains, and panicle weight. Data collected were analyzed using IRRI STAR software. The result reveal that higher plant extract concentration inhibits the rate of pathogen mycelial growth compared to lower concentration in the selected plants. In the field, some levels of significances were observed among the characters studied though at different levels ($p>0.01$ and $p>0.05$) in all the source of variations. Disease severity and incidence decreases as the concentration level increases in all the selected plants. Moreover, others measured characters increases as plant extract concentration increases. The result shows that *Azadirachta indica* is the most efficient among the selected plants followed by *Nicotiana tabacum*, *Aleo Vera and Vernonia amygdalina* respectively. It is recommended that farmers should use the aqueous leaf extract from any of these plants that is available in their locality to manage rice blast as it is economical and ecofriendly.

Keywords: Antifungal potential, aqueous plant extract, disease severity, disease incidence, rice, rice blast

Introduction
Rice (*Oryza sativa* L.) is a cereal crop that is widely grown and consumed across the globe (Shahriar *et al*., 2020) [26]. It is rated as the most consumed staple food globally (Kumar *et al.,* 2020) [21]. More than half of the world human's population rely on rice for their survival directly or indirectly through its consumption, production, processing or marketing (Asante *et al*., 2019). The by-products from rice is used as feed in livestock industry. The level of rice consumption in Nigeria has risen greatly (10.3% and above per year) since the mid-1970s due to increasing human's population growth rate (+2.8% per annum), increasing *per capita* consumption (+7.3% per annum) and shifting consumer preference (Idris *et al.,* 2013) [17]. In Nigeria, rice is well adapted to all the agro-ecological zones (Ajijola *et al.,* 2012) [1]. Despite its favourable environmental and soil production conditions, its production rate is far below the consumption rate. The gap in the production and consumption rate of the product has led to its scarcity and unaffordable for the poor citizens due to the competition that do arose in acquiring the product (Agbowuro *et al.,* 2020a) [8]. To meet the consumers demand, there is a need to bridge the production and consumers gap. Hence, importation of rice grain from other nations where there is sufficient rice production becomes necessary (Kamai *et al.,* 2020) [20]. High import duties followed by Federal Republic of Nigeria government ban on rice importation has make the scenario worse than ever before (Ayinde, 2013) [5]. Enhancing domestic rice production, processing and marketing for food and malnutrition security and sustainability should be a focus point for national development at this critical time.
Various factors responsible for low rice production include biotic and abiotic pressure, inadequate agricultural inputs supply, poor road network, poor funding and inaccessible credit facilities to the rural farmers, poor post-harvest processing and marketing facilities. Of the biotic factors affecting rice production, rice blast disease caused by a fungus named *Magnaporthe oryzae* is regarded as the most dangerous and destructive disease of rice

globally (Miah *et al.*, 2014) [23]. Rice blast disease infect all the plant parts at different stages of its growth and development except the roots (Idowu *et al.*, 2013) [14]. According to Agbowuro *et al.*, 2020b [31], the strategy adopted in managing the disease, field inoculum load, varietal susceptible and prevailing environmental condition of the area play a major role in its severity. Various authors has reported that the disease can cause up to 70 – 100% grain losses if proper management approach is not adopted or in an epidemic growing season (Dean *et al*., 2012) [10]. *Magnaporthe oryzae* infected plants tissues are disorganized, and nutrients and water movement within the plant are disrupted. Thus, this can lead to complete or partial sterility and grain filling failure (Zhu *et al*., 2005; Ram *et al.*, 2007) [29, 25] and general poor performance of the crop. Majority of the rice growers are not aware of the level of damage rice blast is causing on their farm, thus they don't care in managing it. However, those that are managing the disease are using synthetic pesticides while few are sourcing for resistant rice varieties (Mossini *et al.*, 2004) [24]. These chemical pesticides such as (80% of Mancozeb), (50% of Benomyl) etc are not readily affordable to poor resources farmers and has adverse effects on the farmer's health due to improper handling of the chemicals because of the level of their formal education and to the ecosystem at large.

Extracts from plant parts has been reported for their antimicrobial activities since the ancient days and so many authors have confirmed the report Jabeen, (2006) [19] and Lalitha, (2010) [22]. These plant extracts are cheaply available in nature, cost effective, relatively safe for users, biodegradable and ecofriendly. This offer a great opportunity for the farmers and the environment at large. The objectives of this work was to evaluate the efficacy of selected plant extracts against *Magnaporthe oryzae* and also to determine the suitable concentration for effective management for the benefit of the farmers and the environment at large.

