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EVALUATION OF DIFFERENT AQUEOUS PLANT EXTRACTS AGAINST RICE BLAST DISEASE FUNGUS (*Magnaporthe oryzae*)

[1*]Agbowuro G. O., [2]Aluko M., [2]Salami A. E., [3]Awoyemi S. O.

[1]Department of Biological Sciences, Elizade University, Ilara-Mokin, Ondo State.

[2]Department of Crop, Horticulture and Landscape Design, Ekiti State University, Ado-Ekiti.

[3]Department of Crop Production Technology, Federal College of Agriculture, Akure.

* Corresponding author's email: agbowuro@yahoo.com

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ABSTRACT

The antifungal potentials of some medicinal plant leaf extracts have been established against fungal diseases. This research work was conducted to evaluate the effects of aqueous plant leaf extracts of five plants: Apple of Sodom (*Calotropis procera*), Neem tree (*Azadirachta indica*), Thorn Apple/Angel's trumpet (*Datura metel*), Aleo plant (*Aleo vera*) and Siam weed (*Chromolaena odorata*)) at different concentration (25, 50, and 100%) against rice blast disease (*Magnaporthe oryzae*) in-vitro and in-vivo. The research work 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, number of tillers per plant, number of filled grains, the weight of 1000 grains, and panicle weight. The data collected were analyzed using IRRI STAR software (IRRI, 1979). Percentage inhibition was significantly higher at higher concentrations for all the aqueous plant extracts as compared to lower concentrations. The field trial result shows that there were significant differences among all the studied traits though at different levels for all the sources of variation. The leaf plant aqueous extracts at all the varied concentrations reduced the rate of disease severity and incidence while the number of tillers per plant, the number of filled grains, the weight of 1000 seeds, and panicle weight increased compared to control. The result revealed that Apple of Sodom (*Calotropis procera*) is the most efficient in combating rice blast disease followed by Neem tree (*Azadirachta indica*), Thorn Apple/Angel's trumpet (*Datura metel*), Aleo plant (*Aleo vera*), and Siam weed (*Chromolaena odorata*) in that other. These aqueous plant extracts can be used to manage rice blast disease at a low cost and it is ecofriendly compare to chemical fungicides.

Keywords: Disease severity, *Magnaporthe oryzae*, Medicinal plants, Plant extracts, Rice.

INTRODUCTION

Rice is a cereal crop that is commonly grown globally (Shahriar *et al.*, 2020). Rice as a staple food occupies a prominent place in the human diet as the grain forms a rich calorie source (Agbowuro *et al.*, 2020). It was estimated by the International Rice Research Institute, Philippines that rice production has to increase by 33.3% by the year 2020 to meet the demand of the world population. Shortages of annual increase of rice grain globally have been estimated to be from 400,000 tons in the year 2016 to 800,000 tons by 2030 (Thirze, 2016). Its production rate in Nigeria has been reported to be lesser than its consumption rate; four millions metric tons were produced in Nigeria while the consumption rate was seven million metric tons in 2018 thereby resulting in the scarcity of the product, leading to competition among the consumers and an increase in price. In some developing countries like Nigeria, rice is now a golden meal amidst low-class citizens. Nigeria relies on the importation of rice from Asian countries which is estimated to be over three million tons per year (Kamai *et al.,* 2020).

Various biotic and abiotic pressures limit rice production across the globe (Zhang and Xie, 2014). Rice blast disease is recognized as the most destructive fungal disease among the biotic factors affecting rice production (Miah *et al.,* 2014. Nasruddin and Amin, 2013). This disease has been reported to cause up to 70 - 80% grain loss whereas some authors reported yield losses of 100%, that is, total loss during the epidemic growing season (Zhu *et al.*, 2005, Prasad *et al.*, 2006 and Dean *et al.*, 2012). Rice blast disease infects all rice plant parts except its roots, and the highest losses in rice grain yield are associated with neck blast (Zhu *et al.*, 2005).

The severity of the grain yield loss from rice blast disease depends on the susceptibility of the variety grown, prevailing environmental condition of the area, the degree of infection, inoculum load on the field, and timing of controlling the disease (Agbowuro, 2020). Blast destroys rice that can feed over 60 million people for a year

(Scheuermann *et al*., 2012). Rice blast has been recognized as a threat to rice production, its sustainability, global food insecurity, and humanity in general. The knowledge of rice blast disease management is essential to avoid a reduction in biomass accumulation, grain yield loss, and unwanted expenses by the growers or farmers in managing the disease. This knowledge will not only bridge the wide gap between rice supply and its demand but also reduce the cost of production, reduce rice grain price, and sustain food security. The use of resistant variety is another promising way of managing the disease but the varieties are not readily available for the growers in developing nations. The objective of this work was to evaluate different aqueous plant extracts and also to determine its appropriate concentration against rice blast disease fungi *Magnaporthe oryzae*.

