PLANT EXTRACT EFFECT ON SEED-BORNE PATHOGENIC FUNGI FROM SEEDS OF PADDY GROWN IN SOUTHERN INDIA

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Abstract: A total of 40 seed samples of different cultivars of paddy, collected from southern part of India, were mycologicaly analyzed by agar plating method on Czapek's-Dox-Agar (CzA) medium and Standard Blotter Method (SBM), revealed the occurrence of 33 diverse fungal species belonging to 16 genera. The species of *Drechslera oryzae* (RP 82.5%) was recorded with the incidence and relative preponderance (RP), followed by *Curvularia lunata* (RP 67.5%) and *Aspergillus niger* (RP 65.0%) respectively. The data on the diversity and incidence of fungal species would be a great importance in the region for predicting the extent of pre-and post-infections.

In vitro antifungal activity assay of methanol extract of 12 plants belonging to different families were tested against eight pathogenic fungal species viz., *Alternaria alternata*, *Aspergillus flavus*, *Curvularia lunata*, *Drechslera oryzae*, *D. halodes*, *Fusarium moniliforme*, *Pyricularia oryzae* and *Trichoconis padwickii* by poisoned food technique. The results revealed that, methanol extract of *Acacia nilotica*, *Caesalpinia coriaria*, *Decalepis hamiltonii*, *Emblica officinalis*, *Lawsonia inermis* and *Mimosops elengi* showed significant antifungal activity at 3500 μg/ml concentration. Comparative evaluation of the extracts with the synthetic fungicides viz., bavistin, blitox, captan, dithane M-45 and thiram at the recommended dosage revealed that, antifungal activity of methanol extract of *D. hamiltonii*, *L. inermis* and *M. elengi* was almost equivalent. These plants can possibly be exploited in the management of seed-borne pathogenic fungi and prevention of biodeterioration of paddy in an eco-friendly way.

Key words: paddy grown, plant extracts

INTRODUCTION

Rice (*Oryza sativa*) is one of the most widely grown important cereal crops of the world. In India, rice occupies the first place both in area of about 42.24 million hectares and produces about 82 million tones (Krishnamurthy *et al*. 2005). Rice is prone to several diseases caused by fungi, bacteria, viral and mycoplasmal pathogens (Agrios 1997). Seed serves as an important microcosm for saprotrophic and pathogenic microorganisms and paddy seeds are no exception to this (Agrios 1997; Domijan *et al.* 2005). More than 50 fungal pathogens have been reported to be seed-borne in paddy (Agrawal 1999). Of the fungi involved, species of *Alternaria, Aspergillus, Ceratobasidium, Cercospora, Cochliobolus, Curvularia, Dreschslera, Fusarium, Gaeumannomyces, Microdochium, Penicillium, Pyricularia, Pythium, Rhizoctonia, Rhizopus, Sclerophthora, Trichoderma* and *Tricoconella* are most common associates in paddy all over the world, causing pre- and post-infections and considerable quality losses viz., seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity, seedling damage and their nutritive value have been reported (Miller 1995; Janardhana *et al*. 1998; Kavitha *et al*. 2005).

Seed treatment is the safest and the cheapest way of control of seed-borne fungal diseases and to prevent biodeterioration of grains (Chandler 2005; Bagga and Sharma 2006). A large number of fungicides are being used in the form of dusting, slurry and soaking treatment (Agrios 1997). Even though effective and efficient control of seed borne fungi can be achieved by the use synthetic chemical fungicides, the same cannot be applied to grains for reasons of pesticide toxicity (Harris *et al*. 2001). It is now realized that chemical fungicides cause serious environmental problems and are toxic to non-target organisms (Anon 2005). The toxic effect of synthetic chemicals can be overcome, only by persistent search for new and safer pesticides accompanied by wide use of pest control methods, which are eco-friendly and effective.

Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides (Varma and Dubey 1999). Extracts of many higher plants have been reported to exhibit antifungal properties under laboratory trails (Parekh *et al*. 2006; Aliero and Afolayan 2006;

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Buwa and Staden 2006; Ergene *et al*. 2006; Mohana *et al*. 2008). Exploitation of plant metabolites in crop protection and prevention of biodeterioration caused by fungi appear to be promising.

In view of these, the authors screened a large number of paddy samples in the laboratory for identification of the seed-borne fungi which are responsible for many diseases and biodeterioration of paddy. Authors also screened some plants extracts for management of some of these pathogenic fungi commonly associated with paddy. All these data's were presented in this paper.

MATERIALS AND METHODS

Isolation, identification and determination of percent incidence and relative preponderance of associated seed-borne fungi in paddy seeds samples

Collection of paddy seeds samples

A total of 40 seeds samples of different cultivars of paddy (IR-20, IR-60, IR-64, IET-8116, Jaya, Rasi, Sona masuri, Jyothi, Mandya vijaya, Bilihamsa, Mysore malligae and Intan cultivar) (approximately 1 kg) were collected from markets, local stores, agricultural co-operatives and farm fields (immediately after the harvest) of different agro-climatic regions of states of Karnataka and Tamil Nadu (25 samples from Karnataka and 15 samples from Tamil Nadu), during the harvest seasons of 2007 and 2008. Samples were brought to the laboratory in sterile plastic bags and kept at 4°C. All the samples were subjected to mycological analysis.

Mycological analysis of paddy seeds samples

The collected 40 seeds samples were plated on Czapek's-Dox-Agar medium by agar plating method and Standard Blotter Method to isolate frequently occurring important seed-borne pathogenic field and storage fungi associated with these seed samples. Four hundred seeds from each sample were surface sterilized with 2% sodium-hypochlorite solution for 3 min. and rinsed twice with sterile distilled water. The sterilized two hundred seeds of each of the samples were subjected to SBM method and another two hundred seeds were subjected to agar plating method (ISTA 1996). The plates were incubated under alternating periods of 12 hours darkness and 12 hours of light at 25±2°C for 7 days.

The fungal colonies expressed on paddy grains in both SBM and agar plate method, were visualized using stereo-binocular microscope. Each representative isolate of seed-borne fungal species was again transferred onto CzA medium to study the growth characteristics, colony morphology, mycelial structure and morphology, chlamydospore morphology, conidiophore and conidia morphology in pure culture form. All the isolates were identified up to the species level by using fungal keys (Booth 1977; Leslie and Summerell 2006; Nagamani *et al*. 2006). The percent incidence and relative preponderance of seed-borne fungi were determined using the formula:

Percent Incidence (PI) = number of seeds on which the fungus was encountered in each sample x 100/total number of seeds tested in each samples,

Relative Preponderance (RP) = Number of seed samples on which the fungus was encountered x 100/total number of seed samples tested (Agrawal 1999)

The fungi viz., *Alternaria alternata*, *Aspergillus flavus, Curvularia lunata, Drechslera oryzae*, *D. halodes*, *Fusarium moniliforme*, *Pyricularia oryzae* and *Trichoconis padwickii* which were frequently associated in higher percentage in paddy were selected and served as test fungi for antifungal activity assay.

Antifungal activity assay of plant extracts and synthetic fungicides

Collection of plant materials

Fresh leaves of *Acacia nilotica* (Mimosaceae), *Argemone mexicana* (Papaveraceae), *Caesalpinia coriaria* (Caesalpinaceae), *Emblica officinalis* (Euphorbiaceae), *Euphorbia tirucalli* (Euphorbiaceae), *Lawsonia inermis* L (Lythraceae), *Leucas aspera* (Lamiaceae), *Mimosops elengi* (Sapotaceae), *Phyllanthus amarus* (Euphorbiaceae), *Tinospora cordifolia* (Menispermaceae), *Tribulus terrestris* (Zygophyllaceae) and rhizome of *Decalepis hamiltonii* (Asclepiadaceae), free from disease were collected from Bangalore region of Karnataka, washed thoroughly 2–3 times with running tap water and once with sterile distilled water, shade-dried, powdered and used for extraction.

