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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 8 (9): 1284-1289 2005
ISSN 1028-8880

Antifungal Effects of Plant Extracts on Seed-borne Fungi of Wheat Seed Regarding Seed Germination, Seedling Health and Vigour Index

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Abstract: Ten plant extracts, such as rhizome of *Zingiber officinale*, bulb of *Allium sativum*, *Allium cepa*, leaves of *Adhatoda vesica*, *Lawsonia alba*, *Azadirachta indica*, *Achyranthes aspera*, stem of *Cuscuta reflexa*, root of *Vinca rosea* and seeds of *Nigella sativa* were tested for their efficacy *in vitro* against seed-borne fungi of wheat. All the plant extracts significantly reduced the incidence of seed-borne fungi; increased seed germination; number of healthy seedlings and vigour index. Alcoholic extracts of neem (*Azadirachta indica*) and garlic (*Allium sativum*) completely controlled the intensity of *Bipolaris sorokiniana*, *Fusarium* sp., *Aspergillus* sp., *Penicillium* sp. and *Rhizopus* sp. after the treatment on wheat seeds. Next to garlic and neem, *V. rosea* extract showed good inhibition and 1.40% intensity of *B. sorokiniana* followed by bulb extract of *A. cepa* and leaf extract of *A. aspera* (1.53 and 1.53%). Water extract of test plants also showed good antifungal effects against seed-borne fungi of wheat. Treated seeds of wheat by the extract of *A. indica* gave 99.33% seed germination, which is 16.86% higher over untreated control. The highest vigour index (1936, 2194; 1826, 2223 and 1871, 2174) was recorded after the application with alcoholic and water extracts of rhizome of ginger, bulb of garlic and leaf of neem on seeds of wheat while the lowest was in untreated control. The highest (86.95%) and the lowest (13.05%) healthy and infected seedlings were recorded when the seeds were treated with neem extract. Next to neem extract, garlic extract treated wheat seeds gave 84.70% healthy seedlings and 15.13% infected seedlings, respectively.

Key words: Plant extracts, wheat, seed borne fungi, germination, healthy seedling, vigour index

INTRODUCTION

Wheat (*Triticum aestivum* L.) is considered as one of the most important cereal crop in the world and second most important cereal crop in Bangladesh. Wheat suffers from as many as 120 different diseases in the world, of which 42 are seed-borne and 35 are caused by fungi[1,2]. In Bangladesh, 120 diseases are known to occur on wheat by different organisms and eight of them are caused by seed-borne fungi[3-5]. Among these diseases, leaf blight caused by *Bipolaris sorokiniana* Sacc. is the most common and severe disease in Bangladesh[6]. Seed borne infection of fungal pathogens are important not only for its association with the seeds which cause germination failure and/or causing disease to the newly emerged seedlings or growing plants, but also contaminate the soil by establishing its inocula permanently. It was therefore necessary to search for control measures that are cheap, ecologically sound and environmentally safe to eliminate or reduce the incidence of these economic important pathogens, so as to increase seed germination and to obtain healthy and vigorous plant as well as better yield of wheat. In recent years much attention has been given to non-chemical substances for seed treatment to protect them against seed-borne pathogens. Chemical fungicides can control the plant diseases, but it has bad effects on human health, plants, fishes and other animals etc which is harmful to our environment. On the other hand, several higher plants and their constituents have shown success in plant disease control and are proved to be harmless and non-phytotoxic unlike chemical fungicides[7-12]. Plant extracts have played significant role in reducing the incidence of seed-borne pathogens and in the improvement of seed quality and emergence of plant seeds in the field. Parimelazhagan and Francis[13] established that leaf extract of *Clerodendrum viscosum* increased seed germination and improved seedling developments of rice seeds. Allice and Rao[14] also observed that *Allium sativum* extracts significantly reduced seed infection by *Drechslera oryzae* and treated seeds had significantly higher viability. The extracts of plants exhibited marked effect on germination of fungal spores as well[15-17] and it inhibited the fungal growth[18]. In this study, water and alcoholic extracts of plants were

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tested for their efficacy against seed-borne fungi of wheat seeds to see seed germination, vigour index and number of healthy and diseased seedlings.

