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In vitro Evaluation of Antifungal Activity of Botanical Extracts Against Post-harvest Fungal Pathogens of Maize (*zea mays* l.)

Shakshi Singh[1]* and Asha Sinha[2]

[1]*College of Agricculture, Powarkheda, Narmadapuram (M.P.)*
[2]*Mycology and Plant Pathology, IAS, Banaras Hindu University, Varanasi, (U.P.), India*
** Corresponding author E-mail: singh.bhu683@gmail.com*

Abstract: The present investigation was conducted to evaluate the occurrence of seed-borne fungal pathogens associated with maize (*Zea mays* L.) variety Vivek 27 and to assess the antifungal efficacy of selected plant extracts against these pathogens under in vitro conditions. Seed mycoflora were detected using agar plate and standard blotter paper techniques. A total of 14 fungal species were isolated, including *Aspergillus niger*, *A. flavus*, *Rhizopus stolonifer*, *Curvularia lunata*, *Alternaria alternata*, *Fusarium moniliforme*, and *Rhizoctonia solani*. Among the isolated fungi, *Aspergillus niger* exhibited the highest values of relative density, relative frequency, and relative abundance by both methods. The antifungal activity of five botanicals, namely neem (*Azadirachta indica*), tulsi (*Ocimum sanctum*), onion (*Allium cepa*), ginger (*Zingiber officinale*), and garlic (*Allium sativum*), was evaluated against the seed-borne fungi at 1:1 (w/v) concentration. Considerable suppression of fungal growth was observed after seed treatment with plant extracts. Garlic and neem extracts showed comparatively greater inhibitory effects against several storage fungi including *Aspergillus*, *Penicillium*, and *Rhizoctonia* species. The findings suggest that plant-based extracts may serve as eco-friendly alternatives to synthetic fungicides for the management of seed-borne pathogens in stored maize.

Keywords: Maize, seed-borne fungi, botanical extracts, antifungal activity, agar plate method, blotter method, biodiversity indices

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereal crops cultivated worldwide because of its high productivity, nutritional value, and wide adaptability under different agro-climatic conditions. In India, maize ranks third after rice and wheat and is utilized for food, feed, fodder, and several industrial applications. Despite its economic importance, maize production and storage are severely affected by seed-borne fungal pathogens. Stored grains are highly susceptible to fungal contamination during storage, resulting in quantitative and qualitative losses. Several storage fungi, particularly species of *Aspergillus*, *Fusarium*, and *Penicillium*, are known to produce mycotoxins that reduce grain quality and pose serious health hazards to humans and animals. Excessive use of chemical fungicides for disease management has created environmental concerns due to toxic residues, development of resistance, and adverse effects on non-target organisms. In recent years, attention has shifted toward eco-friendly and sustainable disease management strategies. Plant-derived extracts have emerged as promising alternatives because they possess natural antifungal compounds, are biodegradable, and are comparatively

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safer for the environment. Several medicinal and aromatic plants have demonstrated inhibitory effects against plant pathogenic fungi.

Therefore, the present study was undertaken with the following objectives:

1. To detect and identify seed-borne fungi associated with maize variety Vivek 27 using agar plate and blotter paper methods.
2. To evaluate fungal diversity using ecological indices.
3. To assess the antifungal potential of selected botanical extracts against seed-borne fungi of maize.

MATERIAL AND METHODS

Collection of seed samples: Maize seeds of variety Vivek 27 were collected from local markets and stored in sterilized cloth bags at room temperature until further use.

Determination of seed Mycoflora

(A) Agar plate method (Muskett, 1948)

Potato Dextrose Agar (PDA) medium was poured aseptically into sterilized Petri-dishes was allowed to cool and settle down. Ten seeds were placed in each Petri-plate containing solidify PDA. There were two replications each having 50 seeds. All the Petri-plates containing seeds were incubated at 25± 1˚C for a week under 12 hours alternating cycles of light and darkness. Fungi growing on seeds were isolated and identified under microscope.

