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EFFECT OF PLANT EXTRACT ON SEED GERMINATION AND SEED-ASSOCIATED FUNGI OF BRRI-28 AND BRRI-50 RICE VARIETIES

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Keywords: Rice seeds, Blotter test, Plant extracts, Seed germination, Seed associated fungi.

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Abstract

The present investigation aimed to evaluate the antifungal effects of *Adhatodavasica* L., *Tagetes patula* L., *Vinca rosea* L., *Lowsoniainermis* L. and *Andrographis paniculuta* L. on seed germination and seed- associated fungi of two rice varieties viz.BRRI-28 and BRRI-50 in *In vitro*conditions following the Blotter method. The highest seed germination was recorded as 58.67% and it enhanced 68.18% over control at 1:1 dilution of *A. vasica*L. in surface sterilized seeds of BRRI-28 variety. Total of five fungal genera namely *Aspergillus niger*, *Fusarium* sp., *Aspergillus flavus*, *Trichoderma viride*, *Penicillium* sp. and *Rhizopus* sp. were isolated from treated and controlled rice seeds. *A. niger*, *Rhizopus* sp., *Fusarium* sp. and *T. viride* were totally controlled at all dilutions of the tested plant extracts in the BRRI-28 variety while in BRRI-50 variety *A. niger*, *Rhizopus* sp., *Fusarium* sp. and *T. viride* were also remarkably controlled with all dilutions of *L. inermis* L. and *A.paniculuta* L. extracts. Among these plant extracts *A. paniculuta*extractswas the most potential followed by *A. vasica* L., *T. patula* L., *V. rosea* L.and *L.inermis* L. for reduction seed-associated fungi.

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Introduction

Rice (*Oryza sativa* L.) is an annual cereal grass widely cultivated for its seed, used for human food and it constitutes about 90% of the total food grain production. In Bangladesh rice is the major important group of food crops and especially honored as a high carbohydrate source. In Bangladesh 20 major diseases occurred on rice by different organisms such as bacteria (2), fungi (13), viruses (2) and nematodes (2) and create micronutrient deficiency problem (Miah et al., 2008). In Bangladesh, the total production (summer and winter) area 2,89,12,000 acres were used for rice cultivation with an average yield of 1,301 kg/acre and the total production is 37,607 metric tons during the 2020-2021 growing season (BBS-2021). Seed-associated fungi are main agents reducing yield and grain quality of rice. Beside this they produced toxic substances which cause diseases on human or animals. Most of the storage fungi species are *Aspergillus* spp. *Penicillium* spp., *Mucor* spp., *Fusarium* spp. and *Rhizopus* spp. Chemical fungicides are used to prevent the seed borne disease of rice that cause serious environmental pollution and are toxic to human. The plant extracts act as inducers of nematicides effects (Coltro-Roncato et al., 2016) and especially, fungicide effect for the control of pathogenic organisms (Meena et al., 2020) and inhibit fungal growth under laboratory condition (Yassinet al., 2012). Moreover, plant metabolites showed promising results against seed borne and associated fungi. Therefore, searching for a more sustainable agriculture, plant extracts have also been tested as an important tool for seed treatment (Chandel and Kumar, 2017; OJO et al., 2020). Hence present research has been conducted to know the efficacy of some plant leaf extracts on seed germination and vigour index and to control seed associated fungi in BRRI-28 and BRRI-50 rice varieties in *in vitro* condition.

Materials and Methods

The experiment was carried out at the Plant Pathology and Mycology Laboratory of the Department of Botany, University of Rajshahi, Bangladesh.

Collection of seeds and health testing

Samples of rice seeds (BRRI-28 and BRRI-50) were collected from Regional Rice Research Center, Shaympur, Rajshahi (RWRC) during June 2013 to March 2014. Standard blotter method was used for health testing according to ISTA (1996) rules.

Plant materials used

Five plant materials namely *Adhatodavasica*L., *Tagetes patula*L., *Vinca rosea* L., *Lowsoniainermis*L. and *Andrographis paniculuta*L. Plant leaves were used which were Rajshahi University campus.