MATERIALS AND METHODS

Seed treatment and isolation of fungi: Seed samples of wheat were collected from Regional Wheat Research Institute, Shympur Rajshahi, Bangladesh during the season 2004-2005. Samples were preserved in plastic container at 28°C for three months. Hundred seeds of tested varieties were immersed at 2.5% concentration of different plant extracts for 3 min. The treated seeds were dried in sunlight. Seeds were grown and the fungi were collected using blotter method as described by International Seed Testing Association[19]. Twenty-five seeds were placed per petri dish containing moist filter paper. The Petridishes containing seeds were incubated at 28°C for 7 days. The incubated seeds were examined under microscope in order to record the incidence of different seed borne fungi on seeds.

Preparation of plant extracts: Rhizome of *Zingiber officinale*, bulb of *Allium sativum* and *Allium cepa*, leaf of *Adhatoda vesica*, *Lawsonia alba*, *Azadirachta indica* and *Achyranthes aspera*, stem of *Cuscuta reflexa* (Dodder), root of *Vinca rosea* and seeds of *Nigella sativa* were extracted with alcohol and water following the method described by Mahadevan and Sridhar[20]. Five gram tissues were cut into pieces and immediately plunged in ethyl alcohol/water in a beaker and allowed to boil for 5 to 10 min. Used 5 to 10 mL of alcohol or water for every gram tissue. The extraction was done on top of a steam bath. The extracts were cooled in a pan of cold water. The tissues were crushed thoroughly in a mortar with a pestle and then passed through two layers of chess cloth. The ground tissues were reextracted in ethyl alcohol/distilled water of the said plant materials. Both the extracts were cooled and filtered through Whatman's No. 1 filter paper. The volume of the extract was evaporated on a steam bath to dryness and adequate amount of distilled water was added for 5 g of tissues to adjust the ratio of the plant extracts at 2.5% and used for seed treatment before sowing of wheat seeds.

Germination test and determination of vigour index by paper towel method: This study was done by using rolled paper towel method[21]. Two hundred seeds for each treatment were tested. Fifty seeds were placed in rows on two layered moistened paper towel and then the towels were rolled and closed both ends by using rubber bands and placed it in an upright position for 5 to 7 days at 28°C and in light for 12 h each day. The size of each towel paper was taken 45x28 cm. Three replications of 50 seeds in each towel band set were used for this experiment. Then the germination was counted. For determination of seedling vigour, 25 seedlings were randomly selected from each towel band and their individual shoot and root length were measured. Seed vigour for every treatment was determined according to the following formula after Abdul-Baki and Anderson[22].

Vigour index = Mean of root length (cm) + Mean of shoot length (cm) x percentage of seed germination

Analysis of data: The data on different characters were subjected to statistical analysis of variance to find out the variation resulting from experimental treatments. Treatments were compared by LSD (Least Significant Difference) test.

RESULTS AND DISCUSSION

Effects of ten plant extracts viz., *Zingiber officinale*, *Allium sativum*, *Allium cepa*, *Adhatoda vesica*, *Achyranthes aspera*, *Azadirachta indica*, *Lawsonia alba*, *Cuscuta reflexa*, *Vinca rosea* and *Nigella sativa* were tested against seed-borne fungi of wheat var. Kanchan (Table 1). All the plant extracts have inhibitory effect and reduced the incidence of seed-borne fungi *Bipolaris sorokiniana*, *Fusarium* sp., *Aspergillus* sp., *Penicillium* sp. and *Rhizopus* sp. when treated with the plant extracts in the wheat var. kanchan. The incidence percentage of seed-borne fungi varied with the types of plants and medium of extraction (water and alcohol) used. Alcoholic extracts of neem and garlic completely inhibited the presence of *Bipolaris sorokiniana*, *Fusarium* sp., *Aspergillus* sp., *Penicillium* sp. and *Rhizopus* sp. respectively on treated wheat seeds, whereas the highest percentage of *B. sorokiniana* (11.67%), *Fusarium* sp. (24.33%), *Aspergillus* sp. (17.07%), *Penicillum* sp. (7.50%) and *Rhizopus* sp. (17.67%) were recorded form untreated seeds (Table 1). Misra and Dixit[23] have shown that garlic could be used as a potent fungicide against plant pathogens *in vitro*. *Allium sativum* has also been found effective against seed borne pathogens of jute[24]. Allice and Rao[14] also observed that *Allium sativum* extracts significantly reduced seed infection by *Drechslera oryzae* and treated seeds had significantly higher viability. Next to garlic and neem, alcoholic extracts of *V. rosea* showed 1.40% incidence of *B. sorokiniana* on tested wheat seeds followed by bulb extract of *A. cepa* and leaf extract *A. aspera*. This result indicates that leaf extract of neem, bulb extract of garlic, rhizome extract of ginger, bulb