(B) Standard blotter technique(de Tempe, 1953)

The blotting paper was sterilized and then three pieces of sterilized blotting papers in folds moistened with sterilized distilled water were placed in each sterilized Petri dish of 9 cm diameter. Ten seeds were placed equal distance on blotter in each Petri dish.There were two replications each having 50 seeds. The Petri-plates were incubated at 25 ± 1˚C under 12 hours alternating cycle of light and darkness. Plated seeds were observed from time to time for the presence and growth of fungal species on the seeds.

The seed mycoflora were identified with the help of literature (Thom and Raper, 1945; Raper and Thom, 1949; Barnett, 1962; Klich, 2002; Leslie and Summerell, 2008; Visagie *et al.*, 2014; URL 1 and 2).Based on the individuals fungi recorded in the distinct seed samples were analysed for density, frequency, abundance, relative density, relative frequency, relative abundance, importance value index, Simpson index of Dominance, Shannon- Weaver Index of Diversity and evenness. The importance value index of seed sample was determined as the sum of relative frequency, relative density and relative dominance (Curtis and McIntosh, 1950).

Density is calculated by the equation:

$$\text{Density} = \frac{\text{Total number of individuals of a species in all Petri plate}}{\text{Total number of Petri plate studied}}$$

Frequency (%) is calculated by the equation:

$$\text{Frequency (\%)} = \frac{\text{Number of Petri plate in which the species occurred X 100}}{\text{Total number of Petri plate studied}}$$

Abundance- It is the study of the number of individuals of different species in the community per unit area. It is represented by the equation:

$$\text{Abundance} = \frac{\text{Total number of individuals of a species in all Petri plate}}{\text{Total number of individuals of a species in all Petri plate}}$$

Relative density, relative frequency and relative abundance were calculated as:

$$\textbf{Relative density} = \frac{\text{Number of individuals of a species X 100}}{\text{Number of Petri plate studied}}$$

$$\textbf{Relative frequency} = \frac{\text{Number of occurrence of the species X 100}}{\text{Number of occurrence of all the species}}$$

$$\textbf{Relative abundance} = \frac{\text{Total Petri plate of the species X 100}}{\text{Total Petri plate of all the species}}$$

Importance Value Index (IVI)- It was calculated by equation (Burlakoti and Karmacharya, 2004): **IVI = Relative frequency + Relative density + Relative dominance,**

The maximum importance value for any one genus is 300 (100 + 100 + 100). It is useful, as it provides an overall picture of the density, frequency and cover of a genus in relation to community.

Simpson's Dominance Index (D) -The Simpson's index (D) is calculated using the following equation (Simpson, 1949):

$$H' = \sum_{i=1}^{s} \frac{n_i}{n} \ln \frac{n_i}{n}$$

Where 'ni' is the proportion of individuals of the with species in the community. Simpson's index gives relatively little weight to the rare species and more weight to the common species. It weighs towards the abundance of the most common species. It ranges in value from 0 (low diversity) to a maximum of (1-1/s), where s is the number of species. In nature the value of d ranges between 0 and 1. With this, index 0 represents infinite diversity and 1, no diversity. The bigger the (D) value, the smaller the diversity.

Shannon-Wiener Index (H): This is a widely used method of calculating biotic diversity in aquatic and terrestrial ecosystems and is expressed as SWI (Shannon and Weaver, 1963):

$$H' = \sum_{i=1}^{s} \frac{n_i}{n} \ln \frac{n_i}{n}$$

Where, H= index of species diversity s= number of species ni= proportion of total sample belonging to the ith species.

Evenness Index (E): This is relative distribution of individuals among taxonomic groups within a community and is expressed (Pielou, 1966) as:

$$E = H'/\log S$$

Where, H' = Shannon –Wiener diversity index, and log S= Natural log of the total number of species (S defined as **Species Richness**) recorded.

