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Efficacy of Selected Plant Extracts against *Pyricularia grisea*, Causal Agent of Rice Blast Disease

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Abstract

Rice blast disease, caused by a seed-borne fungus *Pyricularia grisea*, is an important and serious disease of rice (*Oryza sativa* L.) worldwide. The disease has been reported to cause yield losses of up to 40% in Tanzania. Studies were conducted to determine the effect of aqueous extracts of *Aloe vera*, *Allium sativum*, *Annona muricata*, *Azadirachta indica*, *Bidens pilosa*, *Camellia sinensis*, *Chrysanthemum coccineum*, processed *Coffee arabica*, *Datura stramonium, Nicotiana tabacum* and *Zingiber officinalis* for control of rice blast disease (*Pyricularia grisea*) *in-vitro* and *in-vivo*. The results indicate that processed *C. arabica* at 10% and 25% (v/v) had the highest (81.12%) and (89.40%) inhibitory effect, respectively, against *P. grisea*. Aqueous extract from *N. tabacum* at 10% concentration ranked third (80.35%) in inhibiting *P. grisea*. These were followed by extracts from 25% *A. vera* (79.45%) and 25% *C. coccineum* flower (78.83%). The results also indicate that, extracts from *A. indica*, *A. vera*, *A. sativum*, *C. arabica*, *D. stramonium*, *C. sinensis*, *Z. officinalis* and *N. tabacum* did not have any phytotoxic effect on seed germination, shoot height, root length, dry weight, seedling growth and seedling vigour index. These plant extracts can thus be used for rice seed treatment to manage rice blast disease.

Keywords

Plant Extracts, *Pyricularia grisea*, Rice

1. Introduction

Rice blast disease, caused by a seed-borne fungus *Pyricularia grisea*, is an important and serious disease of rice

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(*Oryza sativa* L.) worldwide [1]-[3]. The disease has been reported to cause yield losses of up to 40% in Tanzania [4]. Rice blast disease is one of the major factors causing losses in quality and quantity of rice [5]. Small-scale farmers in Tanzania keep their harvested rice seeds for sowing in the following season. This practice facilitates multiplication of *P. grisea* within the seed and becomes a source of inoculum for new infection [6].

Management of rice blast is difficult because the pathogen is seed-borne. Management approaches have mainly focused on the use of synthetic chemicals and resistant rice varieties [7]. In Tanzania, rice blast management has been limited to foliar spray with fungicides such as Dithane M-45 (80% of Mancozeb) and Benlate (50% of Benomyl) [8]. However, their efficacy can be limited by the rapid development of resistance of the pathogen to these chemicals. Chemicals applied as either seed dressing or spray such as Abendazim, Benomyl, Benomyl + Copper sulphate, probenazole, thiabendazole and pyroquilon fungicides have been used in different rice growing countries to manage rice blast disease [9]. However, such chemicals are expensive, not easily available to small-scale farmers and have detrimental effects on the environment, farmer and consumer health, beneficial predators and parasitoids [10]. These fungicides have also been reported to affect useful organisms used in biological control programs [11] [12]. Amadioha [13] reported water and ethanol leaf extracts and oil extract of seeds of *Azadirachta indica* to be effective in reducing radial growth of *P. grisea in-vitro* and development and spread of blast disease in rice plants. Therefore, natural plant products (botanicals) are becoming a new source of agricultural chemicals [13] to manage plant diseases.

Plant extracts have been known for their medicinal and antimicrobial properties since ancient times [14] [15]. They offer a greater scope than synthetic chemicals as they are relatively safe, easily biodegradable and eco-friendly [2] [11] [16] [17]. Gurjar *et al.* [17] reported that plant extracts from *Azadirachta indica*, A. Juss., *Allium sativum*, Linn., *Eucalyptus globulus*, Labill., *Curcuma longa*, Linn., *Nicotiana tabacum*, Linn and *Zingibar officinale*, Rose inhibited growth of pathogens such as *Phytopthora infestans*, *Alternaria alternate*, *Rhizoctonia solani* and *Curvularia lunata*. Natural chemicals from plants are cheap, readily available and cost-effective in developing countries where synthetic fungicides are scarce and expensive for resource-poor farmers [7].

The main focus of the present study was to evaluate efficacy of plant extracts against *P. grisea in-vitro* and *in-vivo* and phytotoxicity of plant extracts on rice plant development.