Materials and Methods

The study was conducted *in vivo* and *in vitro*. The field experiment was carried out at the Biological Garden of Elizade University, Ilara-Mokin, Nigeria for three consecutive growing seasons (Early and late season of 2020, and early season of 2021). The sites at each growing season had been previously used for the cultivation of arable crops and left to fallow for the period of two years. The prevailing vegetation in the sites include Siam weed, Goat weed, African sun flower and few stands of spear grass. The research state lies on rainforest agro-ecological zone of Nigeria. The laboratory aspect of the work was conducted at the Biotechnology and Microbiology laboratory of Elizade University, Ilara-Mokin, Nigeria. The composite soil sample in the experimental sites were randomly collected at the beginning of each season with sterilized soil auger at 0-15 cm depth before the soil preparation for soil physiochemical analysis and to determine the inoculum load in the soil. Moreover, different locations of about 1,000 meters distance were used for the experiment at each season.

The rice seeds used for this study were FARO 52 and FARO 61 collected from the Africa Rice Centre through the International Institute of Tropical Agriculture, Ibadan Nigeria and two landrace accessions; named after the communities they were sourced from (Igbemo and Mokwa) for the purpose of this study. The two communities (Igbemo, Ekiti State and Mokwa, Niger State) are popular rice cultivated town in Nigeria. *Magnaporthe oryzae*, was isolated from an infected rice plant leaves demonstrating a typical rice blast disease symptom from a rice field from Ijare Forest Reserve Settlement, Ijare Ondo State. Four plants that were readily available in so many part of Nigeria were selected for evaluation of their leaf extracts. The plants includes the Neem tree *(Azadirachta indica)*, Aleo (*Aleo vera*), Bitter leaf (*Vernonia amygdalina*) and Tobacco (*Nicotiana tabacum*). The choice of using water for extraction of the plant extract was its readily available without cost.

Plant Aqueous Extract Preparation

Disease free leaves of the selected plants [Neem tree *(Azadirachta indica)*, Aleo (*Aleo Vera*), Bitter leaf (*Vernonia amygdalina*) and Tobacco (*Nicotiana tabacum*)] were collected at Ijare Reserve Forest, Ijare Ondo State Nigeria. The fresh leaves were properly rinsed with distilled water and air dried for 1 hour, sliced into pieces using sterilized knife and grinded with clean blender to obtain 1 kg of paste. The grinded leaf paste was thoroughly homogenized for 5 min and filtered using a four folds sterilized cheese cloth. The filtrate was centrifuged at 5000 rpm for 15 min and the clear supernatant was collected and designated as standard(s) (Dar *et al*., 2018) [6]. The designated standard solution(s) (25, 50, and 100%) were diluted with distilled water to get the desired concentrations. Mancozeb was prepared at 500ppm concentration within 625 mg of 80% Mancozeb WP in 1000ml of distilled water and mixed very well for homogeneity to be used as control.

Isolation of the *Magnaporthe oryzae* Inoculum

The *Magnaporthe oryzae* infected rice plant leaves collected were sliced with sterilized knife and sterilized with 0.1% mercuric chloride for 30 sec and washed with distilled water. The sterilized infected rice plant leaves were gently transferred aseptically into Potato Dextrose Agar (PDA) containing streptomycin (40 µgL) medium contained in Petri dishes. The streptomycin (40 µgL) was added to prevent bacteria contamination. Incubation of the Petri dishes were carried out at room temperature (28±2ºC). 72 hours after incubation, radiating mycelia growth was observed at the edges of the infected bits. Edge of the fungal colonies was transferred to PDA medium slants in a refrigerator at 10 ºC. The causal organism was identified as *Magnaporte oryzae* based on morphological and cultural characteristics (Tuite, 1969) [28]. The pathogen was sub-cultured to obtain a pure culture. The Petri dishes containing the pathogen "*Magnaporte oryzae"* inoculum were stored in the refrigerator at 5 ˚C for future usage (Harlapur *et al.*, 2007) [12].

In vitro assay of plant extracts on *Magnapothe oryzae* at a varied concentration

The antimicrobial effects of the selected plant extracts on the radial growth of *Magnapothe oryzae* in culture was assessed by growing the fungus on a PDA medium containing different concentration (25, 50 and 100%) of the selected plant extract in a 19 cm diameter Petri dish.