Screening of plant extracts

Abundantly use of synthetic pesticides and commercial antibiotic for human and crop protection is harmful to environment, ecosystem as well as human health. This study was carried out to know the efficacy of different plant extracts on seed-borne fungi of maize. Evaluation of five plants extracts *viz.*, neem, tulsi, onion, ginger and garlic were tested against the seed borne fungi of maize in **Table 1**. Botanicals were collected from the local area and market. Plant extract prepared by macerating leaves /bulb /rhizomes /cloves in ratios weight / volume (1:1) in distilled sterilize water or methanol and termed as standard extract (SE).

Table 1: The particulars of botanicals used for seed treatment is given below

S. No.	*Common name*	*Botanical Name*	*Family*	*Plant parts used*	*Dilutions (w/v)*
	Neem	*Azadirachta indica* A.Juss	Meliaceae	Leaves	1:1
	Tulsi	*Ocimum sanctum* L.	Lamiaceae	Leaves	1:1
	Onion	*Allium cepa* L.	Amary llidaceae	Bulbs	1:1
	Ginger	*Zingiber officinale* Roscoe	Zingi beraceae	Rhizomes	1:1
	Garlic	*Allium sativum* L.	Amary llidaceae	Cloves	1:1

Table 2: Estimation of seed borne fungi of maize variety Vivek 27 by Agar plate method

Fungal species	*Dn*	*F (In %)*	*Ab*	*RD (In %)*	*RF (In %)*	*RA (In %)*	*IVI (In %)*	*D=Pi*Pi*	*H=-{(pi) × ln(pi)}*	*E={H/ln(S)}*
Rhizopus stolonifer	2.900	100	0.163	14.309	10.638	16.292	41.239	0.0189	0.273	0.110
Mucor globosus	1.167	60	0.039	5.756	6.383	3.933	16.072	0.0029	0.157	0.063
Aspergillus flavus	2.700	100	0.152	13.322	10.638	15.169	39.129	0.0170	0.266	0.107
Aspergillus fumigatus	1.625	80	0.073	8.018	8.511	7.303	23.832	0.0063	0.201	0.081
Aspergillus niger	5.100	100	0.287	25.164	10.638	28.652	64.454	0.0462	0.330	0.133
Penicillium notatum	0.600	100	0.034	2.960	10.638	3.371	16.970	0.0032	0.162	0.065
Penicillium versicolor	0.500	60	0.017	2.467	6.383	1.685	10.535	0.0012	0.118	0.047
Alternaria alternata	1.300	100	0.073	6.414	10.638	7.303	24.356	0.0066	0.204	0.082
Curvularia lunata	1.625	80	0.073	8.018	8.511	7.303	23.832	0.0063	0.201	0.081
Fusarium moniliforme	1.000	100	0.056	4.934	10.638	5.618	21.190	0.0050	0.187	0.075
Microdochium fisheri	1.250	40	0.028	6.168	4.255	2.809	13.232	0.0019	0.138	0.055
Rhizoctonia solani	0.500	20	0.006	2.467	2.128	0.562	5.157	0.0003	0.070	0.028

Note: Dn=Density, F= frequency, A= Abundance, RD=Relative Density, RF= Relative frequency, RA= Relative abundance, IVI= Importance value index, D= Simpson index of Dominance, H= Shannon- Weaver Index of Diversity, E= Evenness

Table 3: Estimation of seed borne fungi of maize variety Vivek 27 by Blotter paper method