2. Materials and Methods

2.1. Seed Samples

Rice (*Oryza sativa*) seed samples of nineteen different rice genotypes were used in this study. Among the collected seed samples, ten (Mbawambili, Masantula, Bishori, Domo la fisi, Kisegese, Mzambia, Kaniki, Karamata, Dongo la Songea and Usiniguse) were local accessions, while Supa, Kihogo red and Afaa Mwanza were varieties commonly grown in many areas. Released varieties were IR 64, Kalalu, Mwangaza, TXD 85, TXD 88 and Saro 5. The collected seed samples were transported to the African Seed Health Centre (AfSHC) laboratory at Sokoine University of Agriculture and stored at 4˚C for further testing.

2.2. Isolation and Preparation of *Pyricularia grisea* Inoculum

Cultures of *P. grisea* used in this study were isolated from infected rice seeds collected from Mvomero, Kilosa and Kilombero districts, Morogoro region, Tanzania. The germinating spores and mycelia were picked with a slightly bent inoculating needle and transferred to the Petri dishes containing Potato Dextrose Agar (31.2 g Potato Dextrose Agar powder and 800 ml of distilled water) at 4 equidistant points [18]. The Petri dishes were sealed with masking tape and incubated at alternating temperatures of about 28˚C - 32˚C for five days in alternating cycles of 12 h light and darkness to induce growth of *P. grisea* and were kept upside down. The pure cultures of the isolates were grown on V8 agar (3 g Calcium Carbonate, 200 ml V-8 juice and 20 g agar in 800 ml of distilled water) for 14 days to induce sporulation. The V-8 agar was used for producing *P. grisea* conidia. The petri dishes containing *P. grisea* inoculum were stored in the refrigerator at 5˚C [19] for further use.

2.3. Sources of Plant Extracts

Plants used as sources of extracts in this study are listed in **Table 1**. Fresh leaves (about 3 kg) of each plant species were harvested using a sterilized pair of scissors and put in perforated sacs and labeled properly for location, date and name of plant species. The samples were sent to the African Seed Health Centre (AfSHC) screen house

Table 1. Plants used as sources of antifungal extracts in the study.

Common name	Scientific name	Area (region) collected
Neem	*Azadirachta indica*	Morogoro
Aloe	*Aloe vera*	Morogoro
Garlic	*Allium sativum*	Iringa
Black jack	*Bidens pilosa*	Morogoro
Soursop	*Annona muricata*	Morogoro
Coffee	*Coffee arabica*	Morogoro
Thorn apple	*Datura stramonium*	Mbeya
Pyrethrum	*Chrysanthemum coccineum*	Iringa
Tobacco	*Nicotiana tabacum*	Iringa
Ginger	*Zingiber officinalis*	Iringa
Tea	*Camellia sinensis*	Mbeya

for further processing.

2.4. Preparation of Plant Extracts

Leaves of plants presented in **Table 1** were washed with running tape water to remove soil materials, and rinsed with Sterile Distilled Water (SDW) three times. The leaf samples were then cut into small pieces and placed on benches in the screen house at 25˚C - 28˚C to dry for 3 to 4 weeks. The dried leaves of each plant species were made into powder separately using a sterilized mortar and pestle and then sieved with one millimeter sieve. The powder of each plant species was packed in water proof plastic bags and labeled appropriately as described by Akinbode and Ikotun [20] and stored at 4˚C until used [21]. Crude plant extracts were obtained by infusing 50 g of each plant material in 100 ml SDW to give 50% w/v in a 500 ml conical flask and the mixture was incubated at 25˚C - 28˚C for 20 hours [22] [23]. The infusion was filtered separately through sterile double-layered cheese cloth into a sterile 400 ml beaker and the resulting stock solution was collected and stored at 25˚C - 28˚C until used [24].

2.5. Amendment of PDA with Plant Extracts

Four different concentrations (1%, 5%, 10% and 25%) of each plant extract were prepared and added directly to the sterile PDA through sterile micro-filters in the laminar flow chamber. The stock solutions were removed and measured by a 10 ml syringe without a needle. To prepare these concentrations, two, 10, 20 and 50 ml of the stock solutions were mixed in 198 ml, 190 ml, 180 ml and 150 ml, respectively, of autoclaved PDA as described by Kamalakannan and Shanmugam [25]. The PDA medium with plant extracts (PDAPE) were poured into Petri dishes (20 ml) and allowed to solidify and then stored at 5˚C until used.

