

Original Research Article

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***In vitro* Antifungal Potency of Plant Extracts against Post-Harvest Storage Fungal Pathogens of *Zea mays* L.**

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ABSTRACT

Keeping in view the immense importance of seed the present investigation was carried out to detect 14 seed borne mycoflora of maize variety Vivek 27 by using Agar plate method and Blotter paper method. Per cent maximum relative density, frequency and abundance values recorded in *Aspergillus niger* (25.164, 10.638 and 28.652) and (23.538, 9.434 and 27.174) followed by *Rhizopus stolonifer* (14.309, 10.638 and 16.292) and (13.652, 9.434 and 15.761), *Aspergillus flavus* (13.322, 10.638 and 15.169) and (13.652, 9.434 and 15.761), *Aspergillus fumigatus* (8.018, 8.511 and 7.303) and (7.061, 7.547 and 6.522), *Curvularia lunata* (8.018, 8.511 and 7.303) and (4.708, 9.434 and 5.435), *Alternaria alternata* (6.414, 10.638 and 7.303) and (6.120, 9.434 and 7.065) by Agar plate method and Blotter paper method, respectively. 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 at ratio of 1:1 (w/v). Fungi suppressed after seed treatment with selected botanicals were *Rhizopus stolonifer*, *Mucor globosus*, *Aspergillus candidus*, *A. flavus*, *A. fumigatus*, *A. niger*, *Penicillium citrinum*, *P. expansum*, *P. notatum*, *P. versicolor*, *Rhizoctonia solani*, *Alternaria alternata*, *Fusarium oxysporum*, *F. roseum* and *Curvularia lunata*.

Keywords

Seed borne mycoflora,
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Introduction

Demand for food poses major challenges to humankind due to rising population. For facing these challenges humans used enormous amount of chemically synthesized fungicides because of their enormous use, easiness and extreme effectiveness to control plant diseases. Due to their harmful effects on human being as well as soil health, nowadays focus is shifting in the direction of biological methods to manage plant diseases as they have

no adverse consequence on humans as well as environment. Maize (*Zea mays* L.) is the very resourceful crop with wider adaptability in varied agro-ecologies. United States of America, Argentina, China, Brazil, Mexico and India are major maize growing country in world. Maize is the 3rd most significant food crops in India after rice and wheat (the average productions in India are 2.43 t ha⁻¹). It is cultivated for diverse purposes in different parts of the country throughout the year as grain, fodder, green cobs, sweet corn, baby corn, popcorn and industrial purposes etc. The

major maize growing states are Andhra Pradesh (20.9 %), Karnataka (16.5%), Rajasthan (9.9 %), Maharashtra (9.1%), Bihar (8.9%), Uttar Pradesh (6.1 %), Madhya Pradesh (5.7 %) and Himachal Pradesh (4.4 %), which contributes more than 80% of the total maize production (Barupal and Sharma, 2015).

It has got immense potential and hence called as 'Miracle crop' and also called as 'Queen of cereals'. Seeds are a gift of nature, of past generation and diverse culture. It is our inherent duty to protect them and to pass them on to future generation. Deterioration of stored seeds and grains by fungus is a major problem in India. During storage, grains are colonized by various fungal species undergo quantitative and qualitative losses due to mycotoxin production (Wahegaonkar and Shirurkar, 2013 and Ganesh and Patel, 2014).

There are restrictions on use of fungicides and pesticides (Pal and Gardener, 2006). So, it is very essential to develop control measures as alternative to chemicals (Raghavender *et al.*, 2009 and Singh *et al.*, 2014). Plant metabolites and plant based antifungal formulation appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in comparison to the synthetic pesticides (Verma and Dubey, 1999). Some traditionally useful plants have been shown to exhibit fungi-toxic property (Awuah, 1996).

Plant extracts as control measures are cost effective and non-toxic methods. Plant based antifungal formulation are now being used (Omer, 2010). In present investigation, to detect seed borne mycoflora of maize variety Vivek 27 by using Agar plate method and Blotter paper method, and antifungal activity of various plant extracts on growth of seed borne fungi had been carried out.

Materials and Methods

Collection of seed samples

The seed samples were collected from local market and preserved in cloth bags at room temperature during the studies as described by Neergaard (1973).

Determination of seed Mycoflora

Agar plate method (Muskett, 1948)

Sterilized 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 $25 \pm 1^\circ\text{C}$ for a week under 12 hours alternating cycles of light and darkness. Fungi growing on seeds were isolated and identified under microscope.