Fungal species	*Dn*	*F (In %)*	*Ab*	*RD (In %)*	*RF (In %)*	*RA (In %)*	*IVI (In %)*	*D=Pi*Pi*	*H=-{(pi) × ln(pi)}*	*E={H/ln(S)}*
Rhizopus stolonifer	2.900	100	0.158	13.652	9.434	15.761	38.847	0.0168	0.265	0.100
Mucor globosus	1.250	40	0.027	5.885	3.774	2.717	12.376	0.0017	0.132	0.050
Aspergillus flavus	2.900	100	0.158	13.652	9.434	15.761	38.847	0.0168	0.265	0.100
Aspergillus fumigatus	1.500	80	0.065	7.061	7.547	6.522	21.130	0.0050	0.187	0.071
Aspergillus niger	5.000	100	0.272	23.538	9.434	27.174	60.146	0.0402	0.322	0.122
Penicillium expansum	1.000	60	0.033	4.708	5.660	3.261	13.629	0.0021	0.140	0.053
Penicillium notatum	0.600	100	0.033	2.825	9.434	3.261	15.519	0.0027	0.153	0.058
Penicillium versicolor	0.500	60	0.016	2.354	5.660	1.630	9.645	0.0010	0.111	0.042
Alternaria alternata	1.300	100	0.071	6.120	9.434	7.065	22.619	0.0057	0.195	0.074
Curvularia lunata	1.000	100	0.054	4.708	9.434	5.435	19.576	0.0043	0.178	0.067
Fusarium moniliformae	1.625	80	0.071	7.650	7.547	7.065	22.262	0.0055	0.193	0.073
Fusarium oxysporum	0.667	60	0.022	3.138	5.660	2.174	10.973	0.0013	0.121	0.046
Microdochium fisheri	0.500	40	0.011	2.354	3.774	1.087	7.214	0.0006	0.090	0.034
Rhizoctonia solani	0.500	40	0.011	2.354	3.774	1.087	7.214	0.0006	0.090	0.034

Note: Dn=Density, F= frequency, A= Abundance, RD=Relative Density, RF= Relative frequency, RA= Relative abundance, IVI= Importance value index, D= Simpson index of Dominance, H= Shannon- Weaver Index of Diversity, E= Evenness

Table 4: Occurrence of mycoflora on maize seeds after treatment with methanolic plant products by Agar plate method

List of Fungi	*Control*	*Neem*	*Tulsi*	*Onion*	*Ginger*	*Garlic*
Rhizopus stolonifer	+	+	+	+	-	-
Mucor globosus	+	-	+	-	+	-
Aspergillus candidus	-	-	-	-	-	+
Aspergillus flavus	+	-	+	+	-	+
Aspergillus fumigatus	+	+	+	+	+	-
Aspergillus niger	+	+	+	+	-	-
Penicillium notatum	+	-	-	+	+	-
Penicillium rubrum	-	-	-	-	-	+
Penicillium versicolor	+	-	-	-	-	+
Alternaria alternata	+	+	+	+	+	-
Curvularia lunata	+	+	+	+	+	-
Fusarium moniliforme	+	+	+	+	+	+
Microdochium fisheri	+	+	+	+	+	+
Rhizoctonia solani	+	-	+	-	-	-

+ = Present
- = Absent

Table 5: Occurrence of mycoflora on maize seeds after treatment with methanolic plant products by Blotter paper method

List of Fungi	*Control*	*Neem*	*Tulsi*	*Onion*	*Ginger*	*Garlic*
Rhizopus stolonifer	+	+	+	+	-	-
Mucor globosus	+	-	+	-	+	-
Aspergillus candidus	-	+	-	+	-	+
Aspergillus flavus	+	-	+	+	+	+
Aspergillus fumigatus	+	+	+	+	+	-
Aspergillus niger	+	+	+	+	-	-
Penicillium citrinum	-	-	-	-	+	+
Penicillium expansum	+	+	+	-	+	-
Penicillium notatum	+	-	-	+	+	+
Penicillium versicolor	+	-	-	-	-	+
Alternaria alternata	+	+	+	+	+	-
Curvularia lunata	+	+	+	+	+	-
Fusarium moniliformae	+	+	+	+	+	+
Fusarium oxysporum	+	-	+	+	-	+
Fusarium roseum	-	+	+	-	-	-
Microdochium fisheri	+	+	+	+	+	+
Rhizoctonia solani	+	-	+	-	-	-