Mycelia segments of 5 mm were made from vigorously growing periphery of a five days old colony of *M. oryzae* on PDA using a sterilized corn borer. Each of the mycelial segment made was transferred one after the other to the

centre of each of the prepared and sterilized PDA with the selected plant extracts at 25, 50 and 100% concentrations (v/v) in Petri dishes. The PDA in Petri dishes without plant extract serve as the control. The PDA in Petri dishes with Mancozeb serve as control while the one with distilled water serve as absolute control. The inoculated PDA media in the Petri dishes were sealed with masking tape, well labelled with permanent marker, and incubated at 25±1 °C for seven days. The experiments were laid out randomized complete block design with a split-plot arrangement with eight replications in three phases. 7 days after incubation, the colony diameter of the *M. oryzae* was cautiously measured for each of the treatments. The inhibition of mycelial growth was measured according to Ogbebor and Adekunle (2005) [30].

$$\% \text{ mycelial inhibition} = \frac{\text{Mycelial growth diameter in control} - \text{Mycelial growth diameter in treatment}}{\text{Mycelial growth diameter in control}} X\ 100$$

Field Trial of Selected Plant Extracts on *M. oryzae*

The study was laid out in Split-Split plot arrangement using a Randomized Complete Block design (RCBD) with three replications. The seeds were sterilized with 0.5% sodium hypochloride solution for 60 sec and rinsed with distilled water before planting. Excellent agronomy practices were adopted to raise a good crop. Two weeks after seedling emergence, the plants were inoculated with a spore suspension (10^6 spores per ml of distilled water) of the *M. oryzae* with 0.02% Tween 20 in 0.25% gelatin per plot uniformly using rechargeable knapsack sprayer. Spraying was done carefully to achieve uniformity at 18:00 hours of the day and left for overnight. At about 15 hours after the plant inoculation, Distilled water was spray at 3 hours interval for five consecutive times to maintain high humidity (Agbowuro *et al*., 2021) [9]. The spore suspension were determined using a haemocytometer. At six weeks after seedling emergence, spraying of the selected aqueous plant extracts at varying concentration was done at 18:00 hour of the day using knapsack sprayer. Control plants were also sprayed with 0.75 ml per litre of 80% Mancozeb WP while absolute control were sprayed with distilled water.

Data Collection and Analysis

Data were collected from 12 randomly selected rice plants per plot for disease incidence (DI) (at
50% day to ripening), disease severity (DS) (at 50% day to ripening), number of tillers per plant (NTP^{-1}) (at 12 weeks after planting), number of filled grains (NFG), the weight of 100 grains (W100G) and panicle weight per plant (PWP^{-1}). Data were subjected to analysis of variance using IRRI STAR software (2014). Scoring for disease severity and incidence were conducted using the International Rice Research Institute standard evaluation system scale (0–9 scale) (IRRI, 2013) [15]. Means were separated by Duncan's Multiple Range Test. The disease incidence and disease severity was calculated using equation i and ii respectively.

$$\text{Disease Incidence (\%)} = \frac{\text{No. of samples affected with disease}}{\text{No. of Sample observed}} \text{ x } 100$$

$$\text{Disease Severity (\%)} = \frac{\text{Sum of the score of diseased samples}}{\text{No. of samples scored}} \text{x } \frac{1}{\text{highest score}} \text{x } 100$$

Results and Discussion

Soil Properties of the Experimental Site

The soil physiochemical properties in the experimental site for the three seasons were presented in Table 1. The values obtained for the soil pH ranges from 5.61 to 5.78. This shown that the soil is slightly acidic. Soil nutrients availability for plants is still possible at this pH level (Golla, 2019) [32]. The total nitrogen ranges from 1.0 to 1.10 g kg^{-1} for the three seasons, these values were lower than the soil nutrient critical levels of 1.5-2.0 g kg^{-1}. The result shown that the soils are deficient in soil nitrogen (Sobulo and Osiname, 1981) [27]. The available phosphorus fall within the range of the soil nutrient level of 10-15 mg kg^{-1} (Agboola and Corey, 1973) [2]. This shows that the soil is not deficient in phosphorus. The soil textural class is sandy loam for the three seasons. This class of soil have the capability to hold water and nutrients. The microbial test conducted in determining the present of the inoculum in the soil shows negative.

Table 1: The soil physiochemical properties of the soil sample.