2.6. *In-Vitro* Assay of Plant Extracts against *Pyricularia grisea*

The fungitoxic effect of different plant extracts *in-vitro* were studied by inoculating PDA with 14-day-old pure cultures of *P. grisea* [19]. Small mycelia segments (5 mm) were made using a sterile 5 mm diameter cork borer. Each mycelial segment was then transferred singly onto the centre of each PDAPE Petri dishes at 1%, 5%, 10% and 25% concentrations (v/v). The PDA Petri dishes without plant extracts were used as negative control and PDA mixed with Apron Star® 42 WS contained 20% Thiamethoxam, 20% Metalaxyl-M and 2% Difenoconazole, trade names Cruiser and Actara were included as positive controls. The experiments were laid out in a split plot in a randomized complete block design with rice blast as main plot and plant extracts as a subplots with 50 treatments (12 plant extracts with 4 different concentrations and 2 controls: Apron Star® 42 WS and distilled water). The inoculated Petri dishes were sealed with masking tape and incubated at 28˚C - 32˚C for five days in alternating cycles of 12 hours light and darkness to induce growth of *P. grisea*. Radial growth of *P. grisea* was then recorded after every three days for up to 21 days after inoculation by measuring mycelial growth diameters along two diagonal lines previously drawn on the reverse side of each Petri dish to serve as a reference, using a 30 cm plastic ruler to determine the effectiveness of plant extracts. Calculation of percent inhibition of fungal

growth was estimated based on Ogbeborand Adekunle [26] methods as follows: Percentage mycelial inhibition = 100 (Mycelial growth diameter in control—Mycelial growth diameter in treatment)/Mycelial growth diameter in control. The treatments that showed high percentage control in *in-vitro* by reducing *P. grisea* growth were selected and the experiments were repeated twice.

2.7. *In-Vivo* Assay of Plant Extracts against *P. grisea*

2.7.1. Seed Inoculation with *P. grisea*

Four hundred seeds of a susceptible rice variety Supa were spray-inoculated with 1×10^5 spores/ml of *P. grisea* strain following procedures of Namai and Ehara [27]. Inoculated seeds were dried in the laminar flow chamber on three layers of blotter papers in Petri dishes for 2 h. Seeds were stored at 4˚C until used.

2.7.2. Treatment of Inoculated Seeds with Plant Extracts

The most promising concentrations of plant extracts in *in-vitro* were used in *in-vivo*. Twenty five per cent suspensions of extracts of *Azadirachta indica*, *Aloe vera*, *Allium sativum*, *Coffee arabica*, *Camellia sinensis*, *Datura stramonium*, *Nicotiana tabacum* and *Zingiber officinalis* were prepared by soaking 15 g of plant parts (ground leaves and seeds) in 60 ml of SDW. Ten per cent suspension of *Nicotiana tabacum* extracts was also prepared by soaking 6 g of leaves in 60 ml of SDW for 20 h [22]. The extracts were filtered using double-layered cheesecloth and the volume was adjusted to 60 ml with SDW and put in 80 ml scotch bottle with cover. The extracts were then kept at 4˚C until used.

Rice seeds (pre-inoculated with *P. grisea*) were soaked in suspensions of each pre-determined concentrations of plant extract overnight and then dried on sterile blotter papers for 2 h on the laminar flow chamber. The seeds were placed in a beaker and 40 ml of each plant extract suspension was added. The seeds were gently stirred by a stirring glass rod to ensure that they were completely immersed and evenly distributed. Beakers containing inoculated seed were covered by aluminum foil to reduce external contaminations and placed at the constant temperature of 25˚C for 20 h.

Two hundred seeds in 4 replicates (50 seeds per replicate) were tested per plant extract. Treated seeds were planted in pots (25 seeds/pot) containing a mixture of sandy-loam soil:farmyard manures (3:1) and kept under screen house conditions (25˚C - 30˚C and 85% - 95% RH). The efficacy of plant extract treatments in the control of *P. grisea* incidence in rice seedling was obtained by assessing rice seedlings for disease incidence and severity at 15, 20, 25, 30 and 35 days after sowing [28]. Scores for disease severity were done using the international scale (0 - 9 scale) developed by International Rice Research Institute [4]. In addition, the height of seedlings and leaves were evaluated. The weight of seedlings was evaluated 35 days after sowing. The height of the seedlings was determined using a ruler by measuring aerial part of the seedling from the soil surface to the terminal node of the developing leaf. To determine the fresh weight, rice seedlings were carefully cut at the bottom of the stem using a pair of scissors and the seedlings were placed on a weighing balance.

