Efficacy of herbal powders on seed mycoflora and seed quality of oilseeds

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ABSTRACT

The present study involved seed health testing of four oil seeds (mustard, niger, groundnut and sunflower) for seed-borne fungi and seed vigor, and the evaluation of neem and tulsi leaf powders as seed treatment biofungicides. Standard blotter, agar plate and paper towel methods were employed. All the seed samples tested yielded several seed borne fungi. The herbal powders were screened individually and in combination (1:1) at the concentrations of 2, 5 and 10% by seed treatment. The incidence of seed borne fungi from oilseeds was substantially reduced by neem and tulsi powder on seed treatment in comparison to untreated control seeds. The leaf powders also increased seed germination and seedling emergence with increased shoot and root length. These plant powders could possibly be exploited in the management of seed-borne pathogenic fungi to prevent deterioration of oil seeds and to increase their vigor in an eco-friendly way.

Keywords: Oil seeds, neem, tulsi, leaf powders, fungitoxicity, phytotoxicity, seed treatment, biofungicides

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INTRODUCTION

Oil seeds are the largest source of vegetable oils (Gunstone, 2002) and oils and fats are an important source of energy in the human diet. Oilseeds are also used in animal feed because of their high protein content. These seeds contain energy for the sprouting embryo mainly as oil whereas cereals contain the energy in the form of starch (Lucas, 2000). India is one of the largest producers of oilseeds in the world and oil seeds occupy an important position in the Indian agricultural economy (Madhusudhana, 2013). Indian vegetable oil economy is the fourth largest in the world. The country accounts for 6-7% of vegetable oils production next to U.S.A, China and Brazil (Rai *et al*., 2016). Oil seeds constitute the second largest agricultural commodity after cereals in India occupying 13% of gross cropped area and are cultivated in an area of 25.73 million hectares with a production of 26.67 million tons (Subhash Reddy *et al*., 2016). Nine oil seeds namely groundnut, raspseed-mustard, soybean, sunflower, safflower, sesame, niger, castor and linseed are extensively cultivated in India (Madhusudhana, 2013). India ranks first in castor and sunflower production in the world, second in the production of groundnut and sesame, third in linseed and rapeseed, fifth and sixth in soybean and sunflower, respectively (Subhash Reddy *et al*., 2016).

Despite the rapid increase in cultivated area of the oilseed crops, a discouraging aspect is that the productivity is going down. In recent years the yield levels of the country are recording lowest in the world due to the influence of several biotic and abiotic factors. Among the several biotic limiting factors susceptibility to disease is the major constraint. Several diseases are known to affect oilseed crops resulting in yield loss and many of such diseases are caused by seed borne mycoflora viz., *Aspergillus niger, Aspergillus flavus, Rhizopus* sp., *Fusarium* sp., *Mucor* sp., *Alternaria* sp., *Colletotrichum* sp., *Macrophomina phaseolina, Penicillium* sp., *Botrytis* sp. etc. (Ghosh *et al*., 2018). Seeds in

the field as well as in ill storage conditions undergo deterioration on interaction with several microbes, both qualitatively and quantitatively. Such seeds are not fit for human consumption and are also not acceptable to the industries (Kakde and Chauhan, 2011). Seed borne mycoflora produce mycotoxins and these mycotoxins may get transferred to the oil produced (Bordin *et al*., 2014).

Seed treatment with synthetic fungicides is the oldest, cheapest and most effective means of controlling most seed-borne pathogens (Saroja *et al*., 2012). Even though effective and efficient control of seed-borne fungi can be achieved through the application of synthetic fungicides, chemical fungicides cause serious environmental problems and are toxic to non-target organisms (Ahmad and Zaidi, 2018). Seed treatment with bio-protectants provides an economical and relatively non-polluting delivery system (Ghosh *et al*., 2018). Plants are a rich source of secondary metabolites having antiviral, antibacterial and antifungal activities (Fathima *et al*., 2009; Girish, 2016). Plant derived biofungicides are less phytotoxic, more systematic and easily biodegradable (Essien *et al*., 2008). Neem (*Azadirachta indica* A. Juss) is a well-known medicinal plant native to India and has great potential in the fields of pest management, environment protection and medicine. Neem is reported to have antifungal activity against many phytopathogenic fungi (Girish and Shankara Bhat, 2008). *Ocimum sanctum* L., (holy basil), called tulsi in India, is a plant recognized with its medicinal values since time immemorial (Singh *et al*., 2007). Owing to these aspects plant powders have been successfully used for the management of seed borne fungi (Rochalsk, 2009; Kandhare, 2018).