Properties		Values	
	Season I	Season II	Season III
Sand (g kg^{-1})	590.75	600.34	592.30
Clay (g kg^{-1})	200.18	201.64	198.73
Silt (g kg^{-1})	209.07	198.02	208.97
Textural Class	Sandy loam	Sandy loam	Sandy loam
pH (H_2O)	5.78	5.61	5.66
Total Carbon (g kg^{-1})	9.10	9.22	9.20
Organic Matter (g kg^{-1})	10.55	9.86	10.12
Total Nitrogen (g kg^{-1})	1.0	1.10	1.05
Available Phosphorus (mg kg^{-1})	10.15	10.20	10.25
Ca^{2+} (cmol kg^{-1})	1.20	1.21	1.26
Mg^{2+} (cmol kg^{-1})	0.61	0.70	0.66
K^{+} (cmol kg^{-1})	0.12	0.22	0.18
Na^{+} (cmol kg^{-1})	0.15	0.17	0.16

The laboratory results shown that the selected aqueous plant leaf extracts at different concentrations against *M. oryzae* indicated that the aqueous plant extracts inhibits the pathogen mycelia growth on the PDA. It was observed that the percentage inhibition of mycelial growth of *M. oryzae* increases as the plant extract concentration increases. This is an evidence that the selected aqueous plant extract had antifungal properties against *M. oryzae*. It was observed that *A. indica* leaf extract exhibited the highest level of the mycelial growth inhibition of the pathogen among the selected plant extracts followed by *N. tabacum*, *A. Vera* and *V. amygdalina* respectively. However, the control 500 ppm of Mancozeb had the highest level of the mycelial growth inhibition on the pathogen while absolute control (distilled water) did not inhibit the mycelial growth. There is high level of significances (P>0.05) among all the selected plants at every concentration Table 2.

Table 2: Inhibition of Radial Growth of *M. oryzae* on PDA treated with aqueous Plant Extracts at Different Levels (v/v) of Concentrations in three separate experiments.

Aqueous Plant Extracts and Controls	% inhibition of mycelial growth			
	25%	50%	100%	
Aleo	40.36[c]	45.16[c]	53.44[b]	
Azad	50.53[a]	59.12[a]	64.86[a]	
Nico	43.87[b]	49.67[b]	55.87[b]	
Verno	36.87[d]	41.35[d]	50.15[c]	
Control (Mancozeb)				75.68
Absolute Control, (Distilled water)				0.00

*Values are means of eight replications in three separate experiments.

Means with the same letter on column are not significantly different ($p<0.05$) using Duncan's Multiple Range Test. Note: Aleo: *Aleo Vera*, Azad: *Azadirachta indica*, *Nico. Nicotiana tabacum* and Verno: *Vernonia amygdalina*.

The mean squares for all the parameters measured are shown in Table 3. The mean squares due varieties for all the measured characters were significantly different though at different levels.

The weight of 100 seeds and panicle weight were significant at ($p<0.05$) while others were significant at ($p<0.01$). The level of the significances could be as a result of different in the genetic components of the rice varieties (Akinyosoye *et. al*., 2017). For seasons, all the measured parameters were significantly different though at different levels ($p>0.05$ and $p>0.01$). The slight differences in soil properties and environmental variables within the three cropping season although could contribute to the levels of significances among the parameters measured. For plant extracts, the measured parameters were significant ($p>0.01$) for the measured parameters except weight of 100 seed that is not significant. This could be an evidence of different constituents in the plant extracts. The different levels of interaction studied that serves as source of variation ranging from varieties, season, plant extract and concentrations exhibit different level of significances ($p>.001$ and $p>0.05$) among the characters measured while some where not significant for these interaction.

Table 3: Analysis of variance for all the measured character across three seasons.

So V	DF	DS	DI	NTP	FG (%)	X100SW	PW
Rep.	2	2228.98	409.56	201.60	593.78	288.02	3.69
Var.	3	854.26**	770.69**	330.24**	300.51**	251.85*	6.68**
Season.	2	3.03*	2.08**	0.009**	0.23**	0.08*	1.10*
Var. x Season	6	3.03*	2.10**	0.01**	0.21**	0.08*	1.01**
PE	3	8611.73**	5231.47**	918.94**	3397.47**	2176.19	62.14**
Var. x PE	9	42.72	55.86**	12.60**	18.82**	27.63	0.27**
Season x PE	6	3.26	2.08**	0.02**	0.13	0.03	0.05
Var. x season x PE	18	3.84	2.85**	0.02**	0.12	0.04	0.06
Conc.	2	23362.54	8946.53	3190.02**	3059.25**	4654.67*	100.81**
Var. x Conc	6	29.86	9.58**	2.16**	13.32**	8.61	0.10*
Season x Conc	4	0.29	0.17**	0.03**	0.04	0.02	0.004
PE x Conc	6	181.08**	232.52**	12.77**	19.09**	55.33	2.02*
Var. x Season x Conc	12	0.29	0.17*8	0.03	0.04	0.02*8	0.005
Var. x PE x Conc	18	23.24**	31.17**	11.82**	5.64**	7.13**	0.09**
Season: PE:CONC.	12	0.31	0.17**	0.02	0.11	0.05	0.007
Var: LOC:PE:CONC.	36	0.30	1.79*	0.02	0.11	0.04	0.006
Error	431						