MATERIALS AND METHODS

The Study Location

The research work was conducted at Microbiology Laboratory and Biological Garden of Elizade University, Ilara Mokin, Ondo state, Nigeria between January to August 2020. A composite soil sample of the experimental site was randomly taken at the depth of 0-15cm before land preparation and the soil sample was analyzed at the Soil Science Laboratory of Ekiti State University, Ado-Ekiti.

Experimental Materials

The plant materials used for this research work comprised of five rice genotypes collected from the Africa Rice Centre through the International Institute of Tropical Agriculture, Ibadan Nigeria. The rice genotypes were N-L-43, FARO 52, KOGONI 91-1, LUSItano, and FARO 61. *Magnaporthe oryzae*, a fungus was isolated from the rice plant showing typical symptoms from the Faculty of Agricultural Sciences of Ekiti State University rice field. Five plants–selected for evaluation, of their leaf extracts for the study are the Apple of Sodom (*Calotropis procera*), Neem tree *(Azadirachta indica)*, Thorn Apple/Angel's trumpet (*Datura metel*), Aleo (*Aleo vera*), and Siam weed (*Chromolaena odorata*). The plants were selected for the research work based on previous research works conducted on the plants on their anti-fungal properties and their availability throughout the year in the study area.

Plant Extract Preparation

Healthy leaves of the selected plants were collected from Ilara-Mokin and its environs in Ondo state, Nigeria. Fresh leaves of the selected plants: Apple of Sodom (*Calotropis procera*), Neem tree *(Azadirachta indica)*, Thorn Apple/Angel's trumpet (*Datura metel*), Aleo (*Aleo vera*), and Siam weed (*Chromolaena odorata*) collected were properly washed with distilled water to remove the dirt, inside the laboratory. One (1) kg of each selected plant leaves was cut into pieces using a disinfected knife and macerated using grinder in 1 l of distilled water. The macerated plant material was homogenized for 5 min and filtered using a muslin cloth. The filterate was centrifuged at 5000 rpm for 15 min and the clear supernatant was collected and designated as standard(s) (Dar *et al*., 2018). The standard solution(s) was diluted with distilled water to get the desired concentrations (25, 50, and 100%).

Isolation of the *Magnaporthe oryzae* Inoculum

Rice plant leaves typically showing the symptoms of leaf blast (*Magnaporthe oryzae*) were collected from the Faculty of Agricultural Sciences of Ekiti State University Rice Field. The diseased leaves were sliced into small pieces and sterilized with 0.1 percent mercuric chloride for 30 sec and later washed several times using sterilized water. The sterilized, sliced infected rice leaves were transferred aseptically into potato dextrose agar (PDA) medium contained in Petri dishes. Before the pouring of the potato dextrose agar into the Petri dishes, streptomycin (40 μgL^{-1}) was added to prevent contamination by bacteria. The Petri dishes were incubated at room temperature (28±2 ºC). Three days after incubation, radiating mycelia growth was noticed at the edges of the infected bits. Edge of the fungal colonies was transferred to potato dextrose agar medium slants in a refrigerator at 10 ºC and periodical sub-culturing for all the studies. The causal organism was identified as *Magnaporte oryzae* based on morphological and cultural characteristics (Tuite, 1969). The Petri dishes containing *Magnaporte oryzae* inoculum were stored in the refrigerator at 5 °C (Harlapur *et al.,* 2007).

In-vitro Assay of Plant Extracts on *Magnapothe oryzae* at a Varied Concentration

Mycelia segments (5 mm) were made from actively growing periphery of a 5-day old colony of *Magnaporthe oryzae* on potato dextrose agar using a sterile 5 mm diameter cork borer. Each of the mycelial segment made was then transferred one by one aseptically into the centre of each of the prepared and sterilized potato dextrose agar with plant extracts at 25, 50 and 100% concentrations (v/v) in Petri dishes while the potato dextrose agar Petri dishes without plant extract serve as the control. The inoculated Petri dishes were properly sealed with masking tape, well labelled, and incubated at 25±1 °C for 7 days. The experiments were laid out in a split-plot arrangement in a randomized complete block design with rice blast as the main plot while five plant extracts at three different concentrations and control serve as treatments with seven replications in two phases. Seven days after incubation, the colony diameter of the fungus was carefully measured for each of the treatments and the inhibition of mycelial growth was estimated based on the method of Ogbebor and Adekunle (2005).