In the present study, potential of herbal powders of neem and tulsi in inhibiting the seed mycoflora and in improving the seed quality of oilseeds was investigated with the aim of developing an ecofriendly seed treatment strategy.

METRIALS AND METHODS

Isolation of seed borne mycoflora

Random samples of four varieties of oilseeds (mustard, niger, groundnut and sunflower) were collected from local market (Neergaard, 1973). Two standard methods were followed namely Standard Blotter Method (SBM) and Agar plate method (ISTA, 1993) for the isolation of fungi from oilseeds.

The selected seeds were first surface sterilized with distilled water and then immersed in 0.2% sodium hypochlorite solution for 1-2 min., and again rinsed with distilled water and dried for almost 1 min. Sterile blotter papers were placed in sterile 9.0 cm Petri plates and moistened with sterile distilled water to provide moist condition. Ten surface sterilized oilseeds of groundnut and sunflower and 25 surface sterilized oil seeds of mustard and niger were placed at equidistance on moistened filter paper in Petri plates separately.

In Agar plate method, 15 ml of sterilized Potato Dextrose Agar medium (PDA, Himedia) was poured into the sterilized glass Petri plates of 9.0 cm diameter. After solidification of medium, ten surface sterilized oilseeds of groundnut and sunflower and 25 surface sterilized oil seeds of mustard and Niger were placed at equidistance onto the PDA Petri plates separately.

All the plates (standard blotter method and agar plate method) were incubated for a week at 26 ± 2^{o}C under 12 h, alternating cycle of light and darkness. The SBM Petri plates were watered frequently with sterile distilled water to provide moist condition. After incubation period, the number of seeds germinated and number of seeds infected were recorded. The percentage of germination and of infection was calculated by using the formula: % of germination = (Total number of seeds germinated / Total number of seeds plated) X 100; % of infection = (Total number of seeds infected / Total number of seeds plated) X 100.

The fungi occurring on each and every seed in the plates were identified preliminarily on the basis of sporulation characters like sexual or asexual spores with the help of stereoscopic ~~binocular~~ microscope. The identification and

further confirmation of seed borne fungi was made by observing them under compound microscope and with the help of standard manual (Barnett and Barry, 1972).

Preparation of dry leaf powders of neem, tulsi and the seed treatment

Leaf powders of two plants (neem and tulsi) were assessed for their effects on oilseeds by seed treatment. Leaves of neem and tulsi were air dried at room temperature (26 ± 2°C) for 4-5 days. The dried leaves were powdered and preserved in glass bottles separately. The oilseeds were treated with the plant powders at 2%, 5% and 10% concentrations (2g, 5g, 10g / 100g of seeds) with neem, tulsi and combination of neem and tulsi (1:1) powders separately by thorough mixing for 15 min. The treated seeds were left as such overnight. Untreated seeds served as control.

Seed quality testing

The treated seeds were blotted dry and subjected to a germination test by the standard blotter method and paper towel method (ISTA, 1993). One hundred treated and untreated seeds of each oilseed (mustard, niger, groundnut and sunflower) were drawn randomly and were allowed to germinate on moistened blotter papers in Petri plates and between two layers of autoclaved paper towels at 26 ± 2°C for 8 days to investigate the influence of herbal powders on seed quality. The Petri plates and paper towels were watered frequently with sterile distilled water to provide moist condition. After incubation, the number of germinated seeds, number of infected seeds, root length and shoot length were recorded. Seed quality evaluation parameters like percentage germination, percent infection, and vigor index were calculated as per the method advocated by Abdul Baki and Anderson (1973).