Preparation of plant extracts

Fresh clean leaves of selected five plants were brought to the laboratory and cut into small pieces. After that plant extracts were prepared following the procedure of Ahmed et al. (2013). In this method, the chopped leaf samples weighted 50 gm each of plants and grounded in a mortar and pestle by adding sterile distilled water at 1:1, 1:2 and 1:3 w/v ratios (1:1=50 gm plant material crushed in 50 ml distilled water). The extracts were filtered through cheese cloth. These were centrifuged at 3000 rpm for 20 min and stored in a refrigerator at 4°C until used.

Seed treatment

All seeds are sterilized with 1% Sodium hypo chloride (NaOCl) solution for 2 minutes while in control, the seeds were dipped in distilled water only. Then the seeds were dipped for 30 minutes by the prepared plants extract (at 1:1, 1:2 and 1:3 dilutions) separately. After 30 min. the treated seeds were soaked on blotting paper and placed on moist blotter to determine seed germination, vigor index and seed associated fungi.

Testing of seed germination

For seed germination test blotter method was followed. Three layers' moist blotter papers were placed each 9 cm petridish. In each petridish, randomly selected 25 seeds of BRRI-28 and BRRI-30 varieties were placed at same distance. The petridishes were then incubated at room temperature for seven days. After thatnumber of germinations of the seed were counted, shoot and root length were measured and vigour index were calculated using the following formula:

Seed germination (%)= Number of seeds germinated/Total number of seeds used×100

Vigour index= (Mean of root length + Mean of shoot length) × Percentage of seed germination

Measurement of seed associated fungi and infection percentage

In blotter test, after seven days number of fungi per seeds and infected seeds were counted. Infection percentage was calculated following the formula:

Infection %= No. of infected seeds by individual fungus /Total number of seeds used× 100

Identification of the fungi were confirmed by studying their morphological and microscopic characteristics following the standard methods of Booth (1971) and Alexopoulos (1979).

Statistical analysis

Experiments were conducted in triplicate for consistency of results and statistical purposes. The data were expressed as mean and standard error (M±SE) using Microsoft Excel software 2013. $P<0.05$ was considered statistically significant in ANOVA test.

Results and Discussion

Efficacy of plant extracts on seed germination and vigor index of rice

Five plant leaf extracts were tested on the seeds of BRRI-28 and BRRI-50rice varieties and the results are presented in Table 1. The maximum germination percentage (58.67%) was recorded at 1:1 dilution of *A. vasica* L. in the seeds of BRRI-28varietycompared toBRRI-50variety (28%).These results completely agree with Akter et al. (2022) who observed higher germination of all cucumber seed after treatment with 25% aqueous extracts of the ten medicinal plants over control. Among them, royal poinciana (*Delonix regia*Boj. ex Hook.) Raf. performed very well to increase germination and growth as well. The shoot and root lengths were decreased (0.30 and 1 cm) at 1:1 dilution of *L. inermis*L. extract over control (12.40 and 16.40) in BARI-28 variety, respectively. The highest reduction of vigour index (39.87) was

observed at 1:1 dilution of *V. rosea* L. extract over control. These results completely disagree with Akter et al. (2022) who showed Bougainvillea

(*Bougainvillea spectabilis*), False daisy (*Eclipta alba*), Drumstick (*Moringaoleifera*), Sisso (*Dalbergia sissoo*), Mimosa (*Mimosa pudica*), Mint (*Mentha spicata*), Devil's cotton (*Abroma augusta*), Asthma plant (*Euphorbia hirta*), Ivy gourd (*Coccinia grandis*) and Royal poinciana (*Delonixregia*) increased shoot and root length and also agree Ismail et al. (2022) who presented the potential use of plant extracts with germination-inhibiting properties.

Efficacy of plant extracts on seed-associated fungi of rice

Tested different plant extracts showed different levels of anti-fungal activity in controlling the seed-associated fungi of the seeds of rice varieties (BRRI-28 and BRRI-50). Tested plant extracts showed different levels of anti-fungal activities against the seed-associated fungi.Total of five fungal genera namely *Aspergillus niger*, *Fusarium* sp., *Aspergillus flavus*, *Trichoderma viride*, *Penicillium* sp. and *Rhizopus* sp. were identified from the seeds of BRRI-28 and BRRI-50 varieties. Among them *Rhizopus* sp. was the most dominant fungus.