Table 1: Effect of plant extracts on seed borne fungi of wheat var. Kanchan

	Alcoholic extract					Water extract				
	Percentage of associated fungi									
Treatments	*Bipolaris sorokiniana*	*Fusarium* sp.	*Aspergillus* sp.	*Penicilliu* sp.	*Rhizopus* sp.	*Bipolaris sorokiniana*	*Fusarium* sp.	*Aspergillus* sp.	*Penicilliu* sp.	*Rhizopus* sp.
Zingiber officinale	2.267de	2.000g	0.00g	0.00d	0.000d	0.000e	5.000cd	00.00f	0.00d	0.000c
Allium sativum	0.000f	0.000h	0.00g	0.00d	00.000d	0.000e	1.667e	00.00f	0.00d	0.000c
Alliumcepa	1.533de	8.167d	5.83e	4.50c	0.000d	0.000e	7.167c	5.83de	0.00d	0.000c
Adhatoda vesica	4.667c	12.170b	4.00f	0.00d	0.000d	5.833c	5.667cd	00.00f	0.00d	4.667b
Lawsonia alba	2.333d	6.333e	13.77b	0.00d	4.167c	4.840d	11.33b	17.50b	0.00d	0.000c
Azadirachta indica	0.000f	0.000h	0.00g	0.00d	0.000d	0.000e	0.000e	00.00f	0.00d	0.000c
Achyranthes aspera	1.533de	2.333g	9.76c	5.50b	5.833b	7.167b	6.500cd	11.83c	2.83c	0.000c
Cuscuta reflexa	6.333b	11.670bc	0.00g	0.00d	0.000d	0.000e	5.833cd	6.50d	5.50b	0.000c
Vinca rosea	1.400e	3.167f	3.83f	0.00d	0.000d	5.900c	4.833d	5.50e	0.00d	0.000c
Nigella sativa	5.33 3c	11.33c	7.00d	4.33c	0.000d	00.000e	9.833b	11.50c	0.00d	4.667b
Control	11.670a	24.33a	17.50a	7.50a	17.67a	11.670a	23.67a	24.00a	7.50a	7.667a
LSD (p=0.01)	0.8215	0.7937	0.6041	0.700	0.4847	0.3535	2.001	0.6892	0.4899	0.4899

extract of onion and stem extract of dodder have some fungicidal properties that inhibit the growth of the seed borne fungi. Hashmi *et al.*[25] reported that seed treatment with neem (*A. indica*) extracts control the seed borne mycoflora. The extracts from *N. sativa* and *A. vesica* resulted 5.33 and 4.66% incidence of *B. sorokiniana* from treated seeds of wheat. Alcoholic extracts of rhizome of *Z. officinale* gave only 2% incidence of *Fusarium* sp., next to garlic and neem extracts which was statistically identical to leaf extract of *A. aspera*.