RESULTS

Isolation of seed borne mycoflora

All the four oilseed samples (mustard, niger, groundnut and sunflower) procured from the local market showed high incidence of mycoflora. The percentage of germination and infection of the four oilseeds are presented in Table 1.

Table 1. Percentage germination and infection of oilseeds

Oilseeds	No. of seeds plated	No. of seeds germinated	% germination	No. of seeds infected	% infection
Mustard	100	31	31	37	37
Niger	100	22	22	25	25
Groundnut	100	21	21	46	46
Sunflower	100	20	20	44	44

A number of different seed borne fungi were isolated by standard blotter method and by agar plate method. The results of this study indicated the dominance of *Aspergillus* sp., viz *Aspergillus flavus, A. ochraceus* and *A. niger* followed by *Alternaria alternata, Cladosporium cladosporioides, Helminthosporium oryzae, Rhizopus stolonifer, Mucor mucedo, Curvularia lunata* and *Fusarium oxysporum*. Seed mycoflora of mustard showed variation in their composition. *Rhizopus stolonifer, Curvularia lunata, Aspergillus flavus* and *A. niger* were isolated. The prevalence of mycoflora was comparatively low in the niger seeds, *Cladosporium* sp., *Fusarium* sp., and *Mucor mucedo* were isolated. The prevalence of mycoflora was high in the groundnut seeds compared with others. *Aspergillus flavus, A. niger, A. ochraceus, Rhizopus stolonifer, Mucor mucedo* and *Alternaria alternata* were isolated from the seeds. Sunflower seeds yielded *Aspergillus niger, A. ochraceus, Rhizopus stolonifer* and *Helminthosporium* sp.

Effect of neem herbal powder treatment

Oilseeds treated with neem powder exhibited significant inhibition of the fungal growth and enhanced percentage of germination (Fig. 1; Table 2).

Table 2. Effect of herbal powders on seed quality of oilseeds

Oilseed	Con (%)	No. of seeds plated	Herbal powder from neem			Herbal powder from tulsi			Mixture of neem and tulsi (1:1)		
			Ger (%)	Inf (%)	V.I	Ger (%)	Inf (%)	V.I	Ger (%)	Inf (%)	V.I
Mustard	0	100	22	38	3011.2	22	38	3011.2	22	38	3011.2
	2	100	36	19	5101.1	31	22	4887.2	33	20	5026.4
	5	100	59	10	8229.9	51	13	6729.5	57	11	8010.2
	10	100	70	06	14333.3	68	09	11333.0	69	06	11828.5
Niger	0	100	18	32	2512.0	18	32	2512.0	18	32	2512.0
	2	100	18	16	3620.0	25	16	397.0	26	18	3603.0
	5	100	33	09	6886.0	48	08	6683.0	50	05	9960.0
	10	100	56	04	10210.0	57	08	9872.0	50	09	8940.0
Groundnut	0	100	20	45	2232.9	20	45	2232.9	20	45	2232.9
	2	100	34	30	3546.4	29	27	3103.7	30	29	3228.7
	5	100	55	17	6453.7	51	18	6176.4	54	19	6273.5
	10	100	72	08	10010.1	67	11	9848.6	68	07	9933.1
Sunflower	0	100	22	39	2058.0	22	39	2058.0	22	39	2058.0
	2	100	31	29	2673.4	28	27	2326.3	31	29	2413.2
	5	100	51	18	4226.3	45	15	4103.9	49	17	4213.7
	10	100	70	09	9567.3	64	09	8339.3	67	07	8972.3