In the case of the seeds of BRRI-28variety, *A. niger*, *Rhizopus* sp., *Fusarium*sp. and *T. viride* were totally controlled at all dilutions of the tested plant extracts but at 1:1 dilution was remarkably controlled *A. flavus*. In case of BRRI-50variety, *A. niger*, *Rhizopus* sp., *Fusarium* sp. and *T. viride* were successfully controlled with all dilutions of *L. inermis* L. and *A. paniculuta* L. extracts. *A. flavus* was also totally controlled at 1:1 dilution of all the tested plant extracts and 1:2 and 1:3 dilutions of *T. patula*, *V. rosea* L., *L. inermis* L. and *A.paniculuta* L. extracts in the seeds of BRRI-50variety. Higher dilution performs the best result to eradicate seed-associated fungi. In similar study, Jama et al. (2018) recorded that basak and all amanda extract remarkably reduced *B.sorokiniana*, *A. tenius*, *Culvularia* sp. and *Fusarium* sp. associated with wheat seeds which correlates with the present findings.

Conclusion

From the results, it was observed that higher dilution showed the highest seed germination with *A. vasica* L. extract and higher dilution of *L. inermis* L. extract also showed negative effect to increase the shoot and root length. *A. paniculuta* L. extract showed best antifungal effect of complete elimination of all detected seed associated fungi from rice seeds than other plant extracts. So, plant extracts can be as promising source for enhancement of seed germination and as well as controlling seed associated fungi and may use in better agricultural practices in tomorrow.

Table 1: Effects of plant extracts on seed germination, shoot length, root length and vigour index in BARI-28and BARI-50varieties.

Seed	Treatment	Dilution	Germination (%) Mean+S.E	Increased germination (%) over control	Shoot length (cm) Mean ± S.E.	Root length (cm) Mean ± S.E	Vigour index
BARI-28	*Adhatodavasica* L.	1:1	58.67±3.53	68.18	3.43±0.29	4.20±0.29	447.66
		1:2	49.33±3.53	62.16	1.13±0.03	2.43±0.12	175.62
		1:3	38.67±4.81	51.72	0.57±0.07	1.67±0.22	86.62
	Tagetes patula L.	1:1	44.00±2.31	57.57	0.47±0.07	1.37±0.22	80.96
		1:2	34.67±1.33	46.15	0.57±0.20	1.30±0.12	64.83
		1:3	0.00±0.00	0	0.00±0.00	0.00±0.00	0
	Vinca rosea L.	1:1	41.33±5.33	54.83	1.73±0.27	3.03±0.07	196.73
		1:2	30.67±1.33	39.13	1.93±0.09	3.20±0.06	157.34
		1:3	0.00±0.00	0	0.00±0.00	0.00±0.00	0
	Lowsoniainermis L.	1:1	30.67±3.53	39.13	0.30±0.10	1.00±0.10	39.87
		1:2	0.00±0.00	0	0.00±0.00	0.00±0.00	0
		1:3	0.00±0.00	0	0.00±0.00	0.00±0.00	0
	Andrographis paniculuta L.	1:1	32.00±2.31	41.66	0.40±0.00	0.97±0.12	43.84
		1:2	0.00±0.00	0	0.00±0.00	0.00±0.00	0
		1:3	0.00±0.00	0	0.00±0.00	0.00±0.00	0
Control			18.67±3.53		12.40±0.46	16.40±0.46	537.67
F value ($LSD_{p\leq0.05}$)			67.35(1.893)		60.96(0.364)	120.11(0.364)	
BARI-50	*Adhatodavasica* L.	1:1	0.00±0.00	0	0.00±0.00	0.00±0.00	0
		1:2	0.00±0.00	0	0.00±0.00	0.00±0.00	0
		1:3	0.00±0.00	0	0.00±0.00	0.00±0.00	0
	Tagetes patula L.	1:1	0.00±0.00	0	0.00±0.00	0.00±0.00	0
		1:2	0.00±0.00	0	0.00±0.00	0.00±0.00	0
		1:3	0.00±0.00	0	0.00±0.00	0.00±0.00	0
	Vinca rosea L.	1:1	28.00±6.11	57.14	2.70±0.35	4.17±0.18	192.36
		1:2	0.00±0.00	0	0.00±0.00	0.00±0.00	0
		1:3	0.00±0.00	0	0.00±0.00	0.00±0.00	0
	Lowsoniainermis L.	1:1	0.00±0.00	0	0.00±0.00	0.00±0.00	0
		1:2	0.00±0.00	0	0.00±0.00	0.00±0.00	0
		1:3	0.00±0.00	0	0.00±0.00	0.00±0.00	0
	Andrographis paniculuta L.	1:1	28.00±2.31	57.14	0.53±0.19	1.87±0.15	67.2
		1:2	24.00±6.11	50	1.77±0.20	3.30±0.26	121.68
		1:3	0.00±0.00	0	0.00±0.00	0.00±0.00	0
Control			12.00±2.31		11.20±0.61	14.27±1.16	305.64
F value ($LSD_{p\leq0.05}$)			0.513(1.667)		0.513(0.330)	0.513(0.261)	