+ = Present
- = Absent

RESULTS

Estimation of seed borne fungi by Agar plate method

A total of 12 fungal species were isolated from maize variety Vivek 27 *viz., Rhizopus stolonifer, Mucor globosus, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Penicillium notatum, Penicillium versicolor, Alternaria alternata, Curvularia lunata, Fusarium moniliforme, Microdochium fisheri* and *Rhizoctonia solani* by Agar plate method **(Table 2).** Per cent maximum relative density, frequency and abundance values recorded in *Aspergillus niger* (25.164, 10.638 and 28.652) followed by *Rhizopus stolonifer* (14.309, 10.638 and 16.292), *Aspergillus flavus* (13.322, 10.638 and 15.169), *Aspergillus fumigatus* (8.018, 8.511 and 7.303), *Curvularia lunata* (8.018, 8.511 and 7.303), *Alternaria alternata* (6.414, 10.638 and 7.303), *Microdochium fisheri* (6.168, 4.255 and 2.809), *Mucor globosus* (5.756, 6.383 and 3.933), *Fusarium moniliformae* (4.934, 10.638 and 5.618) and *Penicillium notatum* (2.960, 10.638 and 3.371). Whereas, lowest per cent of relative density, frequency and abundance were recorded in *Penicillium versicolor* (2.467, 6.383 and 1.685) and *Rhizoctonia solani* (2.467, 2.128 and 0.562), respectively. Myco-diversity showed Simpson index of dominance (D) values range from 0.0462 to 0.0003. The Shannon-Weiner diversity index (H) value ranges were from 0.330 to 0.070. Pielou's evenness index (E) of myco-flora showed value ranges from 0.133 to 0.028.

Estimation of seed borne fungi by Blotter paper method

A total of 14 fungal species were isolated from maize variety Vivek 27*viz., Rhizopus stolonifer, Mucor globosus, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Penicillium expansum, Penicillium notatum, Penicillium versicolor, Alternaria alternata, Curvularia lunata, Fusarium moniliforme, Fusarium oxysporum, Microdochium fisheri* and *Rhizoctonia solani*by Blotter paper method **(Table 3).** In Blotter paper method, per cent maximum relative density, frequency and abundance values recorded in *Aspergillus niger* (23.538, 9.434 and 27.174) followed by *Rhizopus stolonifer* (13.652, 9.434 and 15.761), *Aspergillus flavus* (13.652, 9.434 and 15.761), *Fusarium moniliformae* (7.650, 7.547 and 7.065), *Aspergillus fumigatus* (7.061, 7.547 and 6.522), *Alternaria alternata* (6.120, 9.434 and 7.065), *Mucor globosus* (5.885, 3.774 and 2.717), *Curvularia lunata* (4.708, 9.434 and 5.435), *Penicillium expansum* (4.708, 5.660 and 3.261), *Fusarium oxysporum* (3.138, 5.660 and 2.174) and *Penicillium notatum* (2.825, 9.434 and 3.261), respectively. However, minimum per cent of relative density, frequency and abundance were recorded in *Penicillium versicolor* (2.354, 5.660 and 1.630) at par with *Microdochium fisheri* (2.354, 3.774 and 1.087) and *Rhizoctonia solani* (2.354, 3.774 and 1.087), respectively by Blotter paper method on maize variety Vivek 27.Diversity of myco-flora in this study calculated using the Shannon-Weiner diversity index (H') value ranges were from 0.322 to 0.090. Myco-diversity showed Simpson index of dominance (D) values range from 0.0402 to 0.0006. Pielou's evenness index (E) of myco-flora showed value ranges from 0.122 to 0.034.