2.8. Evaluation of Phytotoxicity of Plant Extracts on Rice Seed Germination, Seedling Growth and Vigour

The effect of plant extracts on seed germination, seedling growth and vigour tests were carried out to determine whether the plant extracts were harmful. Seed germination tests were carried out using Between Paper method [29]. Four hundred seeds from three different rice varieties (Mwangaza, Saro-5 and Supa) were transferred separately to a beaker containing 40 ml suspensions of extracts from 25% (v/v) of *Azadirachta indica*, *Aloe vera*, *Allium sativum*, *Camellia sinensis*, *Datura stramonium*, *Nicotiana tabacum*, *Zingiber officinalis* and 10% *Coffee arabica* based on their best performance in the *in-vitro* and *in-vivo* assays. Seeds were soaked in the plant extract(s) for 24 h at 25˚C and dried on sterile blotter papers for 2 h on the laminar flow chamber [30]. Rice seeds soaked in SDW and Apron Star® 42 WS treatments were included in the experiments as negative and positive controls, respectively.

The effect of plant extracts on seed germination was evaluated by counting the number of normal seedlings, abnormal seedlings and dead seeds as recommended byInternational Seed Testing Association [29] Seedling Evaluation Handbook. Four hundred seeds in four replicates of hundred seeds of each sample were placed between two layers of moist germination papers. The germination papers were then folded along one edge and rolled up carefully ensuring that no excess pressure was placed on the seeds. The rolled germination papers were

tied with rubber bands at both ends and put into the transparent plastic bags tied at the top. To prevent the bending, paper towels were transferred in a basket with a flat base (W × L × H = 9.8 cm × 9.8 cm × 9.8 cm) and then placed in the incubation room at 25˚C - 28˚C for 14 days. The shoot length, root length and dry weight of seedlings were measured and recorded 14 days after sowing in order to evaluate the effect of the extracts on seedling growth and vigour [29]. The seedling shoots were cut, kept separately in paper bags and dried in an electric oven maintained at 80˚C temperature for 24 h as described by Chhetri [31]. After drying, the shoot dry weights were recorded by an electric balance. In order to obtain the seedling growth rate (gram per plant) the total weight of dry seedlings per roll of germination paper towels was divided by total number of normal seedlings as described by International Seed Testing Association [29] Seedling Evaluation Handbook.

2.9. Data Analysis

All data collected were analyzed based on the split plot arrangement in a CRD analysis of variance (ANOVA) model using GenStat statistical software. The data for disease incidence and severity of seed-borne fungal pathogens including *P. grisea* and the level of seed infection per sample and percentage germination test (abnormal seedlings, dead seeds and hard seeds) were subjected to square root transformation to normalize the data before analysis. A constant value (0.5) was added to each observation before taking square root [13]. Means were separated by Duncan's multiple range test (DMRT) at P = 0.05.

3. Results and Discussion

Results indicate that the concentrations of the tested plant extracts against *P. grisea* had a positive effect in inhibiting mycelia growth. The results of *P. grisea* growth on PDA amended with plant extracts showed that *A. indica*, *A. vera*, *A. sativum*, *C. arabica*, *C. coccineum*, *D. stramonium*, *C. sinensis*, *Z. officinalis* and *N. tabacum* had antifungal properties against *P. grisea* at high (25%) but not at low (1%) concentrations in *in-vitro* (**Table 2**). For each plant extract studied, the inhibitory effect on *P. grisea* at 10% and 25% concentrations was significantly higher ($P < 0.001$) than 1% and 5% concentrations, except for *B. pilosa* and *A. muricata* where the difference between extract concentrations was not significant ($P < 0.05$). The extracts from *C. arabica* at 25% with 89.40% gave the highest radial growth inhibition of *P. grisea*, while the lowest (0.8%) radial growth inhibition was recorded on PDA amended with plant extracts from *A. muricata* at 5% (**Table 2**). It was generally observed that *A. indica* had fungicidal properties at all tested concentrations unlike other plant extracts tests. Our results are in close agreement to those reported by Amadioha [13] and Kamalakannan *et al.* [32] who observed that neem extract at different concentrations reduced the radial growth of *P. grisea* on PDA medium and incidences and severity of rice blast *in-vivo*. The effects of plant extracts increased with increasing concentration except extract from *N. tabacum*, which showed maximum mycelial inhibition at 10% concentration and thereafter, stimulated mycelial growth at a concentration of 25% (**Table 2**). Taiga and Friday [33] reported that *N. tabacum*, *A. vera* and *A. indica* extracts contain alkaloids and flavonoids; while *N. tabacum* extract that was observed to be more fungi toxic at 10% concentration contained an additional component (tannins), which has been reported to be absent in both *A. vera and A. indica* extracts.