* Mean of three replications.

Table 2: Effects of plant extracts on seed associated fungi in BARI-28 andBARI-50 varieties.

Seed	Treatment	Dilution	Infection (%) of seed associated fungi					
			Aspergillus flavus	***A. niger***	***Fusarium* sp.**	***Rhizopus* sp.**	***Penicillium* sp.**	***Trichoderma viride***
BARI-28	*Adhatodavasica* L.	1:1	0	0	0	0	0	0
		1:2	0	0	0	0	0	0
		1:3	4	4	0	4	4	4
	Tagetes patula L.	1:1	0	0	0	0	0	0
		1:2	0	0	0	0	0	0
		1:3	0	0	4	4	0	0

	Vinca rosea L.	1:1	0	4	0	0	0	0
		1:2	0	8	0	4	0	0
		1:3	0	12	4	8	8	0
	Lowsoniainermis L.	1:1	0	0	0	0	0	0
		1:2	0	0	0	0	0	0
		1:3	4	0	0	0	4	0
	Andrographispaniculuta L.	1:1	0	0	0	0	0	0
		1:2	0	0	0	0	0	0
		1:3	0	0	0	0	0	0
Control			11	28	32	31	32	24
F value ($LSD_{p\leq0.05}$)			1.76(2.90)					
BARI-50	*Adhatodavasica* L.	1:1	0	0	0	0	0	0
		1:2	8	0	0	0	0	0
		1:3	16	4	0	8	8	8
	Tagetes patula L.	1:1	0	0	0	0	0	0
		1:2	0	0	4	0	0	0
		1:3	0	0	8	12	0	0
	Vinca rosea L.	1:1	0	8	0	0	0	0
		1:2	0	12	0	8	16	0
		1:3	0	20	4	16	24	0
	Lowsoniainermis L.	1:1	0	0	0	0	0	0
		1:2	0	0	0	0	0	0
		1:3	0	0	0	0	8	0
	Andrographispaniculuta L.	1:1	0	0	0	0	0	0
		1:2	0	0	0	0	0	0
		1:3	0	0	0	0	0	0
Control			11	31	28	32	32	24
F value($LSD_{p\leq0.05}$)			2.78(4.74)					

* Mean of three replications.

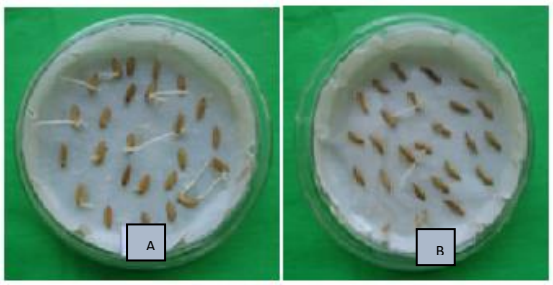

Fig.1:Photograph showing seed germination rate and the infected seed of (A=BARI-28 and B=BARI-50) rice varieties

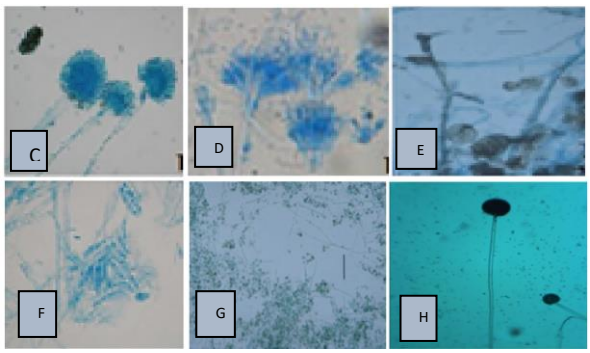

Fig. 2:Photograph showing of different species of fungus.