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

Field Trial of Plant Extracts on *Magnapothe oryzae* at a Varied Concentration

The research work was laid out in Split-Split plot arrangement using a Randomized Complete Block design with three replicates analysis of variance (ANOVA) model. Planting was done on the 10th of March, 2020. Good agronomy practices were adopted in the course of the research work to raise a good crop. The sub-cultured isolated pathogen (*Magnaporthe oryzae*) in the laboratory was mixed with water (an isolate to 10 ml) and introduced to the rice plants uniformly by spraying using a knapsack sprayer two weeks after planting at 18 hours of the day. Thereafter, spraying of the different plant extracts at varying concentrations was done around 18 hours of the day at six weeks after sowing (four weeks after inoculum introduction to plants).

Data Collection and Analysis

Data were collected from 10 randomly selected rice plants on plot basis for disease incidence (at 50% day to ripening), disease severity (at 50% day to ripening), number of tillers per plant (at 10 weeks after planting), number of filled grains, number of unfilled grains, the weight of 1000 grains and panicle weight. The data collected was analyzed using IRRI STAR software (IRRI, 1979). Scores for disease severity and incidence were done by adopting the International Rice Research Institute standard evaluation system scale (0 - 9 scale) (IRRI, 1979). Means were separated by Duncan's multiple range test (DMRT) (P = 0.05).

The disease incidence and disease severity was calculated using equation i and ii respectively.

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

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

RESULTS

Soil Properties of the Experimental Site

The physiochemical properties of the soil (0-15cm) in the experimental site are presented in table 1. The values obtained for the soil pH, carbon (%), organic matter (%), nitrogen (%), Ca^{2+} (C mol kg^{-1}), Mg^{2+} (C mol kg^{-1}), K^{+} (C mol kg^{-1}), Na^{+} (C mol kg^{-1}) are: 5.30, 0.80, 1.39, 0.09, 1.05, 0.55, 0.10, and 0.16 respectively. The soil textural class was sandy loam.

Table 1: Physical and Chemical Characteristics of the Soils in the Experimental Site

Properties	Values
Sand (%)	65
Clay (%)	16
Percentage silt	19
Textural class	Sandy loam
pH	5.30
Carbon (%)	0.80
Organic matter (%)	1.39
Nitrogen (%)	0.09
Phosphorus (mg kg^{-1})	8.80
Ca^{2+} (C mol kg^{-1})	1.05
Mg^{2+}(C mol kg^{-1})	0.55
K^{+} (C mol kg^{-1})	0.10
Na^{+}(C mol kg^{-1})	0.16

Table 2: Percentage Inhibition of Radial Growth of *Magnaporthe oryzae* on Potato Dextrose Agar Mixed with Plant Extracts at Different Levels (v/v) of Concentrations

Plant extracts	(% inhibition of mycelial growth)*		
	25%	50%	100%
METEL	47.02 c	48.11 c	63.80 c
NEEM	50.48 b	57.00 b	69.54 b
PRO	55.30 a	63.88 a	74.62 a
SIAM	40.25 e	43.85 d	56.31 d
VERA	44.19 d	49.60 c	56.37 d

*Values are means of seven replicates in two separate experiments. Means with the same letter on column are not significantly different (P <0.05) using Duncan's Multiple Range Test.

Abbreviations: PRO- *Calotropis procera*, NEEM- *Azadirachta indica*, METEL- *Datura metel*, VERA- *Aleo vera*, and SIAM- *Chromolaena odora.*

Effects of Plant Extracts on the Growth of *Magnaporthe oryzae in-vitro*

The results indicate that tested plant extracts at varied concentrations against *Magnaporthe oryzae* showed a positive effect in inhibiting its mycelia growth. The mycelia growth of *Magnaporthe oryzae* on potato dextrose agar amended with aqueous plant extract showed that all the selected plants had high antifungal properties against *Magnaporthe oryzae* at (100%) but low at 25% concentration *in-vitro* (Table 2). For each plant extracts studied, the inhibitory effect on *Magnaporthe oryzae* at varying concentrations was significantly different ($P<0.05$). The higher the concentration, the more inhibitory effect observed. Extract from Apple of Sodom (*Calotropis procera*) at 100% gave the highest radial growth inhibition (74.62%) while the lowest radial growth inhibition was observed in Siam weed (*Chromolaena odorata*) at 25% concentration (40.25 %) on potato dextrose agar amended with plant extract (Table 2).