*, * * Significant at ($p< 0.05$) and ($p< 0.01$) levels of probability, respectively

Abbreviations: SV: Source of Variation, DI- Disease Incidence, DS- Disease Severity, NTP^{-1} Number of Tillers per Plant, FG- Number of Filled Grains, X100W- Weight of 100 Grains, PW- Panicle Weight., Rep.- Replication, GEN.- Genotype, PE- Plant Extract and CONC.- Concentration.

Figure 1 shows the effects of different aqueous plant extracts at varying concentrations for three cropping seasons against rice blast severity. The result shown that the disease severity reduces as the concentration level increase. For the three aqueous plant extracts concentration levels (25, 50, and 100%), *V. amygdalina* had the highest level of disease severity followed *A. Vera*, *N. tobacum* and *A. indica* respectively. The plants on absolute control (distilled water) plots and the control (Mancozeb) had the highest and the least level of disease severity respectively in all the plots. Figure 2 presents the effects of different aqueous plant extracts at different concentrations for the three cropping seasons against rice blast incidence. Disease incidence follows the same trend with disease severity. The figure 1 and 2 shows the level of significance ($p>0.05$) for plant extract concentration levels for *M. oryzae* severity and incidence across the three seasons.

The effect of the selected plant aqueous extracts with different concentrations across the three seasons on the number of tillers per plant is presented in figure 3. The result revealed the level of significance ($p>0.05$) for number of tillers per plant across the three seasons. The result shows that increase in aqueous plant extracts concentration levels increases the number of tillers per plant across all the selected plants. Plant that received mancozeb had the highest number of tillers per plant while absolute control (distilled water) recorded the least number of tillers per plant. Within the selected aqueous plant extracts, *A. indica* had the highest number of tillers per plant followed by *N. tobacum*, *A. Vera* and *V. amygdalina* respectively.

Figure 4, 5 and 6 presented the effects of selected aqueous plant extracts at different concentrations across the three seasons for number of filled grains, weight of 100 grains and panicle weight. Rice plants that received the highest concentration of aqueous plant extracts (100%) had the highest values (performed better) for the measured characters shown in figure 4 - 6 followed by 50% and 25% concentrations respectively. However, plant that received mancozeb had the highest values for these characters while absolute control (distilled water) recorded the least values except for number of filled grains. Within the selected aqueous plant extracts, *A. indica* had the highest values followed by *N. tobacum*, *A. Vera* and *V. amygdalina* respectively. The figures shows that the level of plant extract concentration for number of filled grains, weight of 100 grains and panicle weight were significantly different (p>0.05) across the three seasons.

The level of mycelial growth inhibition exhibited *in vitro* and the reduction of disease severity and incidence by the selected aqueous plant leaves extracts proved that the plant leaves extract constituent comprises of some secondary metabolites such as alkaloids, phenolic compounds, glycosides and flavonoids that are could be responsible for the inhibition on the pathogen mycelial growth and reduce the disease severity. The result is in agreement with the work of some authors who reported that plant extract exhibits some antimicrobial potential (Iqbal *et al*., 2014; Hubert *et al*., 2015; Agbowuro *et al*., 2020a) [8]. The higher concentration the plant extracts that brings about decrease in disease severity and incidence, and increase in others measured character than the lower concentration is an indication that higher concentration contain more secondary metabolites that are toxic to *M. Oryzae*. This findings is in agreement with the work of Hubert *et al.* (2015), they reported that the efficacy of plant extract relies on it concentration and its chemical makeup of each plant.