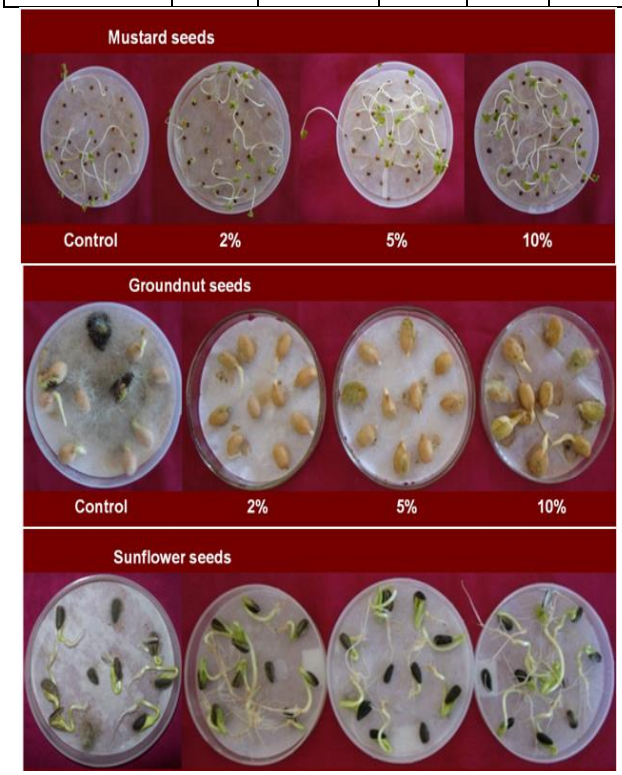

Figure 1. Effect of neem herbal powder on the germination and fungal infection of oilseeds at 2, 5 and 10% concentrations

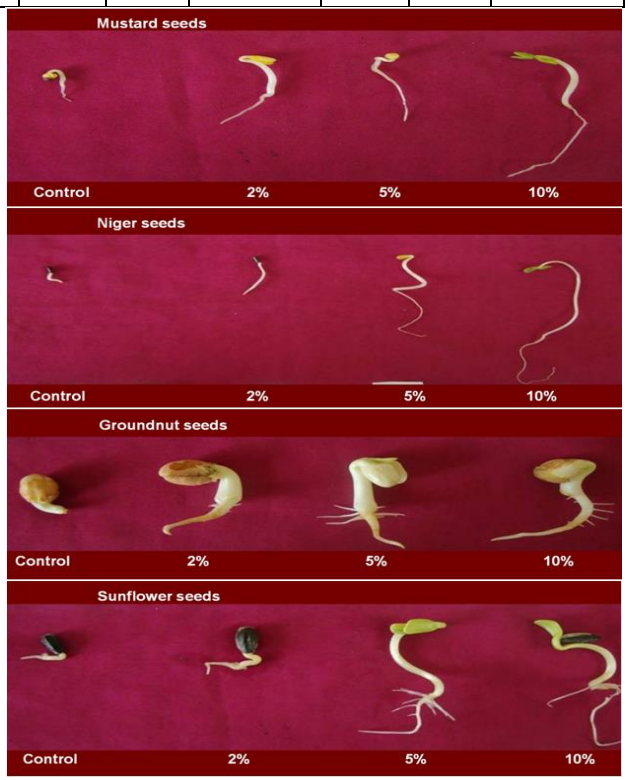

Figure 2. Effect of neem herbal powder on the vigor index of oilseeds at 2, 5 and 10% concentrations

The vigor index of all the oilseeds was also enhanced by the neem herbal powder on treatment (Fig. 2, Table 2). After incubation period, in 10% concentration treatment, comparatively highest germination and seedling vigour index were recorded in mustard seeds followed by groundnut, sunflower and niger seeds. Reduction of fungal infection was 84%, 82%, 81% and 79% in 10% concentration treated mustard, niger, groundnut and sunflower seeds respectively in comparison to the untreated control seeds.