Mean Squares for the Studied Traits in Field Trial

Mean squares for all the studied traits are shown in table 3. The mean squares due genotype for all the studied traits were significantly different for all the traits though at different levels. Disease severity, the weight of 1000 seeds, and pod weight were significant at ($P<0.01$) while others were significant at ($P<0.05$). For plant extract and concentration, all the studied traits were significant at different levels as well. Moreover, genotype by plant extract concentration and plant extract by its concentration interaction was also significantly different at different levels. Genotype by plant extract concentration interaction was significant for all the studied traits at ($P<0.05$).

Table 3: Analysis of Variance for All the Studied Traits

S/V	DF	DS	DI	NT./P	FG	NFG	X1000SW	PW
REP.	2	701.34	212.39	61.11	195.28	445.68	114.96	1.69
GEN.	4	471.25**	270.40 *	104.75*	80.71*	83.99*	154.65 **	2.36 **
PE	4	3344.91*	2448.42 *	533.29 *	1535.02 **	1500.41*	921.53**	29.51**
GEN. :PE.	16	22.33 *	17.12*	3.93*	11.31*	9.17*	9.90*	0.105*
CONC.	2	1213.92*	5069.68*	1776.70*	1498.58*	1516.69*	2189.76 **	45.23**
GEN.:CONC.	8	9.02 **	2.72**	0.51 **	4.09 **	6.11**	3.163**	0.11**
PE.:CONC.	8	64.83*	88.22*	11.58**	9.53 **	11.69**	17.87 *	0.71*
GEN.:PE:CONC.	32	7.60*	8.87*	3.5*	2.41*	3.52*	3.39 *	0.03*
ERROR	148	6.15	2.70	0.92	4.67	4.57	1.75	0.01

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

Abbreviations: SV: Source of Variation, DI- Disease Incidence, DS- Disease Severity, NT./P- Number of Tillers per Plant, FG- Number of Filled Grains, NFG- Number of Unfilled Grains, X1000W- Weight of 1000 Grains, PW- Panicle Weight., Rep.- Replication, GEN.- Genotype, PE- Plant Extract and CONC.- Concentration.

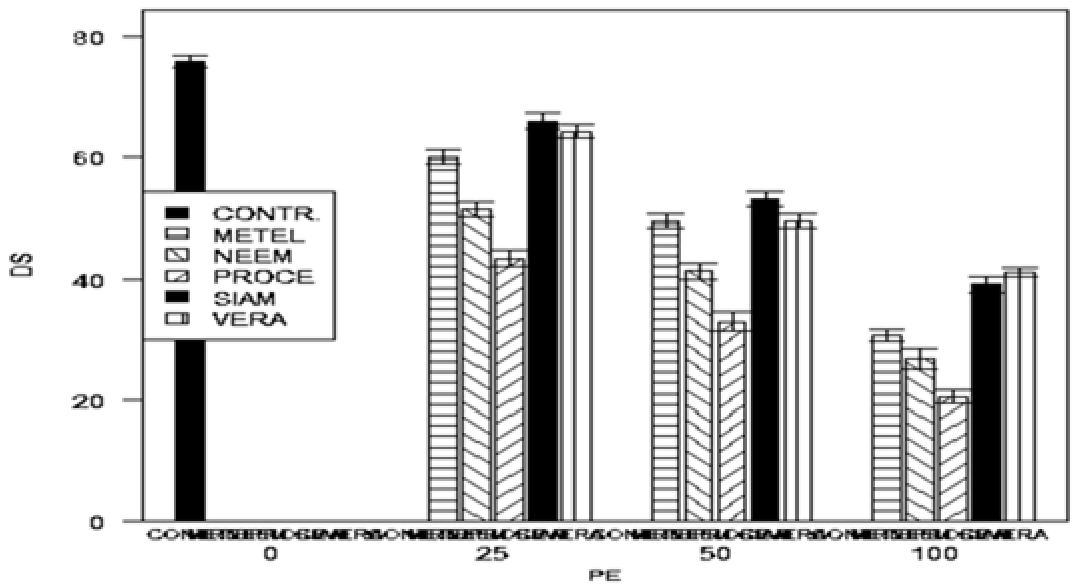

Figure 1: Effects of different Plant Extracts and Its varied Concentration on Disease Severity