Seed extracts of processed *C. arabica* at 10% and 25% had the best inhibitory effect (81.12 and 89.40%), respectively, against *P. grisea* (**Figure 1**). They were followed by extracts from 10% *N. tabacum* (80.35%), 25% *A. vera* (79.45%), 25% *C. coccineum* flower (78.83%), 5% *N. tabacum* (78.33%), 25% *C. arabica* (77.52%), 10% *A. vera* (76.15%), 25% *Z. officinalis* (75.60%), 10% *A. indica* (74.90%), 10% *C. coccineum* (71.18 %), 5% *A. indica* (69.38%), 25% *A. sativum* (68.75%), 10% *A. sativum* (67.25%), 5% *C. coccineum* flower (61.73%) and 25% *N. tabacum* (61.15%) (**Table 2**). Similarly, Hajano *et al.* [34] evaluated garlic, neem and calatropis plant extracts and found that only garlic extract at higher dose successively reduced the infection caused by *Magnaporthe oryzae* on rice. It was also observed that neem extract at high dose 4 ml/15ml medium moderately inhibited the radial growth of *M. oryzae*.

Such results indicate that these plant extracts may have fungicidal properties that can be used as seed treatments for controlling *P. grisea*, the causal agent of rice blast. The antifungal properties of *Z. officinalis* on *Aspergilus flavus*, *Aspergilus niger*, *Fusarium solani* and *Fusarium oxysporum* on post-harvest yam (*Dioscorea alata*, Poir) has been reported by Yeni [35]. Other researchers also reported that *Z. officinalis* has been used for relief from arthritis, rheumatism, coughs, fever and infectious diseases [36]. The highest mycelial growth of *P. grisea* was favored by 5% *B. pilosa* (–10.92%) and 1% (–1.57%) (**Table 2**). The effect of *N. tabacum* on *P. gri-*

Table 2. Percentage inhibition of radial growth of *Pyricularia grisea* on Potato Dextrose Agar mixed with plant extracts at various (v/v) concentrations.

Name of treatment	Plant extracts (% inhibition of mycelial growth)*			
	1%	5%	10%	25%
Control				
SDW	−3.23 h	−3.72 j	−3.47 j	−3.38 j
Apron Star® 42 WS	35.80 ab	50.5 d	82.1 a	89.8 a
Extracts source				
Zingiber officinalis	22.68 d	28.90 f	45.95 e	75.60 c
Azadirachta indica	37.23 ab	69.38 b	74.90 b	77.52 bc
Coffee arabica	34.08 b	50.58 d	81.12 a	89.40 a
Camellia sinensis	7.75 f	16.23 h	35.10 g	52.40 f
Nicotiana tabacum	26.82 c	78.33 a	80.35 a	61.15 e
Aloe vera	16.38 e	27.73 f	76.15 b	79.45 b
Datura stramonium	4.43 fg	23.15 g	39.25 f	49.10 g
Allium sativum	17.35 e	36.25 e	67.25 d	68.75 d
Annona muricata	0.80 gh	1.73 i	2.10 i	3.42 i
Chrysanthemum coccineum (leaf)	19.05 de	20.98 g	23.25 h	26.00 h
Bidens pilosa	−1.57 h	−10.92 k	−7.38 k	−5.43 j
Chrysanthemum coccineum (flower)	39.70 a	61.73 c	71.18 c	78.83 b
Mean	18.38	32.20	47.70	53.04
$LSD_{0.05}$	3.97	3.57	3.13	2.35
F test	***	***	***	***
Variance	7.72	6.28	4.83	2.72

*Values are means of four replicates in two separate experiments. Numbers followed by the same letters in a column are not significantly different at P = 0.05, using Duncan's Multiple Range Test; *** = highly significantly different (P < 0.001).