Effect of tulsi herbal powder treatment

Oilseeds treated with tulsi powder showed substantial inhibition of the fungal growth and improved percentage of germination (Fig. 3, Table 2). Oilseeds also exhibited improved vigor index on treatment with tulsi herbal powder (Fig. 4, Table 2). At 10% concentration treatment, after incubation, comparatively highest germination and seedling vigour index

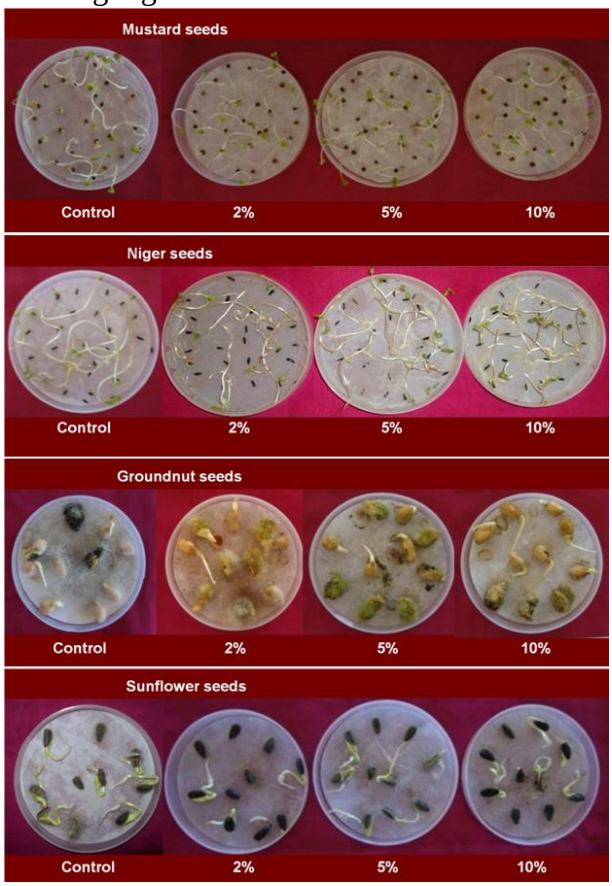

Figure 3. Effect of tulsi herbal powder on the germination and fungal infection of oil seeds at 2, 5 and 10% concentrations.

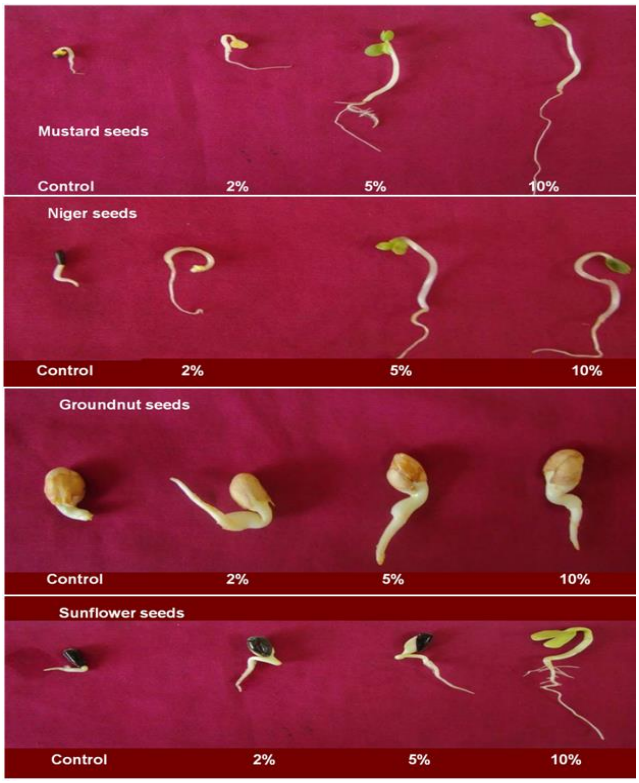

Figure 4. Effect of tulsi herbal powder on the vigor index of oil seeds at 2, 5 and 10% concentrations.

was observed with mustard seeds followed by groundnut, sunflower and niger seeds. The fungal infection was reduced by 76%, 75%, 76% and 77% in treated mustard, niger, groundnut and sunflower seeds respectively at 10% concentration in comparison to the untreated control seeds.