Abbreviations: DS: Disease Severity, PE: Plant Extract

Figure 1 shows the effects of different plant extracts and their different concentrations on disease severity. The result revealed that the disease severity reduces as the concentration increases. Across the three concentration levels (25, 50, and 100 %), the Apple of Sodom (*Calotropis procera*) plant extract had the least level of disease severity followed by Neem tree *(Azadirachta indica)* and Thorn Apple/Angel's trumpet (*Datura metel*) respectively, while rice plants that receive no treatment (control) showed the highest level of disease severity. The effect of different plant extracts and their different concentrations on disease incidence is presented in figure 2. The same trend observed in disease severity was observed in disease incidence. Rice plants that received no treatment (control) had the highest level of disease incidence while plants that were treated with Apple of Sodom (*Calotropis procera*), Neem tree *(Azadirachta indica)*, Thorn Apple/Angel's trumpet (*Datura metel*), Aleo (*Aleo vera*) and Siam weed (*Chromolaena odorata*) extracts had the least level of severity respectively across the three-level of concentrations. The effect of the different plant aqueous extracts across the different concentrations on the number of tillers per plant is presented in table 3. Control had the least number of tiller per plant. As the concentration keeps increasing, the number of tillers per plant keeps increasing. Apple of Sodom (*Calotropis procera*) had the highest number of tillers followed by Thorn Apple/Angel's trumpet

(*Datura metel*), Neem tree *(Azadirachta indica)*, Aleo (*Aleo vera*), and Siam weed (*Chromolaena odorata*).

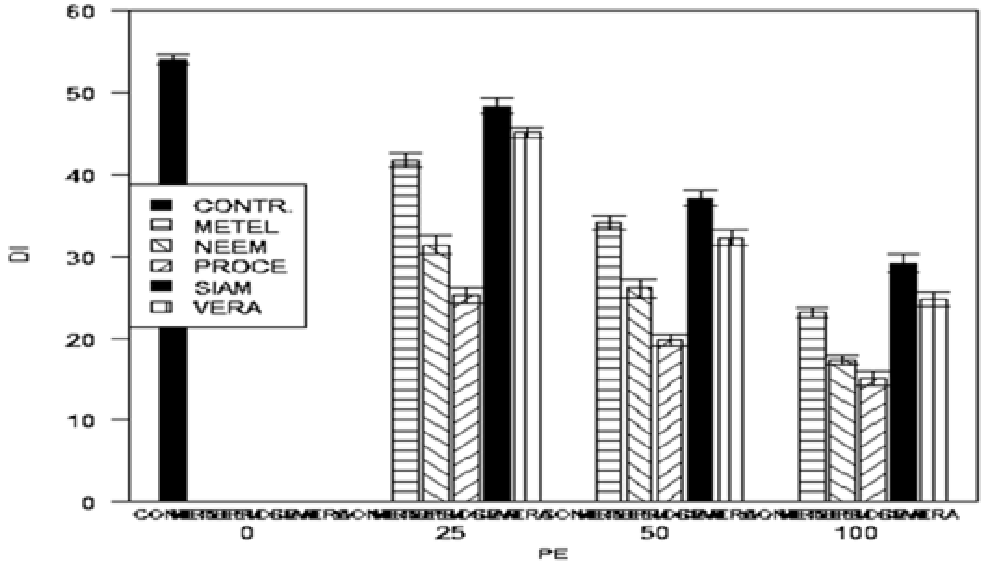

Figure 2: Effects Different Plant Extracts and Its different Concentration on Disease Incidence.