Preparation of methanol extract

Sample (50 g) of the shade-dried powder of each plant materials was filled in the thimble and extracted separately with methanol using Soxhlet extractor until colourless extract was obtained on the top of the extractor. All the extracts were concentrated separately using rotary flash evaporator and preserved at 5°C in an air tight brown bottle until further use (Harborne 1998). All the plant extracts were subjected to antifungal activity assay. The methanol extract of *D. hamiltonii* and *M. elengi* which are recorded highest activity, were further subjected to active fraction separation following the procedure of Roberts *et al*. (1981).

Antifungal activity assay

One gram each of the dried evaporated methanol extract of all the test plants was dissolved separately in 10 ml of methanol. 500 µl of each of the extract was amended with 15 ml of CzA medium per plate before solidification of the medium (total concentration of the extract in CzA medium is 3 500 µg/ml). The medium amended only with 500 µl of methanol served as control. Five mm disc of 7-day-old culture of the test fungi were inoculated. Four replicates were maintained for each extract. The plates were incubated at 22±1°C for seven days. The fungitoxicity of the extracts in terms of percentage inhibition of mycelia growth was calculated by using the formula:

$$\% \text{ inhibition} = dc - dt \times 100/dc$$

where:
dc = average increase in mycelia growth in control,
dt = average increase in mycelia growth in treatment (Singh and Tripathi 1999).

Synthetic fungicides, viz., Bavistin (Methyl benzimidazol-2-ylcarbamate), Blitox (copper oxychloride), Captan (N-(Trichloromethylthio) Cyclo-hex-4-one-1,2, di-carboximide), Dithane M-45 (Zinc ion and Manganese ethylene bisdithio carbamate) and Thiram (Tetramethyl thiuramidisulphide) were also tested at the recommended dosage (2 gm/Lt) for antifungal activity by poisoned food method.

RESULTS

Isolation, identification and determination of percent incidence and relative preponderance of associated seed-borne fungi in paddy seeds samples

A total of 40 seeds samples of paddy collected from different agro-climatic regions of southern part of India (States of Karnataka and Tamil Nadu), when mycologicaly analyzed by agar plating method and SBM revealed the occurrence of thirty-three diverse fungal species belonging to sixteen genera. Data on the percentage incidence and relative preponderance of the seed-borne fungi that occur frequently has been presented in table 1. It was observed that irrespective of the locality and cultivars paddy seeds harboured diverse fungal species. The species of *D. oryzae* (RP 82.5%) was recorded with higher percent incidence, followed by *C. lunata* (RP 67.5%), *A. niger* (RP 65%), *A. flavus* (RP 57.5%), *Penicillium chrysogenum* (RP 55.0%), *F. moniliforme* (RP 52.5%), *D. halodes* (RP 52.5%), *T. padwickii* (RP 47.5%), *A. alternata* (RP 47.5%) and *F. oxysporum* (RP 45.0%). The species *F. equiseti* (RP 12.5%), *F. proliferatum* (RP 15.0%), *Mucor hiemalis* (RP 15.0%), *Chaetomium globosum* (RP 17.5%), *A. nidulans* (RP 20.0%), and *A. versicolor* (RP 20.0%) were recorded

Table 1. Relative preponderance and percent incidence of some important seed-borne fungi in 40 different paddy samples collected from southern part of India