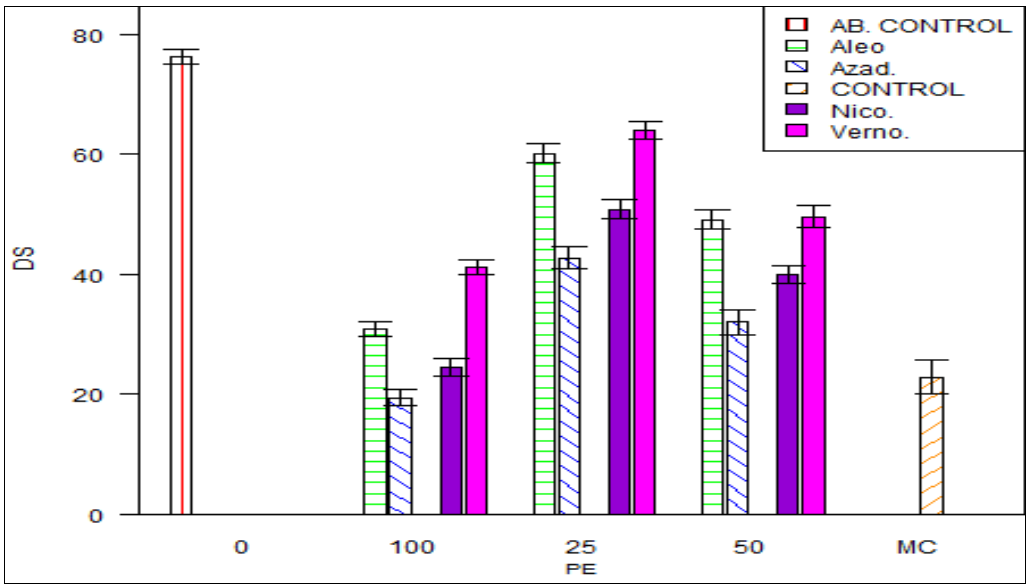

Fig 1: Effects of different aqueous plant extracts at different concentration on disease severity, Abbreviations: DS: Disease Severity, PE: Plant Extract, 0: Absolute control, MC: Control (Mancozeb)

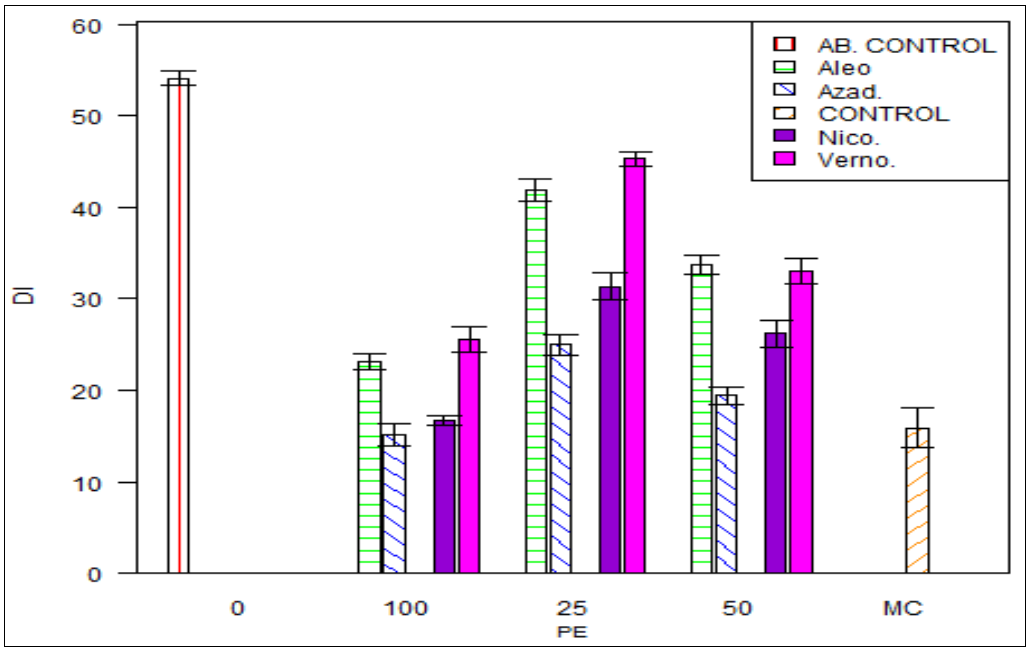

Fig 2: Effects of different aqueous plant extracts at different concentration on disease incidence, Abbreviations: DS: Disease Incidence, PE: Plant Extract, 0: Absolute control, MC: Control (Mancozeb)