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 $25 \pm 1^\circ\text{C}$ 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:

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

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

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

Importance Value Index (IVI)

It was calculated by equation (Burlakoti and Karmacharya, 2004):

$$\text{IVI} = \text{Relative frequency} + \text{Relative density} + \text{Relative dominance}$$

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):

$$D = \frac{\sum_{i=1}^s n_i(n_i - 1)}{n(n - 1)}$$

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 n_i = proportion of total sample belonging to the i th 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).

Results and Discussion

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 moniliforme* (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 moniliforme* (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*.

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)
1.	Neem	<i>Azadirachta indica A.Juss</i>	Meliaceae	Leaves	1:1
2.	Tulsi	<i>Ocimum sanctum L.</i>	Lamiaceae	Leaves	1:1
3.	Onion	<i>Allium cepa L.</i>	Amaryllidaceae	Bulbs	1:1
4.	Ginger	<i>Zingiber officinale Roscoe</i>	Zingiberaceae	Rhizomes	1:1
5.	Garlic	<i>Allium sativum L.</i>	Amaryllidaceae	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)}
<i>Rhizopus stolonifer</i>	2.900	100	0.163	14.309	10.638	16.292	41.239	0.0189	0.273	0.110
<i>Mucor globosus</i>	1.167	60	0.039	5.756	6.383	3.933	16.072	0.0029	0.157	0.063
<i>Aspergillus flavus</i>	2.700	100	0.152	13.322	10.638	15.169	39.129	0.0170	0.266	0.107
<i>Aspergillus fumigatus</i>	1.625	80	0.073	8.018	8.511	7.303	23.832	0.0063	0.201	0.081
<i>Aspergillus niger</i>	5.100	100	0.287	25.164	10.638	28.652	64.454	0.0462	0.330	0.133
<i>Penicillium notatum</i>	0.600	100	0.034	2.960	10.638	3.371	16.970	0.0032	0.162	0.065
<i>Penicillium versicolor</i>	0.500	60	0.017	2.467	6.383	1.685	10.535	0.0012	0.118	0.047
<i>Alternaria alternata</i>	1.300	100	0.073	6.414	10.638	7.303	24.356	0.0066	0.204	0.082
<i>Curvularia lunata</i>	1.625	80	0.073	8.018	8.511	7.303	23.832	0.0063	0.201	0.081
<i>Fusarium moniliforme</i>	1.000	100	0.056	4.934	10.638	5.618	21.190	0.0050	0.187	0.075
<i>Microdochium fisheri</i>	1.250	40	0.028	6.168	4.255	2.809	13.232	0.0019	0.138	0.055
<i>Rhizoctonia solani</i>	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)}
<i>Rhizopus stolonifer</i>	2.900	100	0.158	13.652	9.434	15.761	38.847	0.0168	0.265	0.100
<i>Mucor globosus</i>	1.250	40	0.027	5.885	3.774	2.717	12.376	0.0017	0.132	0.050
<i>Aspergillus flavus</i>	2.900	100	0.158	13.652	9.434	15.761	38.847	0.0168	0.265	0.100
<i>Aspergillus fumigatus</i>	1.500	80	0.065	7.061	7.547	6.522	21.130	0.0050	0.187	0.071
<i>Aspergillus niger</i>	5.000	100	0.272	23.538	9.434	27.174	60.146	0.0402	0.322	0.122
<i>Penicillium expansum</i>	1.000	60	0.033	4.708	5.660	3.261	13.629	0.0021	0.140	0.053
<i>Penicillium notatum</i>	0.600	100	0.033	2.825	9.434	3.261	15.519	0.0027	0.153	0.058
<i>Penicillium versicolor</i>	0.500	60	0.016	2.354	5.660	1.630	9.645	0.0010	0.111	0.042
<i>Alternaria alternata</i>	1.300	100	0.071	6.120	9.434	7.065	22.619	0.0057	0.195	0.074
<i>Curvularia lunata</i>	1.000	100	0.054	4.708	9.434	5.435	19.576	0.0043	0.178	0.067
<i>Fusarium moniliforme</i>	1.625	80	0.071	7.650	7.547	7.065	22.262	0.0055	0.193	0.073
<i>Fusarium oxysporum</i>	0.667	60	0.022	3.138	5.660	2.174	10.973	0.0013	0.121	0.046
<i>Microdochium fisheri</i>	0.500	40	0.011	2.354	3.774	1.087	7.214	0.0006	0.090	0.034
<i>Rhizoctonia solani</i>	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
<i>Rhizopus stolonifer</i>	+	+	+	+	-	-
<i>Mucor globosus</i>	+	-	+	-	+	-
<i>Aspergillus candidus</i>	-	-	-	-	-	+
<i>Aspergillus flavus</i>	+	-	+	+	-	+
<i>Aspergillus fumigatus</i>	+	+	+	+	+	-
<i>Aspergillus niger</i>	+	+	+	+	-	-
<i>Penicillium notatum</i>	+	-	-	+	+	-
<i>Penicillium rubrum</i>	-	-	-	-	-	+
<i>Penicillium versicolor</i>	+	-	-	-	-	+
<i>Alternaria alternata</i>	+	+	+	+	+	-
<i>Curvularia lunata</i>	+	+	+	+	+	-
<i>Fusarium moniliforme</i>	+	+	+	+	+	+
<i>Microdochium fisheri</i>	+	+	+	+	+	+
<i>Rhizoctonia solani</i>	+	-	+	-	-	-