No.	Seed-borne fungal species	Relative preponderance [%]	Number of seed samples in which the fungus was encountered			
			5 to 10%	10 to 25%	25 to 50%	50 to 100%
1	*Alternaria alternata*	47.5	10	08	01	–
2	*Aspergillus flavus*	57.5	05	14	04	–
3	*A. terreus*	35.0	12	02	–	–
4	*A. fumigatus*	25.0	10	–	–	–
5	*A. nidulans*	20.0	08	–	–	–
6	*A. niger*	65.0	12	09	05	–
7	*A. ochraceus*	12.5	05	–	–	–
8	*A. tamari*	30.0	09	03	–	–
9	*A. versicolor*	20.0	08	–	–	–
10	*Chaetomium globosum*	17.5	07	–	–	–
11	*Cladosporium cladosporioides*	47.5	13	6	–	–
12	*Curvularia trifolii*	32.5	12	01	–	–
13	*C. lunata*	67.5	10	13	04	–
14	*C. brachyspora*	22.5	08	01	–	–
15	*Drechslera halodes*	52.5	09	10	2	–
16	*D. hawaiiensis*	22.5	07	02	–	–
17	*D. oryzae*	82.5	10	15	06	02
18	*D. tetramera*	40.0	04	12	–	–
19	*Fusarium equiseti*	12.5	05	–	–	–
20	*F. moniliforme*	52.5	10	09	02	–
21	*F. proliferatum*	15.0	06	–	–	–
22	*F. oxysporum*	45.0	13	04	01	–
23	*F. solani*	35.0	11	03	–	–
24	*Mucor hiemalis*	15.0	06	–	–	–
25	*Nigrospora oryzae*	30.0	12	–	–	–
26	*Penicillium chrysogenum*	55.0	10	12	–	–
27	*Phoma sps.*	22.5	09	–	–	–
28	*Pyricularia oryzae*	25.0	09	01	–	–
29	*Rhizopus stolonifer*	37.5	10	05	–	–
30	*Trichoderma harzianum*	32.5	11	02	–	–
31	*T. viride*	30.0	08	04	–	–
32	*Trichoconis padwickii*	47.5	17	02	–	–
33	*Trichothecium roseum*	30.0	11	01	–	–

Percent incidence is based on 4 replicates with 100 seeds each

with lower percent incidence. *D. oryzae* a known pathogen, was observed in 33 samples and percentage of seed infection varied between 4 to 65%. *C. lunata* was recorded in 27 samples and percentage of seed infection varied between 0.8 to 56%. *A. niger* was found in 26 samples and percentage of seed infection varied between 1.8 to 52%. *A. alternata, A. flavus, P. chrysogenum, T. padwickii* and *F. moniliforme* which are known to produce toxins, were observed in 19, 23, 22, 19 and 23 samples, respectively, and percentage of seed infection varied from 3 to 49%. Another known important seed-borne pathogen *P. oryzae* was found in 10 samples and percentage of seed infection varied from 0.5 to 22%. Tukey HSD statistical data analysis revealed that the fungal diversity and percent incidence was more in seed samples collected from Hassan and Mandya districts of Karnataka.

Antifungal activity assay of plant extract and synthetic fungicides

Among the twelve plants screened, methanol extracts of *A. nilotica*, *C. coriaria*, *D. hamiltonii*, *E. officinalis, E. tirucalli*, *L. inermis* and *M. elengi* have displayed significant activity against all pathogens tested, whereas methanol extracts of *A. mexicana*, *L. aspera*, *P. amarus*, *T. cordifolia* and *T. terrestris* did not show any antifungal activity (Table 2). The Tukey HSD statistical data analysis revealed that, the highest inhibitory activity was observed in *D. halodes* and lowest inhibitory activity was observed in *C. lunata*. Highly significant antifungal activity was observed in extract of *D. hamiltonii* followed by *M. elengi* and *L. inermis* and lowest antifungal activity was observed in extract of *C. coriaria* and *E. tirucalli* respectively. The inhibitory effect of different fractions of methanol extract of *D. hamiltonii* and *M. elengi* revealed that, phenolic compound of *D. hamiltonii* and phenolic and alkaloid compounds of *M. elengi* responsible for antifungal activity.