Water extract of all the tested plants have ability to control seed-borne fungi of wheat var. kanchan which showed hundred per cent inhibition of *B. sorokiniana* with the application of extracts from *Z. officinale*, *A. sativum*, *A. cepa*, *A. indica*, *C. reflexa* and *N. sativa*, whereas the highest incidence (11.67%) *B. sorokiniana* was recorded on untreated seed (control). After treatment with the water extract of *A. aspera* which recorded only 7.16% incidence of *B. sorokiniana* on wheat seeds. Seeds of wheat treated with *A. vesica* and *V. rosea* gave statistically identical results (5.83 and 5.90% incidence of *B. sorokiniana*). All the plant extracts showed significant result in reducing the association of *Fusarium* sp. from the treated seeds. Neem extract showed complete removal of *Fusarium* sp. from the treated seeds and it is statistically similar to garlic extract, while the highest (23.67%) *Fusarium* sp. was recorded in untreated seed (control). Homeopathic drugs, *Filixmasand Blatta orientalis*, completely suppressed the population of *F. oxysporum* in the seed mycoflora of wheat[26]. Next to *A. indica*, *A. sativum* and *V. rosea* root extracts have good antifungal effect against *Fusarium* sp. The rest of the plant extracts showed intermediate effects against *Fusarim* sp. In case of *Aspergillus* sp., *Z. officinale*, *A. sativum*, *A. vesica* and *A. indica* extracts showed good inhibitory effect against *Aspergillus* sp., while the highest percent[24] *Aspergillus* sp. was counted in untreated seeds (control). *V. rosea* treated seeds of wheat also gave good result for controlling *Aspergillus* sp. followed by *A. cepa* bulb extract. Except *L. alba*, other plant extracts have intermediate antifungal effect against *Aspergillus* sp. In case of *Penicillium* sp., except *C. reflexa* and *A. aspera,* all the tested plant extracts have excellent antifungal effect. The plant extracts used for the study also showed good antifungal effect against *Rhizopus* sp. except *A. vesica* and *N. sativa.*

Highest (99.33%) seed germination of wheat was recorded with the application of alcoholic neem extract, while the lowest (85%) seed germination was observed in control, which is 16.86% higher over untreated seeds (control). With the application of *A. sativum* bulb extract on seeds recorded 14.12% increase of seed germination over control followed by *Z. officinale* (12.94%). Treated seeds by other plant extracts also showed good result in respect of increased seed germination and decreased germination failure. On the other hand, lowest germination failure was observed when the seeds were treated with neem extract. The highest (86.95%) healthy and lowest (13.05%) infected seedlings (13.05%) were obtained when the seeds treated with neem extract. Next to neem, garlic extract treated seeds gave (84.70%) healthy and (15.13%) infected seedlings, respectively. But in case of untreated seed, maximum (51.50%) infected and lowest (48.50%) healthy seedlings were obtained. Treated seeds from *Z. officinale* extract also showed good effect to obtained healthy seedlings (78.19%) and least infected seedlings. Rest of the plant extracts *A. cepa*, *A. vesica, C. reflexa*, *L. alba*, *V. rosea*, *A. aspera* and *N. sativa* treated seeds of wheat recorded 76.31, 75.33, 70.97, 66.05, 65.61, 63.59 and 63.39% healthy and 23.69, 24.67, 29.03, 33.94, 34.28, 36.44 and 36.07% infected seedlings, respectively (Table 2).

Plant extracts prepared in water also significantly ($p \leq 0.01$) showed increased seed germination over untreated control. The highest (14.50 and 13.73%)

Table 2: Effect of plant extracts on seed germination, number of healthy and infected seedlings of wheat var. Kanchan

	Alcoholic extract					Water extract				
Treatments	AA	BB	CC	DD	EE	AA	BB	CC	DD	EE
Zingiber officinale	96.00bc	+12.94	4.00f	78.19c	21.78f	94.00b	+10.58	6.00f	71.14cd	28.86e
Allium sativum	97.00b	+14.12	3.00g	84.70b	15.30g	96.67a	+13.73	3.33h	85.57a	12.43h
Allium cepa	88.00e	+3.53	12.00b	76.31d	23.69e	88.67 d	+4.13	11.33b	67.67cd	30.33d
Adhatoda vesica	93.67d	+10.20	6.33de	75.33d	24.67e	95.00ab	+11.76	5.00g	70.19cd	36.48b
Lawsoniaalba	92.92d	+9.31	7.08cd	66.05f	33.94c	89.67d	+5.49	10.33c	74.50bc	25.50f
Azadirachta indica	99.33a	+16.86	0.67h	86.95a	13.05h	97.33a	+14.50	2.67h	84.87a	15.13h
Achyranthes aspera	92.67d	+9.02	7.33c	63.59g	36.07b	93.00bc	+9.41	7.00e	68.10cd	31.90c
Cuscuta reflexa	94.00cd	+10.59	6.00e	70.97e	29.03d	93.33bc	+9.8	6.67ef	75.00bc	25.67f
Vinca rosea	93.33d	+9.80	6.67cde	65.61f	34.28c	91.00cd	+7.05	9.00b	81.67ab	18.33g
Nigella sativa	88.67e	+4.32	11.33b	63.59g	36.41b	93.00bc	+9.41	7.00e	63.33d	35.83b
Control	85.00f	--	15.00a	48.50h	51.50a	85.00e	--	15.00a	48.50e	51.48a
LSD (p=0.01)	2.078		0.7905	1.093	1.151	2.393		0.7516	8.193	1.325