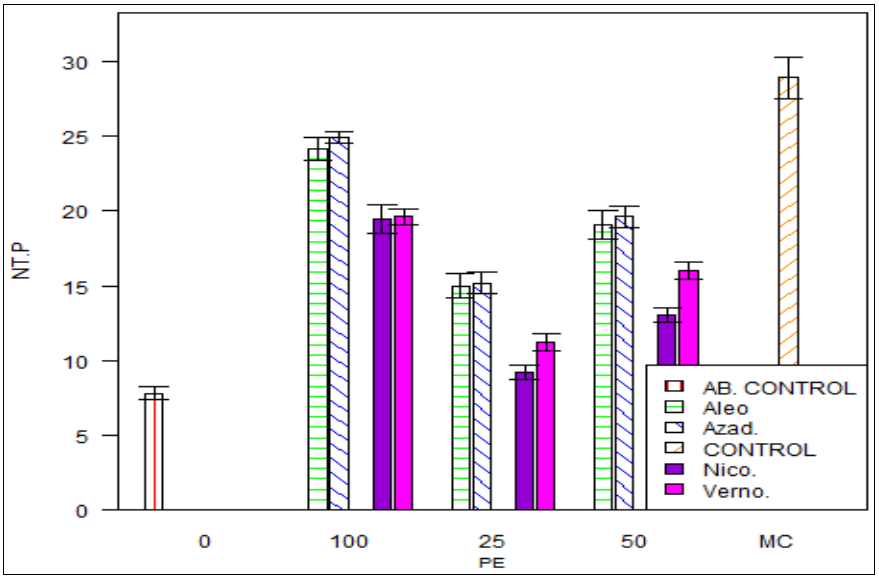

Fig 3: Effects of different aqueous plant extracts at different concentration on number of tiller per plant, Abbreviations: NTP^{-1}: Number of tiller per plant, PE: Plant Extract, 0: Absolute control, MC: Control (Mancozeb)

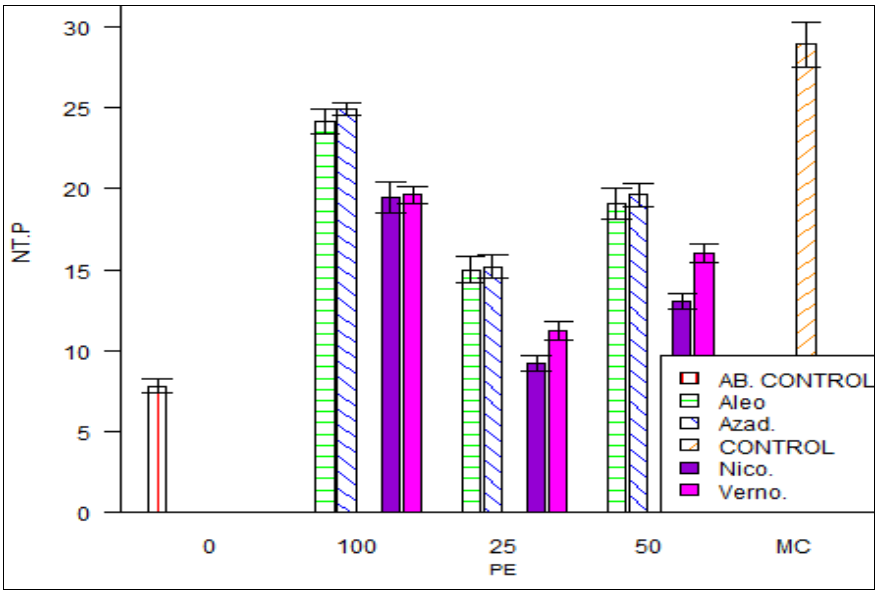

Fig 4: Effects of different aqueous plant extracts at different concentration on disease severity, Abbreviations: DS: Disease Severity, PE: Plant Extract, 0: Absolute control, MC: Control (Mancozeb)

