Environment and Ecology 41 (3D) : 2175—2180, July—September 2023
Article DOI: https://doi.org/10.60151/envec/AVJF4082
ISSN 0970-0420

Effect of Essential Oil against Seed Associated Mycoflora of Wheat Seeds

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Received 22 February 2023, Accepted 12 June 2023, Published on 20 September 2023

ABSTRACT

In the present investigation wheat seed samples were collected from farmers of Bihar and mycoflora associated with wheat seed samples were isolated by standard blotter paper and towel paper methods. Mycoflora i.e. *Bipolaris sorokiniana, Fusarium moniliforme, Alternaria triticina, Aspergillus* sp. and *Penicillium* sp. were isolated from wheat seed samples. Different essential oils (Citronella, Eucalyptus, Lemon grass, Clove and Neem oil) were tested *in-vitro* against isolated pathogens from farmers saved wheat seed samples at different concentrations i.e. 100 ppm, 200 ppm and 300 ppm by "poisoned food technique". Lemongrass oil has shown highest percent inhibition of growth (67.74, 67.74 and 68.81%) among different essential oil tested against *Bipolaris sorokiniana* at all three concentrations 100, 200 and 300 ppm respectively. Neem oil found highest percent inhibition of growth (67.56, 71.17 and 73.87%) among different essential oils tested against *Fusarium moniliforme, Aspergillus* sp. (60.60, 67.67 and 67.67%) and *Penicillium* sp. (38.33, 41.66 and 50%) at all three concentrations 100, 200 and 300 ppm respectively. Eucalyptus oil has shown highest percent inhibition of growth (58.33, 64.58 and 68.75%) among different essential oils tested against *Alternaria triticina* at all three concentrations 100, 200 and 300 ppm respectively.

Keywords Wheat seed, Mycoflora, Essential oil, *In vitro* evaluation.

INTRODUCTION

Seed-borne diseases are major threat to crop production and yield in almost all cultivated crops across the globe by causing pre and post-infections. These have been found to affect the growth and productivity of crop plants (Rabie *et al*. 1997). Diseases, especially caused by seed borne fungal agents, are among the main factors reducing yield and quality of wheat. Wheat crop is affected by approximately 120 different diseases, among them, 42 diseases are seed borne and 35 diseases are caused by fungi (Hasan *et al*. 2005).

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Coincidentally, seed borne diseases of wheat are more destructive in nature and it occurs worldwide. Seeds provide natural substrate for the growth of fungi gets associated with externally or internally or both to the seeds. Fungi associated with seeds as contaminant can cause seed abnormalities, poor germination as well as seedling damage resulting in development of disease at later stages of plant growth by systemic or local infection (Bateman and Kwasna 1999, Khanzada *et al*. 2002). For better crop improvement there is need to produce healthy and disease free seeds.

A number of chemical agents are used to control these pathogens, fungicides are being used in the form of dusting, slurry and soaking treatment (Agrios 1997). Seed treatment is the safest and cheapest practice to control seed-borne fungal pathogens (Chandler 2005). Because of the negative side effects of the fungicides on environment and human health, alternative chemicals or control methods gained importance. Especially in organic agriculture, use of safer chemicals is essential. Plant extracts and essential oils, as safer natural chemicals (Suzuki *et al*. 2015) have long been known to have antimicrobial properties and used to control some plant diseases. (Geraldo *et al*. 2010, Bhardwaj *et al*. 2014). There are also some studies on their use against seed borne fungal diseases of some plant species (Marinelli *et al*. 2012, Ahmed *et al*. 2013). Farmers saved wheat seeds can be infected by many seed borne pathogens. Seed associated mycoflora and seed borne diseases are mainly responsible for low yield and production of poor quality wheat seeds in Bihar. In view of the above facts and importance of seed associated mycoflora, In the current study, effect of Citronella, Eucalyptus, Lemon grass, Clove and Neem oil on the isolated mycoflora from farmers saved wheat seeds was investigated.

MATERIALS AND METHODS

Collection of wheat seed samples

Total 70 seed samples of different variety were collected from the farmers of different region of Bihar state. The collected seed samples were kept in cloth bag and stored in a well ventilated room of the laboratory in Department of Plant Pathology, RPCAU, Pusa, Bihar, India.

Isolation and identification of seed mycoflora associated with wheat seed samples

Seventy seed samples of wheat collected from different locations were used for the isolation of mycoflora by using (i) Standard blotter paper method (ii) Standard agar plate method and (iii) Standard paper towel method (ISTA 2001) were employed for this purpose. After 24 - 48 hrs, fragments of hyphal tip from the growing point were transferred to fresh PDA slants for pure culture preparation. Pure culture was maintained on PDA media slants for further studies.