Comparative efficacy of the extracts, with five synthetic fungicides such as bavistin, blitox, captan, dithane M-45 and thiram (Table 2) revealed that, complete inhibition of mycelia growth of all the test fungi were observed only in thiram even compared to test plants. Dithane M-45 recorded least activity. Highly significant inhibition of mycelia growth of *C. lunata* was observed in methanol extract of *D. hamiltonii, M. elengi*, *L. inermis* and *A. nilotica* compared to blitox and dithane M-45.

Table 2. Antifungal activity of methanol extract of different plants and synthetic fungicides against phytopathogenic fungi isolated from paddy samples

Methanol extracts of different plants	Percentage of mycelium inhibition							
	pathogenic fungi of paddy							
	Alternaria alternata	*Aspergillus flavus*	*Curvularia lunata*	*Drechslera oryzae*	*Drechsclera halodes*	*Fusarium moniliforme*	*Pyricularia oryzae*	*Trichoconis padwickii*
Acacia nilotica	69.7±0.8 de	70.1±0.3 e	62.5±0.8 c	74.6±0.7 d	85.0±0.2 d	83.9±0.7 e	68.5±0.8 de	65.6±0.9 c
Argemone mexicana	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a
Caesalpinia coriaria	28.9± 0.5 c	34.7±0.5 c	13.6±0.3 b	27.6 ±0.3 c	38.2± 0.6 c	29.3± 0.9 c	23.3± 0.5 c	19.3± 0.4 b
Decalepis hamiltonii	81.9±0.4 f	83.9±0.4 f	78.1±0.7 de	82.5±0.3 e	91.3±0.7 e	89.9±0.7 ef	81.2±0.5 f	79.8±0.4 d
Emblica officinalis	65.9±0.8 d	68.6±0.7 d	60.1±0.5 c	70.6±0.4 d	81.5±0.4 d	79.2±0.5 de	64.4±0.4 d	62.8±0.3 c
Euphorbia tirucalli	10.3±0.6 b	12.4±0.4 b	10.1±0.6 b	16.6±0.9 b	22.6±0.3 b	18.7±0.4 b	13.2±0.3 b	11.2±0.6 b
Lawsonia inermis	70.5±0.3 e	74.9±0.5 ef	64.6±0.9 cd	77.8±0.8 de	84.2±0.6 d	81.4±0.5 e	68.3±0.5 de	66.4±0.3 c
Leucas aspera	0.0±0.00 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a
Mimosops elengi	76.4±0.4 ef	78.6±0.5 ef	72.69±0.3 d	83.6±0.7 e	85.6±0.4 d	84.7±0.4 e	70.3± 0.2 e	69.4± 0.2 d
Phyllanthus amarus	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a
Tinospora cordifolia	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a
Tribulus terrestris	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a
Synthetic fungicides								
Bavistin	100±0.0 g	100±0.0 h	95.1±0.5 f	100±0.0 g	100±0.0 f	100±0.00 g	96.5±0.3 gh	98.0±0.7 fg
Blitox	76.3±0.4 ef	93.8±0.3 g	88.9±0.2 e	91.3±0.3 f	100±0.0 f	96.2±0.4 f	86.8±0.9 fg	87.9±0.2 e
Captan	72.2±0.7 e	89.3±0.3 fg	90.7±0.3 ef	87.2±0.4 e	100±0.0 f	86.5±0.7 ef	90.6±0.5 g	91.5±0.2 f
Dithane M-45	66.7±0.7 d	87.7±0.6 fg	59.9±0.4 c	75.6±0.2 d	83.0±0.7 d	73.8±0.2 d	74.9±0.2 e	60.7±0.3 c
Thiram	100±0.0 g	100±0.0 h	100±0.0 g	100±0.0 g	100±0.0 f	100±0.0 g	100±0.00 h	100±0.0 g

Data given are mean of four replicates ±SE; analysis of variance (ANOVA) df = 16 at $p < 0.001$
The value followed by different superscript letters differ significantly when subjected to Tukey HSD (column by column analysis), at 0.5 subset