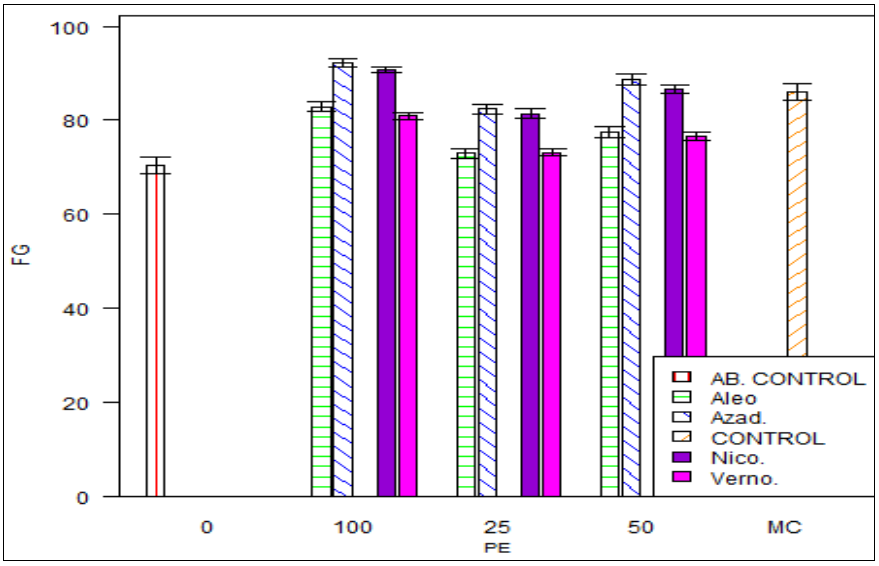

Fig 5: Effects of different aqueous plant extracts at different concentration on number of filled grains, Abbreviations: FG: Number of filled grains, PE: Plant Extract, 0: Absolute control, MC: Control (Mancozeb)

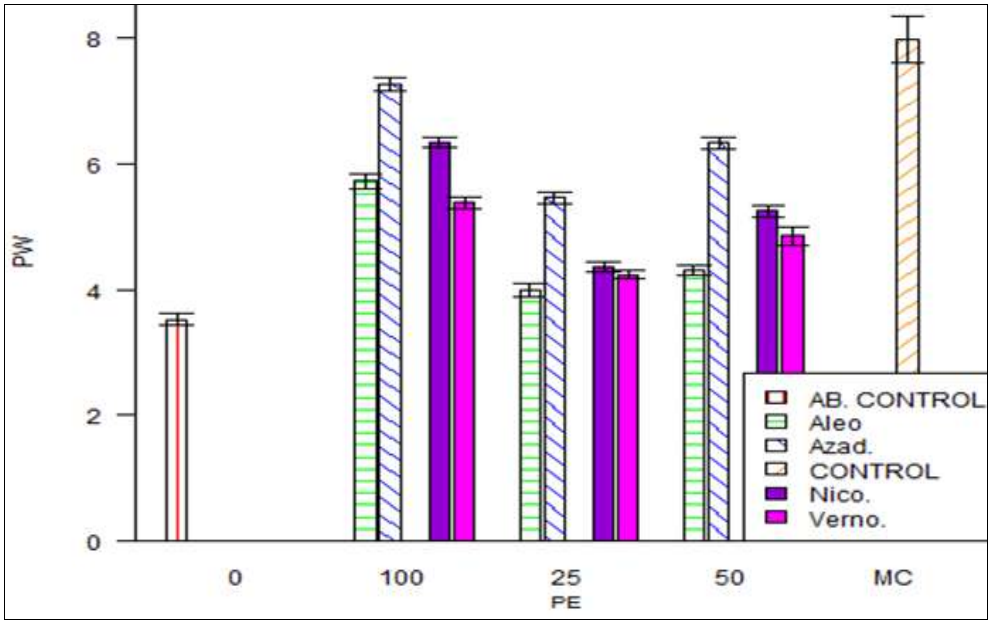

Fig 6: Effects of different aqueous plant extracts at different concentration on panicle weight, Abbreviations: PW: Panicle weight, PE: Plant Extract, 0: Absolute control, MC: Control (Mancozeb).

Conclusion

The result obtained from this work shown that biological control of diseases is coming as a game changer in modern agriculture in reducing disease incidence without causing any hazard to the farmers and the ecosystem at low or no cost. Base on the findings from this work, it was confirmed that aqueous plant extracts of various plants that are readily available could use to manage *M. oryzae*, the most destructive rice plant disease. Moreover, these plant extracts are biodegradable and non-phytotoxic. Thus, the cost of rice production could be reduced by avoiding the cost of buying fungicides. It was observed that *A. indica* outperformed other selected plant extracts followed by *N. tabacum*, *A. vera* and *A. amygdalin* in that order at 25, 50 and 100% concentration levels respectively. It is therefore recommended that farmers should use *A. indica* aqueous leaf extracts to manage *M. oryzae* at 100% concentration. However, in an area where *A. indica* is not readily available, *N. tobacum*, *A. Vera* and *V. amygdalina* aqueous leaf extracts could also be used at 100% concentration depending on their availability.

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