AA: % of germination; BB: % germination increase (+) or decrease (-) over control; CC: % of germination failure; DD: % of healthy seedlings; EE: % of infected seedlings

increased seed germination of wheat over control was recorded when the seeds were treated with *A. indica* leaf and *A. sativum* bulb extract followed by *A. vesica* leaf extracts and lowest germination failure was recorded when the seeds treated with the above mentioned plant extracts. Utikar and Shinde[27] reported that water extract of garlic and *V. rosea* gave more than 80% seed germination compared with 65.30% in untreated control. Treated seeds with the extracts of rhizome of *Z. officinale*, stem of *C. reflexa*, leaf of *A. aspera* and seed of *N. sativa* showed 10.58, 9.8, 9.41 and 9.41% increased seed germination over control, respectively. The ability of the extracts to increase seed germination could be attributed to the suppression of the incidence of seed borne fungi that could have killed the embryo of the seeds. This result is consistent with that of Parimelazhagan and Francis[13] who established that leaf extract of *C. viscosum* increased seed germination and improved seedling developments of rice seeds. The plant extract used for the study showed significant performance in respect of healthy and infected seedlings. They ranged from 48.50 to 85.57% and 12.43 to 51.48%, respectively. The highest (85.57%) and the lowest healthy and infected seedlings of wheat were recorded after treatment with garlic extract and it is statistically similar to neem extract treatment, while the lowest percent healthy seedlings (48.50%) and highest infected seedlings (50.48%) were obtained in untreated seed (control). In case of *V. rosea* root extract treated seeds of wheat, which gave 81.67% healthy seedlings and 18.33% infected seedlings. *C. reflexa* plant extract has significant effect against fungi, where 75% healthy seedlings were recorded with this treatment of seeds. The extract of *A. cepa*, *A. vesica* and *A. aspera* also gave good inhibitory effects against pathogens and to obtain healthy seedlings (67.67, 70.19 and 68.10%) though they are statistically similar to each other. Rests of the plant extracts have intermediate effect on healthy and infected seedlings (Table 2).

Significant results obtained by the plant extracts in respect of root length and shoot length and varied from 6.33 to 11.33 cm and 4.9 to 8.83 cm after 7 days of sowing on paper towel (Table 3). The maximum root and shoot length was recorded when treated with ginger, neem and garlic extract. In case of untreated seed, only 6.33 cm root length was measured. The seeds treated with the extracts of *L. alba*, *A. vesica* and *A. cepa* produced 9.66, 9.00 and 8.66 cm longer root length. The remaining treatments showed intermediate effects in case of root growth in length. On the other hand, 7.66, 6.83, 6.66 and 6.33 cm shoot length were recorded with the treatment of *A. sativum* bulb *L. alba* leaf, *A. cepa* bulb and *A. vesica* leaf extracts, respectively. All the plant extracts have significant ($p \leq 0.01$) effect on vigour index. Highest vigour index (1936,1826 and 1871) was found when the seeds were treated with the extracts of ginger rhizome, garlic bulb and neem leaf. Next to ginger rhizome, garlic bulb and neem leaf extract, *L. alba* and *A. vesica* leaf extracts gave the highest vigour index. But incase of untreated seed, the lowest vigour index was recorded.