DISCUSSION

Southern part of India, with its varied agro-climatic conditions produces a variety of food crops throughout the year. Paddy is one of important food crop in southern part if India. Non-scientific method of agricultural practices, poor storage facilities and unfavourable environmental conditions during pre-and post-harvest handling of crops is responsible for the severe contamination, infection and colonization by fungi (Janardhana *et al*. 1999). There is also a possibility of rejection of the produce for human consumption and cattle feed due to loss in quality by fungi (Lineard *et al*. 1993). Association of variety of fungi, causing significant loss in seed quality and nutritional quality have been reported by Koirala *et al*. 2005. More than 25% of the world cereals are contaminated with known mycotoxins and more than 300 fungal metabolites are reported to be toxic to men and animals (Desjardins *et al*. 2000; Galvano *et al*. 2001). The data on the incidence and diversity of seed-borne fungal species would be of great importance in the region for predicting the extent of post-harvest infection, colonization and subsequent deterioration of cereals. In view of these, throughout the world, much attention has been given to know the diversity, incidence and management of seed-borne and toxigenic fungi. However, data on diversity of seed-borne fungal species on paddy grains is very limited in Southern part of India in general and states of Karnataka and Tamil Nadu in particular.

In the present work species of *D. oryzae*, *C. lunata*, *A. niger*, *A. flavus*, *P. chrysogenum*, *F. moniliforme*, *D. halodes*, *A. alternata*, *T. padwickii* and *F. oxysporum*, known to be pathogenic, were observed in relatively high frequency. These pathogens are disseminated predominantly by seeds. The results indicate the needs for effective management strategies in the field and in transportation and storage to control these seed-borne pathogens of paddy. Such data is of immense value for assessing the possible health hazards in humans and animals upon consumption of such contaminated food grains.

Eventhough effective and efficient control of seed borne pathogenic fungi can be achieved by the use of synthetic fungicides, the same cannot be applied to grains for reasons of pesticide toxicity (Wodageneh *et al*. 1997; Harris *et al*. 2001). Thus, there is a need to search for alternative approaches to store grains/cereals for human consumption without toxicity problems that are ecofriendly and not capital intensive.

Many reports revealed that, plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides (Varma and Dubey 1999; Harborne 1998; Gottlieb et al 2002). Considering these as a first step, in the present investigation twelve plants were screened *in vitro* for antifungal activity against eight important phytopathogenic fungi isolated from paddy. These plants were selected based on traditional medicine knowledge and random choosing from the local flora. The screening revealed that *A. nilotica*, *C. coriaria*, *D. hamiltonii*, *E. officinalis*, *E. tirucalli*, *L. inermis* and *M. elengi* have recorded significant activity against all pathogenic fungal species tested. The finding of the present investigation is an important step towards isolation and characterization of the antifungal agent and its further evaluation for crop protection strategies. *D. hamiltonii* and *E. officinalis* being edible plants possessing significant broad spectrum antifungal activity against important field and storage moulds would probably be an important candidate plants for prevention of biodeterioration of grains during storage. The present investigation suggests the need for further work on identification of the active principle responsible for antifungal activity in these plants.

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REFERENCES

Agrawal R.L. 1999. Seed Technology. 2nd ed. Oxford and IBH Publishing Co., New Delhi: 87–97.

Agrios G.N. 1997. Plant Pathology. 4th ed. Academic Press, California: 245–269.

Aliero A.A., Afolayan A.J. 2006. Antimicrobial activity of *Solanum tomentosum.* Afr. J. Biotechnol. 5 (4): 369–372.

Anon. 2005. Pest control background. Int. J. Pest Control 45 (2): 232–233.

Bagga P.S., Sharma V.K. 2006. Evaluation of fungicides as seedling treatment for controlling bakanae/foot-rot (*Fusarium moniliforme*) disease in basmati rice. J. Mycol. Plant Pathol. 59: 305–308.