+ = 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
<i>Rhizopus stolonifer</i>	+	+	+	+	-	-
<i>Mucor globosus</i>	+	-	+	-	+	-
<i>Aspergillus candidus</i>	-	+	-	+	-	+
<i>Aspergillus flavus</i>	+	-	+	+	+	+
<i>Aspergillus fumigatus</i>	+	+	+	+	+	-
<i>Aspergillus niger</i>	+	+	+	+	-	-
<i>Penicillium citrinum</i>	-	-	-	-	+	+
<i>Penicillium expansum</i>	+	+	+	-	+	-
<i>Penicillium notatum</i>	+	-	-	+	+	+
<i>Penicillium versicolor</i>	+	-	-	-	-	+
<i>Alternaria alternata</i>	+	+	+	+	+	-
<i>Curvularia lunata</i>	+	+	+	+	+	-
<i>Fusarium moniliforme</i>	+	+	+	+	+	+
<i>Fusarium oxysporum</i>	+	-	+	+	-	+
<i>Fusarium roseum</i>	-	+	+	-	-	-
<i>Microdochium fisheri</i>	+	+	+	+	+	+
<i>Rhizoctonia solani</i>	+	-	+	-	-	-
+ = Present						
- = Absent						

Occurrence 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.

Maize seeds were mostly infected by toxin producing fungi such as *Fusarium* spp., *Aspergillus* spp. and *Penicillium* spp. (Mostafa and Kazem, 2011). Frequency occurrence of *Aspergillus niger* and *A. flavus* was very high in maize (Shirurkar and Wahegaonkar, 2013). The most prevalent genus as external seed –borne mycoflora was

Aspergillus spp. (Dawood *et al.*, 2015). The fungi from *Alternaria*, *Fusarium* and *Penicillium* spp. were most frequently isolated from maize grain. These three genera are potential mycotoxin producers (Gulbis *et al.*, 2016). Among the storage fungal pathogens *Aspergillus*, *Fusarium* and *Penicillium* are the most dominant species attacking maize seed. The highest frequency of *Aspergillus* spp. (40.4%) at farmer preserved seed with surface disinfected kernels on agar plate were recorded (Tsedaley and Adugna, 2016). Garlic leaves extract inhibited the growth of 5 fungal species viz. *Aspergillus flavus*, *A. niger*, *A. oryzae*, *Fusarium verticillioides* and *Penicillium* sp. while garlic bulb extract inhibited the growth of 3 fungal species viz. *Aspergillus niger*, *A. oryzae* and *Penicillium* sp. Neem leaves extract showed inhibitory activity against *Aspergillus flavus*, *A. niger*, *A. terreus*, *A. oryzae* and *Penicillium* sp. (Wahegaonkar and Shirurkar, 2013). Garlic extract and neem extract significantly reduce the seed borne fungi of maize (Harris *et al.*, 2001; Irkin and Korukluoglu, 2007; Mossini *et al.*, 2009 and Debnath *et al.*, 2012).

This study indicated that more efficient eco-friendly treatments like botanicals with lesser use of fungicides may provide a better management of the disease.

The infection of seeds due to seed borne fungi and also their toxins are considerable as a threat of human health and animals and by detection of these fungi practical solution to reduce their toxins and hazards. The fungi viz., *Rhizopus stolonifer*, *Aspergillus flavus*, *A. niger*, *A. fumigates*, *Curvularia lunata* and *Alternaria alternata* were most frequently isolated from maize grain in this study. Dry grains to 15% moisture are very important and monitor grain on regular basis during storage to insure that moisture and temperature are maintained at correct levels.

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