Abbreviations: DS: Disease Incidence, PE: Plant Extract

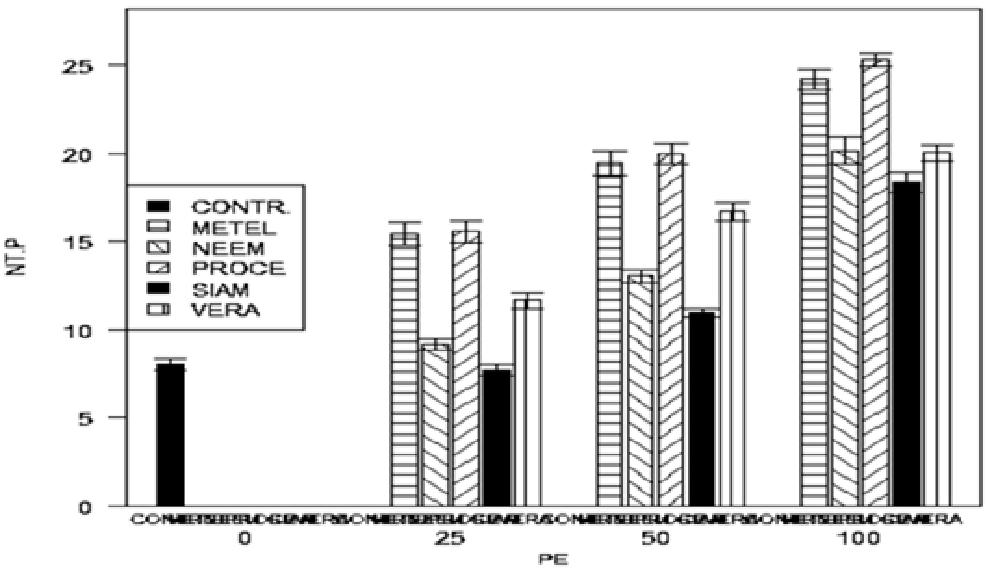

Figure 3: Effects different Plant Extracts and Its Varied Concentration on the Numbers of Tillers per Plant.

Abbreviations: NTP: Numbers of Tillers per Plant, PE: Plant Extract.

Figure 4 shows the effects of different plant extracts and their different concentrations on the number of filled grains. The number of filled grains was close, but analysis of variance revealed the level of significance. Plants that received no treatment had the least number of grain-filled while plants treated with *Calotropis procera* had the highest number of grain-filled followed by *Azadirachta indica*, *Aleo vera*, and *Datura metel* in that order. Similar results were observed for the weight of 1000 seeds and panicle weight as presented in figures 5 and 6. Rice plants that received the highest plant extract concentration (100%) had the highest weight of 1000 seeds and panicle weight followed by 50% and 25 % concentrations.

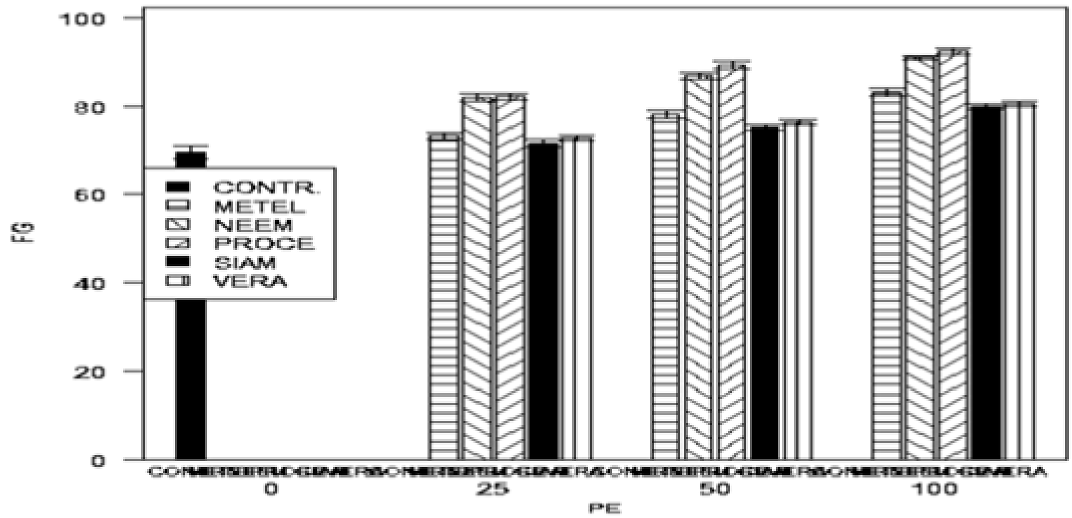

Figure 4: Effects different Plant Extracts and Its Varied concentration on the Numbers of Filled Grains

Abbreviations: FG: Numbers Filled Grains, PE: Plant Extract.