C: *Aspergillus flavus*, D: *Penicillium* sp., E: *Rhizopus*sp., F: *Fusarium* sp., G: *Trichoderma viride*, H: *A. niger*

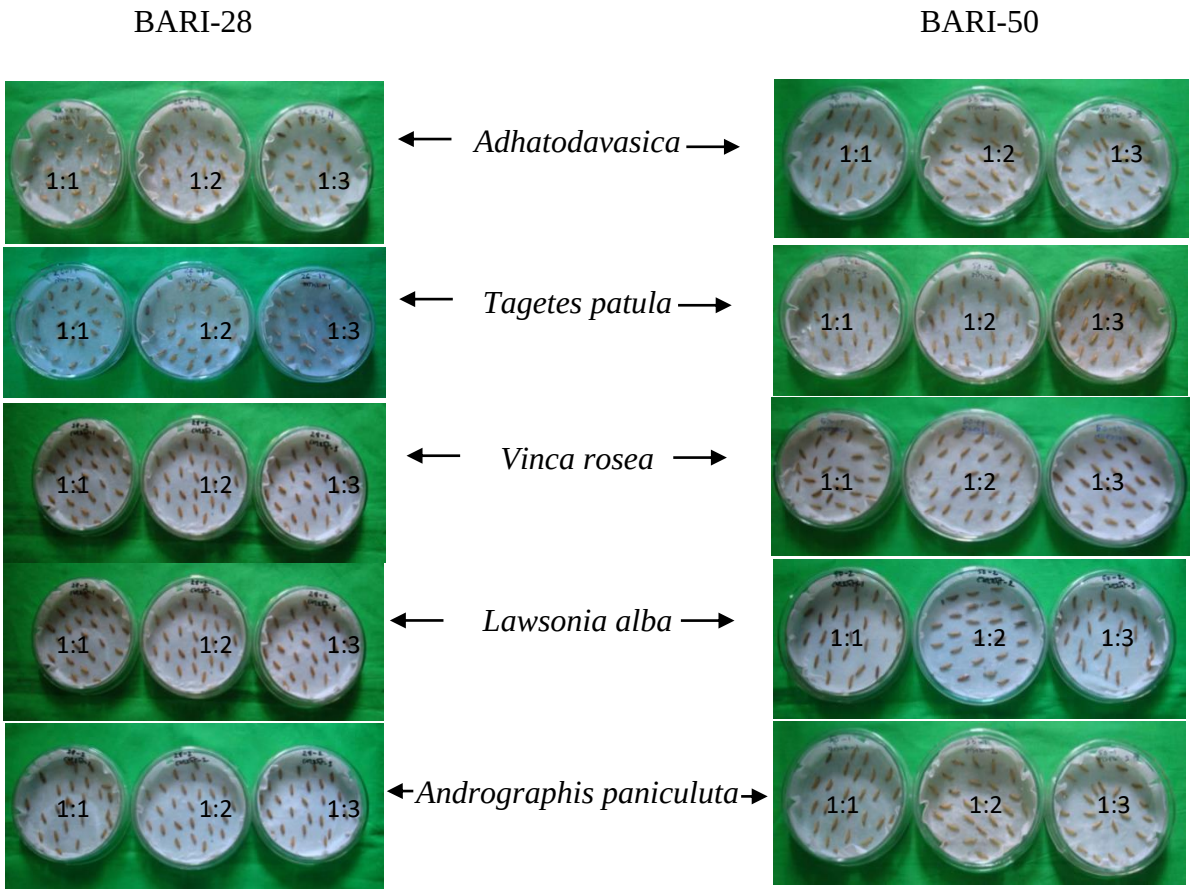

Fig. 3: Photographs showing effect of plant extract on seed germination of BARI-28 and BARI-50 varieties.

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