Effect of herbal powder of neem and tulsi in combination (1:1)

Combination of neem and tulsi powders (1:1) significantly inhibited the fungal growth from all the four oilseeds on treatment in addition to increasing the percentage of germination (Fig. 5, Table 2). The mixture also enhanced the vigor index of all the treated oilseeds (Fig. 6; Table 2). After incubation period, in 10% concentration treatment, comparatively highest germination (69%) and seedling vigour index (11828.5) were recorded in mustard seeds followed by groundnut (68% and 9933.1, respectively) and sunflower (67% and 8972.3, respectively). However in niger seeds the highest germination (55%) and seedling vigour index (9960.0) were observed at 5% concentration.

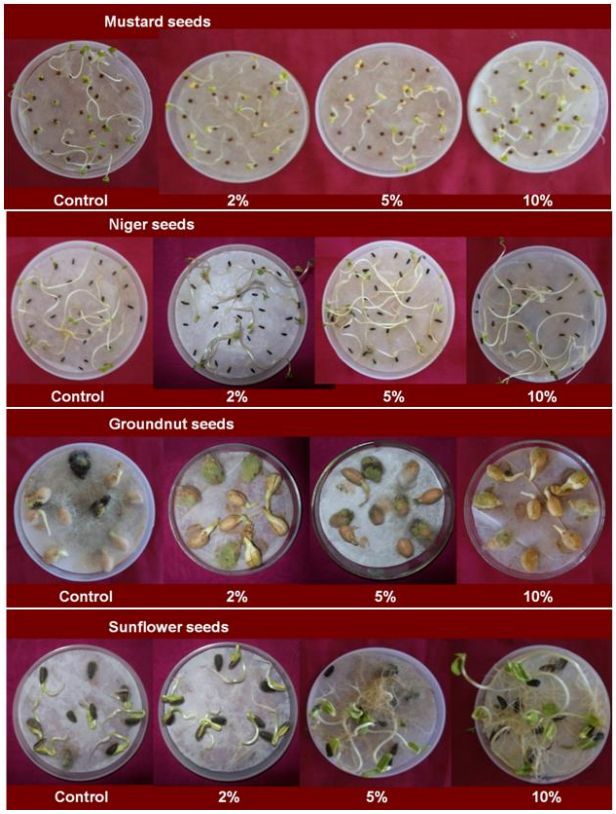

Figure 5. Effect of mixture of neem and tulsi herbal powders (1:1) on the germination and fungal infection of oil seeds at 2, 5 and 10% concentrations.

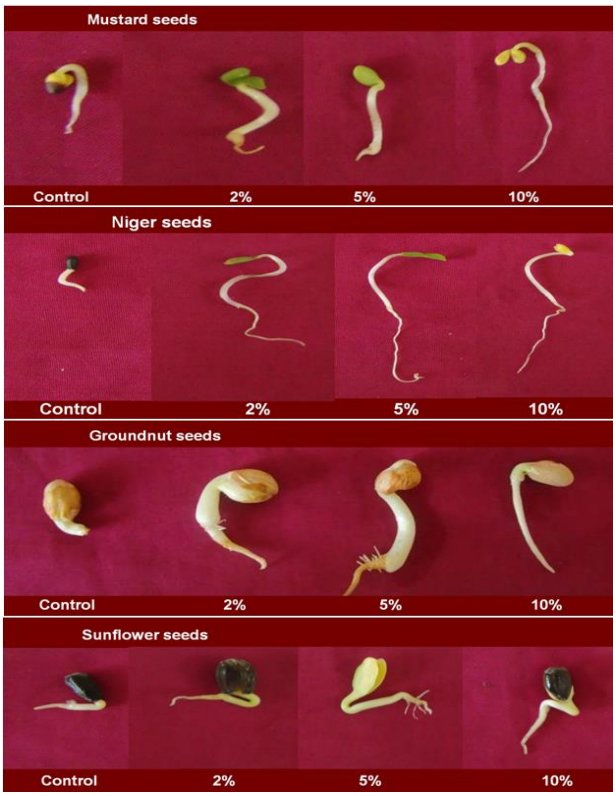

Figure 6. Effect of mixture of neem and tulsi herbal powders (1:1) on the vigor index of oil seeds at 2, 5 and 10% concentrations.