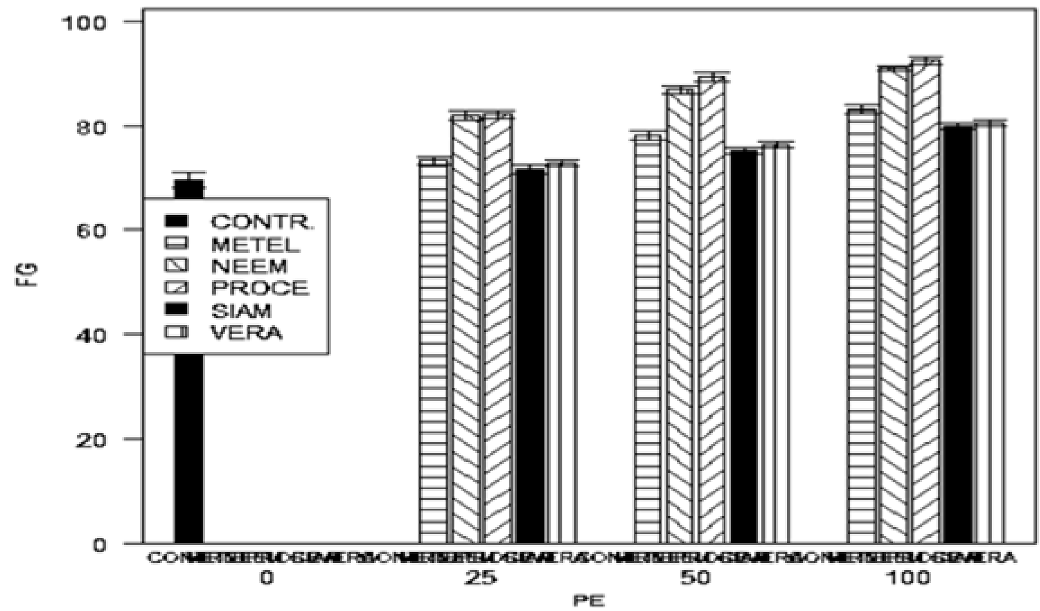

Figure 5: Effects different Plant Extracts and its Varied Concentration on Weight of 1000 Grains

Abbreviations: W100S: Weight of 1000 Grains, PE: Plant Extract.

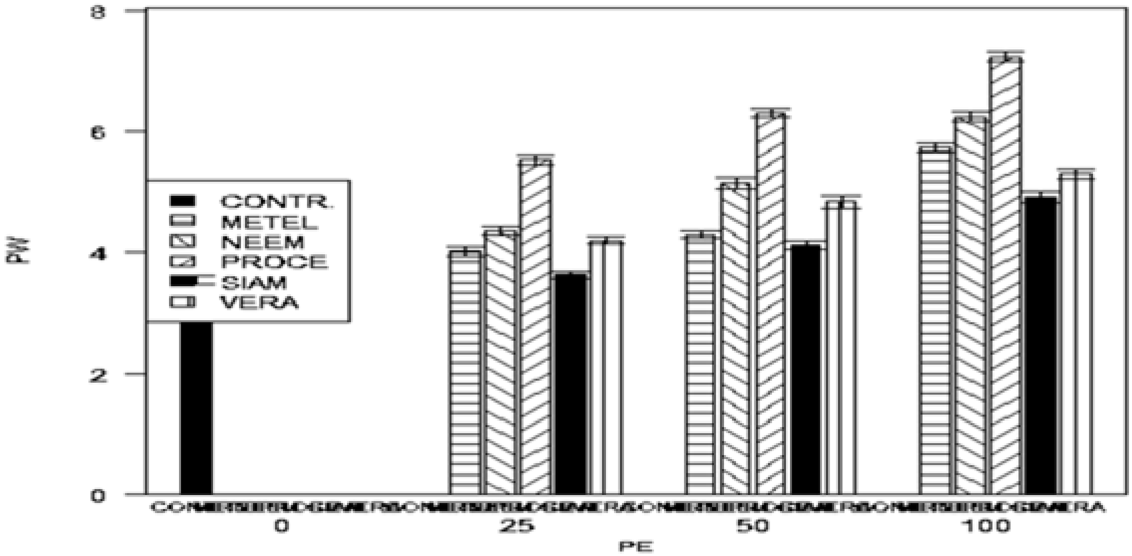

Figure 6: Effects different Plant Extracts and Its Varied Concentration on the Panicle Weight

Abbreviations: PW: Panicle Weight, PE: Plant Extract.