Booth C. 1977. *Fusarium*: Laboratory Guide to the Identification of Major Species. Commonwealth Mycological Institute. Ferry Lane. Kew, Surrey, England: 6–133.

Buwa L.V., Staden J.V. 2006. Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa. J. Ethnopharmacol. 103 (1): 139–142.

Chandler J. 2005. Cost reduction in SIT programmes using exosect auto-dissemination as part of area wide integrated pest management. Int. J. Pest Control 42 (2): 257–260.

Desjardins A.E., Manandhar G., Plattner R.D., Maragos C.M., Shrestha K., McCormick S.P. 2000. Occurrence of *Fusarium* species and mycotoxins in Nepalese maize and wheat and the effect traditional processing method on mycotoxin levels. J. Agric. Food Chem. 48 (4): 1377–1383.

Domijan A., Feraica M., Jurjevic Z., Ivil D., Cvjetkovic B. 2005. Fumonisin B_1, fumonisin B_2, zearalenone and ochratoxin A contamination of maize in Croatia. Food Additiv. Contam. 22 (7): 677–680.

Ergene A., Guler P., Tan S., Mirici S., Hamzaoglu E., Duran A. 2006. Antibacterial and antifungal activity of *Heracleum sphondylium* subsp. a*rtvinense*. Afr. J. Biotechnol. 5 (11): 1087–1089.

Galvano F., Piva A., Ritieni A., Galvano G. 2001. Dietary strategies to counteract the effect of mycotoxins: A review. J. Food Protect. 64 (1): 120–131.

Gottlieb O.R., Borin M.R., Brito N.R. 2002. Integration of ethnobotany and phytochemistry: dream or reality? Phytochemistry 60 (2): 145–152.

Harborne J.B. 1998. *Phytochemical methods*: A guide to modern techniques of plant analysis. 3rd ed. Chapman & Hall Pub., London, UK: 7–8.

Harris C.A., Renfrew M.J., Woolridge M.W. 2001. Assessing the risk of pesticide residues to consumers: recent and future developments. Food Additiv. Contam. 18 (12): 1124–1129.

ISTA. 1996. International rules for seed testing. Seed Sci. Technol. 21 (1): 25–30.

Janardhana G.R., Raveesha K.A., Shetty H.S. 1998. Modified atmosphere storage to prevent mould-induced nutritional loss in maize. J. Sci. Food Agric. 76 (4): 573–578.

Janardhana G.R., Raveesha K.A., Shetty H.S. 1999. Mycotoxin contamination of maize grains grown in Karnataka (India). Food Chem. Toxicol. 37 (8): 863–868.

Kavitha R., Umesha S., Shetty H.S. 2005. Dose dependent impact of dominant seed-borne fungi on seed germination and seedling vigour of cotton seeds. Seed Res. 33 (2): 187–194.

Koirala P., Kumar S., Yadar B.K., Premarajan K.C. 2005. Occurrence of Aflatoxin in some of the food and feed in Nepal. Indian J. Medical Sci. 59 (8): 331–336.

Krishnamurthy C.D., Lokesh S., Shetty H.S. 2005. Occurrence, transmission and remedial aspects of *Drechslera oryzae* in paddy (*Oryza sativa* L.). Seed Res. 33 (2): 195–200.

Leslie J.F., Summerell B.A. 2006. The *Fusarium* Laboratory Manual. 1st ed. Blackwell Publishing Professional, USA: 1–180.

Lienard V., Seck D., Lognay G., Gaspar C., Severin M. 1993. Biological activity of *Cassia occidentalis* L. against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). J. Stored Products Res. 29 (4): 311–318.

Miller J.D. 1995. Fungi and mycotoxins in grain implications for stored product research. J. Stored Product Res. 31 (1): 1–16.