Reduction of fungal infection was 84%, 72%, 84% and 82% in 10% concentration treated mustard, niger, groundnut and sunflower seeds respectively in comparison to the untreated control seeds.

DISCUSSION

The farmer requires healthy quality seeds with a high percentage of germination and purity, free from pathogens to achieve qualitative and quantitative increase in the production of oilseeds (Ghosh *et al*., 2018). Fungi associated with seeds as contaminants cause seed abnormalities, poor germination and seedling damage resulting in development of disease reducing the yields (Ahmad and Zaidi, 2018). This makes it imperative that the seeds must be tested and treated before they are sown in the field (Ghosh *et al*., 2018).

In the present study four oilseeds (mustard, niger, groundnut and sunflower) were considered for treatment with leaf powders of neem and tulsi. The seeds were screened for mycoflora by standard blotter and agar plate methods and a total of eight fungal genera were isolated from these oilseeds. Standard blotter and agar plate methods are recommended by ISTA for the isolation and detection of seed-borne mycoflora. Out of these fungal genera three species were of *Aspergillus*. Among the oilseeds the percentage of contamination in groundnut seed was high. Seed borne mycoflora of oilseeds were previously reported from different parts of the world (Hassan *et al*., 2015; Ghosh *et al*., 2018). The occurrence of storage fungal genera such as *Aspergillus* on seed indicates that the seeds have become contaminated during storage and this can cause low germination in seeds (Shakir and Mirza, 1992). These fungi also produce toxic metabolites that result in reduction of shoot and root elongation (Jain and Pathak, 1996). This indicates that the seed treatment is very important before sowing or storage.

The efficacy of herbal powders (neem and tulsi) against major seed borne fungi of four oilseeds was tested *in-vitro*. The results showed that the two plant leaf powders significantly inhibited the growth of all fungi

with inhibition percentage varying from one powder to another and with concentrations of powders. Comparatively, percentage inhibition of growth of the fungi was highest by neem powder and by mixture (neem + tulsi powders 1:1) and was low by tulsi powder. Plant leaf powder treatments significantly enhanced seed germination and seedling vigor of oilseeds when compared to control. The plant powders used in this study were not phytotoxic even at the higher concentrations tested. The leaf powders rather improved seed germination and seedling emergence of oilseeds significantly, more than the untreated control seeds. The ability of the leaf powder to increase seed germination and seedling emergence could be attributed to the suppression of the incidence of the seed borne fungi that could have killed the embryo of the seeds. Neem and tulsi plant products have been effectively employed for seed treatment against seed borne fungi. Shafique *et al*. (2007) recommended that aqueous extracts of *A. indica* could be used to treat the wheat grains for 10 min., before sowing or storage to reduce the fungal incidence. Treatment of groundnut seeds with neem seed powder was found to be effective for the control of seed borne *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus* sp., (Hassan *et al*., 2015). Black gram seeds treated with *Ocimum basilicum* leaf powder showed less seed mycoflora and increased seed germination than in untreated seeds. Shoot and root lengths were more in treated seeds than in untreated ones (Kandhare, 2018). Evidences from the current and other studies thus show that the neem and tulsi leaf powder and their combinations could be implemented to control seed mycoflora of oilseeds and to enhance their seedlings quality.

The present investigations revealed that neem and tulsi leaf powders have the potential to protect the oilseeds (mustard, sesame, niger, sunflower and groundnut) against fungal infection. Neem and tulsi leaf powders and their synergistic effect enhanced seed germination and vigor index of the oilseeds. These results indicate that both of the leaf powders tested have effective fungitoxicity without phytotoxicity, although their mode of action is not understood very well. From the results of the present study it can be inferred that the herbal powder from neem and tulsi can be used as a natural seed treatments and preservatives for oilseeds. However, additional research is suggested to determine the potential usefulness. The use of botanicals, which are easily available, cost effective and non hazardous, is highly preferred in the recent times for the effective management of seed borne mycoflora and improvement of seed quality.

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