DISCUSSION

Some levels of antifungal activities were found in the plants that were studied all of which are abundantly available around Ondo state, Nigeria. All the plants' aqueous extracts have antifungal activities at different concentrations and were significantly different (<0.05) at different levels in both *in-vitro* and on field trials. Variations were observed in inhibitory effects against the growth of the fungus among plant species selected. Of all the selected plants, the Apple of Sodom (*Calotropis procera*) proved to be the most effective in inhibiting the pathogen growth on the field and *in-vitro* at all concentrations. This result is in agreement with Dar *et al.* (2018) who reported that plant extracts have antifungal properties that can be used against fungal diseases in form of seed treatment or foliar application. Iqbal *et al*., (2014) reported a similar result on the effectiveness of plant extracts on charcoal rot disease of Mungbean when treated. Hubert *et al.* (2015) also reported similar results with aqueous extracts of *Aloe vera, Azadirachta indica,* and *Datura stramonium* for the control of rice blast disease (*Magnaporthe oryzae*) *in-vitro* and on the field.

Taiga and Friday (2009) reported that phytochemicals such as alkaloids, tannins, glycoside, steroids, saponin, medicagenic acid, flavonoids, and phenols present in the plants could be toxic to the fungus thereby causing its growth inhibition (Olufolaji *et al*., 2015). Iqbal *et al*., (2014) explained that a direct relationship exists between concentrations and pathogen growth inhibitions. The pathogen growth inhibitions increase and the rate of blast severity reduces on rice plants as the concentration of the plant extracts increases, evidence that toxic metabolites are present in these aqueous plant extracts; because as the concentration increases, the metabolites also increases. Higher concentrations indicated more toxic components than lower concentrations. This is in conformation with the report Akintobi *et al.* (2016) who concluded that the effectiveness of plant extracts depends on its concentration. The differences recorded among the different five plant extracts might be a result of the different chemical makeup of each plant. Liu *et al*., (2013) and Chen *et al*., (2014) explained that the mechanisms of disease suppression by plant extracts could be that the phytochemicals present in the plant parts may either act and suppress the pathogen directly or it activates the systemic resistance in the plants thereby increasing the plant immunity, hence the disease development is reduced.

The percentage of area damage or lesions caused by the disease on the plant leaves will reduce the area available for photosynthesis in the plant thereby reducing carbohydrate synthesis and grain yield is reduced in return (Agbowuro *et al*., 2020). Many rice growers are not aware that damage done to the plant's leaves affects yield, so they are not bordered whenever they see lesions, a symptom of the leaf blast in their field. Comparing the control (rice plant that received no treatment) with plants that were sprayed with aqueous plant extract at different concentrations, the results show that the plant extract is not toxic to the plants because those treated with varying levels of concentration produce more tillers, gave a higher quantity of grain-filled and panicle weight. If the plant's aqueous extracts were toxic to plants, a concentration at 100% would have recorded the least number of tillers per plant and panicle weight.

Considering the trends of the result, reduction in disease incidence and severity increases the number of tillers per plant and number of filled grains, the higher the number of tillers the more panicle that is produced. Pathogen attack on the rice plant could disorganize the plant tissues and inhibit the movement of water and nutrient (Agbowuro *et al*., 2020). The inhibition of water and nutrient movement could be responsible for the low number of tillers per plant, poor grain fill and low panicle weight in rice plants that received no treatment and those that received low concentration of the plant extracts. This result explains clearly that aqueous plant leaf extract reduces the activities of the pathogen in a concentration-dependent manner.

CONCLUSION

The results obtained from this research work have shown that aqueous plant extract of the selected plants reduced the severity of rice blast disease caused by *Magnaporthe oryzae* and thereby increases yield grain. Aqueous plant extracts obtained in this study are biodegradable, non-phytotoxic, readily available at no cost, hence could reduce the cost of rice production. The result revealed that Apple of Sodom (*Calotropis procera*) extract was the most effective out of the five plant leaf extracts evaluated followed by Neem tree *(Azadirachta indica)*, thorn Apple/Angel's trumpet (*Datura metel*), Aleo (*Aleo vera*), and Siam weed (*Chromolaena odorata*) in that order at 100% concentration level. It is recommended that farmers could use Apple of Sodom (*Calotropis procera*) leaf extract to control rice blast disease on the rice field. In an area where the Apple of Sodom (*Calotropis procera*) is not readily available Neem tree leaves *(Azadirachta indica)* or thorn Apple/Angel's trumpet (*Datura metel*) could be used in place of chemical fungicides.

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