Occurrence of mycoflora on maize seeds after treatment with plant products by Agar plate method

Seeds treatment with five plant products, *viz.* neem, tulsi, onion ginger and garlic were tested against suppression of seed borne fungi of maize seeds variety Vivek 27 by Agar plate method at ratio of 1:1 (w/v) are presented in **Table 4.** Fungi suppressed after seed treatment with neem leaf extract were *Mucor globosus, Aspoergillus flavus, Penicillium notatum, Penicillium versicolor* and *Rhizoctonia solani.* Fungi suppressed after seed treatment with tulsi leaf extract *viz.*, *Penicillium notatum* and

Penicillium versicolor.However, *Mucor globosus, Penicillium versicolor* and *Rhizoctonia solani* were suppressed after seed treatment with onion bulb extract. Ginger rhizomes extract treated maize seeds suppressed *Rhizopus stolonifer, Aspergillus flavus, Aspergillus niger, Penicillium versicolor* and *Rhizoctonia solani*.Garlic cloves extract treated seeds suppressed the *Rhizopus stolonifer, Mucor globosus, Aspergillus fumigatus, Aspergillus niger, Penicillium notatum, Alternaria alternata, Curvularia lunata* and *Rhizoctonia solani.*

Occurence of mycoflora on maize seeds after treatment with plant products by Blotter paper method

Treatment of seeds with five plant products, *viz.* neem, tulsi, onion ginger and garlic were tested against suppression of seed borne fungi of maize seeds variety Vivek 27 by Blotter paper method at ratio of 1:1 (w/v) are presented in **Table 5.** By using Blotter paper method, neem leaf extract treated seeds suppressed *Mucor globosus, Aspergillus flavus, Penicillium notatum, Penicillium versicolor, Fusarium oxysporum* and *Rhizoctonia solani.* The occurrence of two new fungal species were observed after seeds treatment with neem leaf extract by using Blotter paper method *viz*., *Aspergillus candidus* and *Fusarium roseum,* which were absence in control. The fungal species suppressed after treatment with tulsi leaf extract were *Penicillium notatum* and *Penicillium versicolor.* It was observed that the *Fusarium roseum* was observed after seed treatment with tulsi leaf extract, which was absent in control.The fungal species which suppressed after treatment with onion bulb extract were *Mucor globosus, Penicillium expansum, Penicillium versicolor* and *Rhizoctonia solani.*The fungal species suppressed after the treatment with ginger rhizomes extracts were *Rhizopus stolonifer, Aspergillus niger, Penicillium versicolor, Fusarium oxysporum,* and *Rhizoctonia solani*. Both, *Aspergillus candidus* and *Penicillium citrinum* were found after seed treatment with garlic cloves extracts.

DISCUSSIONS

The present investigation revealed that maize seeds were predominantly infected by storage fungi belonging to the genera *Aspergillus*, *Fusarium*, and *Penicillium*. Similar observations have been reported by previous workers who identified these fungi as major contaminants of stored maize grains. The dominance of *Aspergillus niger* and *Aspergillus flavus* observed in this study may be attributed to favorable storage conditions that promote fungal colonization and mycotoxin production. Botanical extracts demonstrated significant antifungal activity against seed-borne fungi. Garlic and neem extracts were particularly effective in suppressing fungal growth, which may be due to the presence of bioactive compounds such as allicin, azadirachtin, and other phenolic metabolites. The findings support earlier reports indicating the antifungal potential of plant-derived extracts against storage fungi and highlight their importance as eco-friendly alternatives to chemical fungicides.

CONCLUSION

The study confirmed the presence of diverse seed-borne fungal pathogens associated with stored maize seeds. *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus stolonifer*, *Alternaria alternata*, and *Curvularia lunata* were the most frequently isolated fungi. Among the tested botanicals, garlic and neem extracts exhibited comparatively higher antifungal activity against major storage fungi. The results suggest that botanical extracts can be used as sustainable and environmentally safe alternatives for managing seed-borne pathogens in maize during storage. Proper drying and storage of maize grains at safe moisture levels are essential for minimizing fungal contamination and maintaining seed quality.

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