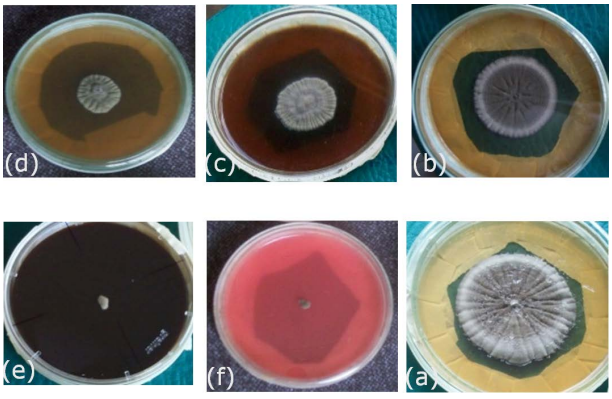

Figure 1. Growth of endophytic fungal mycelia on PDA amended with processed *Coffee arabica*: (a) = PDA amended with sterile distilled water (negative control), (b) = PDA amended with 1 %, (c) = PDA amended with 5 %, (d) = PDA amended with 10%, (e) = PDA amended with 25% and (f) = PDA amended with Apron star Apron Star® 42 WS (positive control) 21 days after incubation.

sea at 1%, 5% and 10% levels showed subsequent increase in percentage of fungal growth inhibition and decrease at 25% (**Table 2**). These results suggest that the optimal concentration of *N. tabacum* leaf extracts was around 10%.

The shoot length of rice seedlings of cultivar Mwangaza were significantly different (P < 0.05) when treated with plant extracts, Apron Star® 42 WS and SDW. Seedlings of rice cultivar Mwangaza treated with Apron Star® 42 WS had the shortest shoot length (10.71 cm) compared with those treated with plant extracts and SDW (**Table 3**). There were no significant differences (P < 0.05) between the root length, dry weight, seedling growth rate and vigour index of cultivar Mwangaza for all treatments (**Table 3**). These results generally indicated that,

Table 3. Effect of treatment with plant extracts on growth and development of rice plants in *in-vitro* using the between paper method.