Mohana D.C., Raveesha K.A., Lokanath R. 2008. Herbal remedies for the management of seed-borne fungal pathogens by an edible plant *Decalepis hamiltonii* (Wight & Arn). Arch. Phytopathol. Plant Protect. 41 (1): 38–49.

Nagamani A., Kunwar I.K., Manoharachary C. 2006. Handbook of Soil Fungi. 1ed. I.K International Pvt. Ltd., New Delhi, 436 pp.

Parekh J., Karathia N., Chanda S. 2006. Evaluation of antibacterial activity and phytochemical analysis of *Bauhinia variegata* L. bark. Afr. J. Biomed. Res. 9: 53–56.

Roberts R.M., Gilbert J.C., Rodewald L.B., Wingrove A.S. 1981. Modern Experimental Organic Chemistry. 3rd ed. Saunders Golden Sunbrust Series, Philadelphia, Holt-Saunders, Japan, Tokyo: 495–506.

Singh J., Tripathi N.N. 1999. Inhibition of storage fungi of blackgram (*Vigna mungo* L.) by some essential oils. Flavour Fragrance J. 14 (1): 1–4.

Varma J., Dubey N.K. 1999. Prospectives of botanical and microbial products as pesticides of tomorrow. Curr. Sci. 76 (2): 172–179.

Wodageneh A., Wulp H.V.D. 1997. Obsolute pesticides in developing countries. Pestic. Information 23 (1): 33–36.

POLISH SUMMARY

WPŁYW WYCIĄGÓW ROŚLINNYCH NA PATOGENICZNE GRZYBY PRZENOSZONE Z NASIONAMI RYŻU UPRAWIANEGO NA POŁUDNIU INDII

Analizie mikologicznej poddano próby nasion 40 odmian ryżu uprawianych w południowych regionach Indii. Zastosowano standardową metodę wykładania nasion na wilgotną bibułę oraz wykładanie nasion na pożywkę agarową Czapek-Dox. Wyniki analizy mikologicznej nasion ryżu wykazały obecność 33 różnych gatunków grzybów należących do 16 rodzajów. Spośród wyizolowanych patogenów dominował gatunek *Drechslera oryzae*, a jego procentowy udział wynosił 82,5%. W dalszej kolejności występowały gatunki *Culvularia lunata* i *Aspergillus Niger*, a ich częstotliwość wynosiła odpowiednio 67,5 i 65,0%. Dane dotyczące zróżnicowania i częstotliwości występowania poszczególnych gatunków grzybów stanowią niewątpliwie, cenną pomoc podczas prognozowania pojawiania się chorób, a także zasięgu ich występowania. W testach *in vitro* oceniano grzybobójcze działanie metanolowych wyciągów z 12 gatunków roślin należących do różnych rodzin przeciwko 8 patogenicznym grzybom: *Alternaria alternata*, *Dreschlera oryzae*, *D. halodes*, *Fusarium moniliforme*, *Pyricularia oryzae*, i *Trichoconis padwickii*, przy użyciu powszechnie stosowanych metod. Wyniki przeprowadzonych testów wykazały, że metanolowe wyciągi z roślin: *Acacia nilotica*, *Ceasalpinia coriaria*, *Decalepis hamiltonii*, *Emblica officinalis*, *Lawsonia inermis* i *Mimosops elengi* wykazały działanie grzybobójcze, w koncentracji 3 500 µg/ml. Porównawcza analiza badanych wyciągów roślin z takimi syntetycznymi fungicydami, jak: bavistin, blitox, kaptan, Dithane M-45 i thiram zastosowanych w zalecanych dawkach, wykazała podobne grzybobójcze działanie metanolowych wyciągów roślinnych z następujących gatunków: *D. hamiltonii*, *L. inermis* i *M. elengi*. Wyciągi z tych roślin można by polecić do zwalczania patogenicznych grzybów przenoszonych z nasionami, a także do zapobiegania biologicznemu rozkładowi nasion w ekologicznych uprawach ryżu.