Water extracts of tested plants showed significant results in respect of root length and shoot length and ranged from 6.33 to 13.33 cm and 6.0 to 11.0 cm. The highest root length (13.33 cm) was measured in the seedlings treated with garlic, ginger and neem extracts followed by dodder extract. The lowest root length (6.33 cm) was recorded in untreated seedlings (control). This result is in accordance with the report of Allice and Rao[14], where they observed that *A. sativum* and *A. indica* extract-treated seeds produced seedlings with longer shoots and roots than those in untreated control. *A. aspera* leaf extract treated seeds did not show good result in respect of root growth in length of seedlings. When the seeds were treated with ginger rhizome extract gave highest shoot length (11.0 cm). Next to ginger rhizome, 9.66 cm shoot length was recorded when treated with garlic bulb extract followed by onion bulb, dodder

Table 3: Effect of plant extracts on seed germination, root length, shoot length and vigour index of wheat var. Kanchan by paper rolled method

	Alcoholic extract				Water extract			
Treatments	% of germination	Root length (cm)	Shoot length (cm)	Vigour Index (VI)	% of germination	Root length (cm)	Shoot length (cm)	Vigour Index (VI)
Zingiber officinale	96.00bc	11.33a	8.76a	1936.0a	94.00b	12.33a	11.00a	2194.0a
Allium sativum	97.00b	10.50ab	7.66b	1826.0a	96.67a	13.33a	9.66b	2223.0a
Allium cepa	88.00e	8.667cde	6.66c	1350.0cd	88.67d	10.17bc	8.66bc	1683.0bc
Adhatoda vesica	93.67d	9.00cd	6.33cd	1436.0bc	95.00ab	9.66c	7.83cd	1663.0bc
Lawsonia alba	92.92d	9.667bc	6.83bc	1518.0b	89.67d	9.16c	7.33d	1480.0cd
Azadirachta indica	99.33a	10.67ab	8.83a	1871.0a	97.33a	13.17a	9.17b	2174.0a
Achyranthesaspera	92.67d	7.76def	5.50def	1229.0def	93.00bc	7.00d	7.06de	1308.0d
Cuscuta reflexa	94.00cd	8.33def	6.50def	1301.0cde	93.33bc	9.33c	8.53bc	1668.0bc
Vinca rosea	93.33d	7.40efg	4.90f	1149.0efg	91.00cd	11.67c	8.50bc	1834.0b
Nigella sativa	88.67e	7.17fg	5.16ef	1094.0fg	93.00bc	9.00c	6.93de	1476.0cd
Control	85.00f	6.33g	6.00cde	1049.0g	85.00e	6.33d	6.00e	1049.0e
LSD (p=0.01)	2.078	1.183	0.9027	148.0	2.393	1.773	1.111	190.40

stem and *V. rosea* root extracts, respectively. On the other hand, lowest shoot length was measured in control.

Highest vigour index (2223, 2194 and 2174) was recorded with the application of garlic bulb, ginger rhizome and neem leaf extracts, while the lowest (1049) was in control. Next to garlic bulb, ginger rhizome and neem leaf extracts, *V. rosea* root extract gave 1834 vigour index followed by *A. cepa* bulb, *A. vesica* leaf and *C. reflexa* stem extracts. The rest of the treatments showed intermediate effects on vigour index of seeds. The present study has therefore shown that extract of neem leaf, garlic bulb and ginger rhizome have antifungal effect and can be used as fungicidal seed treatments for the control of seed-borne fungi of wheat and for increasing seed germination and vigour index.