Rice varieties	Treatment	Means of variables*					
		Germination (%)	Shoot length (cm)	Root length (cm)	Dry weight (g)	Seedling growth rate	Seedling vigour index
Supa	**Control**						
	SDW	96.00 a	10.50 ab	15.44 a	0.13 a	0.006 a	2369 ab
	Apron Star® 42 WS	95.00 a	9.55 b	14.57 a	0.10 b	0.004 b	2100 b
	Extracts source						
	Zingiber officinalis	97.00 a	10.44 ab	15.38 a	0.12 a	0.006 a	2345 ab
	Azadirachta indica	97.00 a	10.47 ab	16.05 a	0.13 a	0.006 a	2380 ab
	Coffee arabica	96.00 a	10.38 ab	15.47 a	0.12 a	0.005 ab	2362 ab
	Camellia sinensis	97.50 a	11.67 a	15.06 a	0.14 a	0.006 a	2435 a
	Nicotiana tabacum	97.00 a	10.09 b	16.18 a	0.13 a	0.005 ab	2424 ab
	Aloe vera	96.00 a	11.67 a	15.06 a	0.14 a	0.006 a	2375 ab
	Datura stramonium	96.50 a	10.62 ab	15.32 a	0.12 a	0.006 ab	2351 ab
	Allium sativum	96.00 a	10.65 ab	15.19 a	0.13 a	0.0060 ab	2376 ab
	Mean	96.40	10.60	15.37	0.12	0.006	2352
	$LSD_{0.05}$	2.21	1.15	1.83	0.01	0.001	280.0
	F test	ns	*	ns	***	ns	ns
	CV (%)	1.6	7.5	8.2	8.2	17.9	8.2
Saro-5	**Control**						
	SDW	95.50 a	11.46 b	14.02 a	0.13 ab	0.006a	2399 a
	Apron Star® 42 WS	91.50 b	9.76 c	13.48 a	0.10 b	0.005a	2011 a
	Extracts source						
	Zingiber officinalis	94.50 a	13.32 a	15.38 a	0.12 ab	0.006 a	2474 a
	Azadirachta indica	95.50 a	12.79 ab	15.53 a	0.11 ab	0.005 a	2448 a
	Coffee arabica	95.50 a	12.39 ab	14.00 a	0.14 a	0.006 a	2480 a
	Camellia sinensis	95.00 a	11.80 b	14.64 a	0.12 ab	0.006 a	2430 a
	Nicotiana tabacum	95.00 a	12.20 ab	14.00 a	0.12 ab	0.005 a	2383 a
	Aloe vera	94.50 a	11.97 b	14.76 a	0.13 a	0.006 a	2436 a
	Datura stramonium	96.00 a	11.99 b	15.36 a	0.13 a	0.006 a	2500 a
	Allium sativum	95.00 a	11.59 b	14.84 a	0.12 ab	0.006 a	2369 a
	Mean	94.80	11.93	14.60	0.12	0.006	2393
	$LSD_{0.05}$	2.56	1.19	2.15	0.02	0.001	264.6
	F test	ns	***	ns	ns	ns	ns
	CV%	1.9	6.9	10.2	12.0	10.9	7.7
Mwangaza	**Control**						
	SDW	95.50 a	11.87 ab	15.24a	0.11 a	0.005 a	2548 a
	Apron Star® 42 WS	94.50 a	10.71 b	12.82b	0.09 a	0.005 a	2200 b
	Extracts source						
	Zingiber officinalis	95.50 a	11.68 b	14.94 a	0.11 a	0.005 a	2472 a
	Azadirachta indica	95.00 a	12.75 a	15.02 a	0.10 a	0.005 a	2434 ab
	Coffee arabica	95.00 a	11.99 ab	14.33 a	0.11 a	0.005 a	2418 ab
	Camellia sinensis	95.00 a	11.74 ab	14.70 a	0.10 a	0.005 a	2499 a
	Nicotiana tabacum	95.00 a	11.63 b	14.53 a	0.12 a	0.005 a	2402 ab
	Aloe vera	95.50 a	11.74 ab	14.95 a	0.10 a	0.005 a	2462 a
	Datura stramonium	95.50 a	11.85 ab	15.48 a	0.11 a	0.005 a	2429 ab
	Allium sativum	95.50 a	11.83 ab	14.69 a	0.11 a	0.005 a	2441 ab
	Mean	95.20	11.78	14.67	0.10	0.005	2431
	$LSD_{0.05}$	2.72	0.92	1.38	0.02	0.001	221.9
	F test	ns	*	ns	ns	ns	ns
	CV%	2.0	5.4	6.5	14.3	12.3	6.3

*Values are means of four replicates. Numbers followed by the same letter in a column are not significantly different at P = 0.05, using Duncan's Multiple Range Test. *** = highly significantly different (P < 0.001); * = significantly different (P < 0.05); ns = not significantly different.

the plant extracts used were not phytotoxic to rice seedlings. These results are in agreement with those reported by Zida *et al.* [23], who showed that Apron Star® 42 WS reduced seedling emergence, plant vigour and grain yield of pearl millet seeds. Seed treatments using ethanol extract and essential oil from *Callistemon*, *Citrinus* and *Ocinum gratissimum* improved the field parameters such as emergence and yield of tested rice varieties against *Bipolaris oryzae* [37]. The current findings suggest that leaf extracts from *A. indica*, *A. vera*, *A. sativum*, *D. stramonium*, *C. sinensis*, *N. tabacum*, *Z. officinalis* and processed *C. arabica*, can be used as seed treatments for the control of *P. grisea* in rice seeds and for improving rice seedling growth. These plant extracts are safe and eco-friendly on rice compared to synthetic chemicals such as Apron Star® 42 WS. More studies are therefore, needed to confirm current findings and to determine the most effective formulation against *P. grisea*.

Identification and characterization of the active compounds from currently tested plant extracts and their role in rice blast disease control is also needed. Research on the range of activity of plant extracts for control of other rice pathogens is recommended.

4. Conclusion

This study has revealed that plant extracts from processed coffee (*C. arabica*) at concentrations of 10% and 25% (w/v) had the highest inhibitory effect (81.12% and 89.40%, respectively) against *P. grisea*—the causal agent of rice blast disease. These results generally indicated that, the plant extracts used were not phytotoxic to rice seedlings. More studies are therefore needed to confirm the current findings and to determine the most effective formulation against *P. grisea*.

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