REFERENCES

1. Richardson, M. J., 1979. An Annotated List of Seed Borne Diseases. 3rd Edn., CAB Publications, CMI, Kew, Surrey, UK., pp: 1-320.
2. Wise, M.V., 1987. Compendium of Wheat Diseases. 2nd Edn., Am. Phytopathol. Soc. St. Paul, pp: 1-31.
3. Talukdar, M.J., 1974. Plant diseases in Bangladesh. Bangladesh J. Agric. Res., 1: 61-86.
4. Fakir, G.A., A.L. Khan, P. Neergaard and S.B. Mathur, 1977. Transmission of *Drechslera* sp. through wheat seed in Bangladesh. Bangladesh J. Agric., 1: 113-118.
5. Fakir, G.A., 1980. An annotated list of seed-borne diseases in Bangladesh. Agricultural Information Service, Dhaka, Bangladesh, pp: 17.
6. Paritosh, K.M., A.R. Mostofa, M.A. Syed and A.S. Mohammad, 2004. Bipolaris leaf blight: A major constraint to sustainable production of wheat grown under humid conditions. 4th International Crop Sciences Congress Brisbane, Australia, 26 September-1 October, 2004.
7. Spencer, D.M., J.N. Topps and R.L. Wain, 1957. Fungistatic properties of tissue. An antifungal substance from the tissue of *Vicia faba*. Nature, 179: 651-662.
8. Appleton, J.A. and M.R. Tansey, 1975. Inhibition of growth of 200 pathogenic fungi by garlic extract. Mycologia, 67: 882-885.
9. Misra, S.B. and S.N. Dixit, 1976. Fungicidal spectrum of leaf extracts of *Allium sativum*. Ind. Phytopathol., 29: 448.
10. Singh, R.N., I.R Sindhu and K. Gupta, 1986. Effect of leaf exudate and extract of spinach on some phylloplane fungi. Acta Bot. Indica, 14: 104-110.
11. Dubey, R.C., 1991. Fungicidal effect of essential oils of three higher plants on sclerotia of *Macrophomina phaseolina*. Ind. Phytopathol., 44: 241-243.
12. Singh, H.N.P., M.M. Prasad and K.K. Shinha, 1993. Efficacy of leaf extracts of some medicinal plants against disease development in banana. Lett. Microbiol., 17: 269-271.
13. Parimelazhagan,T. and K. Francis, 1999. Antifungal activity of *Clerodendrum viscosum* against *Curvularia lunata* in rice seeds. J. Mycol. Plant Pathol., 29: 139-141.
14. Alice, D. and A.V. Rao, 1986. Management of seed borne *Drechslera oryzae* of rice with plant extracts. Intl. Rice Res. News Lett., 11: 19.
15. Singh, H.B. and U.P. Singh, 1981. Effect of volatility of some plant extracts on *Erysiphe*. J. Plant Pathol., 10: 66-67.
16. Singh, Y., R.D. Tripathi, N.N. Tripathi and S.N. Dixit, 1983. The isolation and properties of fungitoxic principle from *Zingiber officinale*. Ind. J. Plant Pathol., 1: 89-96.
17. Singh, B.P., S.P. Singh and A. Mohammad, 1990. Economic efficacy of different fungicides for the control of leaf spot of Cauliflower. Ind. Phytopathol., 43: 207-209.

18. Khair, A., I. Ara, G.K. Joarder and F. Begum, 1995. Effect of clove oil and citral on toxin producing fungal flora of poultry feed. Bangladesh J. Sci. Indust. Res., 30: 191-195.
19. Anonymous, 1976. International rules for seed testing. Seed Sci. Technol., 4: 3-49.
20. Mahadevan, A. and R. Sridhar, 1982. Methods in Physiological Plant Pathology. 2nd Edn., Sivakami Publications. Madraj, pp: 316.
21 Sing, R.A. and M.H.S. Rao, 1977. A simple techniques for detection of *Xanthomonas oryzae* pv. *oryzae* in rice seeds. Seed Sci. Technol., 5: 123-127.
22. Abdul-Baki, A.A. and J.D. Anderson, 1973. Vigour determination of soybean seed by multiple criteria. Crop Sci., 13: 630-633.
23. Misra, S.B. and S.N. Dixit, 1977. Antifungal properties of *Allium sativum* Linn. Sci. Cult., 43: 487-488.
24. Ahmed, N. and K. Shultana, 1984. Fungitoxic effect of garlic on treatment of jute seed. Bangladesh J. Bot., 13: 130-136.
25. Hashmi, R.Y., S. Shahzad, A.K. Khanzada, M. Aslam and S.A.R. Kazmi, 1992. Studies on the seed mycoflora of lentil and its control. Pak. J. Sci. Indust. Res., 35: 345-347.
26. Rake, K., K.K. Khanna, C. Sudhir, R. Khanna and S. Chandra, 1989. Effect of homoeopathic drugs on seed mycoflora of wheat. Natl. Acad. Sci. Lett., 12: 39-41.
27. Utikar, P.G. and P.A. Shinde, 1989. Antifungal effects of phytoextracts vis-a-vis the fungicides on grain mould parameters. Pestology, 13: 23-26.