Evaluation of different botanicals oil against isolated pathogens

Different botanicals oils Citronella, Eucalyptus, Lemon grass, Clove and Neem oils was tested in *in-vitro* against isolated pathogens at different concentrations by poison food technique. Botanical oils were evaluated at 100ppm, 200 ppm and 300 ppm concentrations. Desired concentrations (100 ppm, 200 ppm and 300 ppm) were prepared by thoroughly mixing in sterilized PDA media. These mixtures were poured in to Petri plates aseptically and allowed to solidify. Then the Petri plates were inoculated with 5.0 mm disc of culture by using cork borer. Control plate was inoculated with 5.0 mm disc of culture without adding oil in PDA. Three replications were maintained for each treatment. Inoculated plates were incubated in the BOD at 28±1°C. Observations were recorded after 48 hrs of incubation. Inhibition percentage was calculated by using formula:

$$\text{Inhibition percentage} = \frac{(C-T)\times 100}{100}$$

Where,

C = Radial growth of pathogen in mm in control plate.

T = Radial growth of pathogen in mm in treated plate.

RESULTS

The seed borne mycoflora associated with wheat

seeds were isolated by standard incubation techniques and isolated by culturing on potato dextrose agar medium in petri plates. The plates were incubated at 28±1°C for the fungal growth. Pure cultures thus obtained were maintained for further investigations. The seed borne mycoflora was identified on the basis of cultural and morphological characters were confirmed as *Bipolaris sorokiniana, Fusarium moniliforme, Alternaria triticina, Penicillium* sp., and *Aspergillus* sp. The efficacies of different essential oils were tested against isolated pathogen from wheat seeds samples collected from farmers.

Evaluation of different essential oil against isolated pathogens

Different essential oils (Citronella oil, Eucalyptus oil, Lemon grass oil, Clove oil and Neem oil) were tested in *in-vitro* against isolated pathogens at different concentrations i.e. 100 ppm, 200 ppm and 300 ppm by "poisoned food technique". After 48 hrs of incubation, observations on radial growth of pathogens were recorded and percent inhibition of growth were calculated.

Effect of different essential oils against Bipolaris sorokiniana

Table 1. Effect of different essential oils against *Bipolaris sorokiniana.*

Essential oils	Average growth of *Bipolaris sorokiniana* in mm at different concentration of essential oils					
	100 ppm	I	200 ppm	I	300 ppm	I
Citronella	15.66	49.46	13.00	58.06	12.33	60.21
Eucalyptus	12.33	60.21	12.33	60.21	10.66	65.59
Lemon-grass	10.00	67.74	10.00	67.74	9.66	68.81
Clove	14.33	53.76	14.00	54.83	10.66	65.59
Neem	19.00	38.71	14.33	53.76	12.66	59.14
Control	31.00	0.00	31.00	0.00	31.00	0.00
Factors			CD at 5%		SEm±	
Essential oils (B)			1.09		0.38	
Concentrations (A)			0.77		0.26	
Interaction (A X B)			1.89		0.65	

I = Percentage inhibition over control.

Table 2. Effect of different essential oils against *Fusarium moniliforme.*

Essential oils	Average growth of *Fusarium moniliforme* in mm at different concentration of essential oils					
	100 ppm	I	200 ppm	I	300ppm	I
Citronella	21.00	43.24	18.33	50.45	15.00	59.45
Eucalyptus	13.33	63.96	12.33	66.66	10.33	72.07
Lemon-grass	13.00	64.86	13.00	64.86	11.00	70.27
Clove	14.33	61.26	14.33	61.26	13.66	63.06
Neem	12.00	67.56	10.66	71.17	9.66	73.87
Control	37.00	0.00	37.00	0.00	37.00	0
Factors			CD at 5%		SEm±	
Essential oils (B)			1.1		0.38	
Concentrations (A)			0.78		0.27	
Interaction (A X B)			1.92		0.66	

I = Percentage inhibition over control.

Total five essential oils were tested against *B. sorokiniana* isolated from wheat seed samples. The results were presented in the Table 1, showed that at all three concentrations100, 200 and 300 ppm, Lemongrass oil recorded highest GI (%) i.e. 67.74 %, 67.74 % and 68.817 % respectively, followed by clove (65.54%) and eucalyptus (65.59%), and citronella oil (60.2%) at 300 ppm concentration. The neem oil was found least effective at 300 ppm concentration.

Effect of different essential oils against Fusarium moniliformae

Total five essential oils were tested against *F. moniliformae* isolated from wheat seed samples. The results were presented in the Table 2, showed that at all three concentrations100, 200 and 300 ppm, Neem oil showed highest GI (%) i.e. 67.568%, 71.171% and 73.874% respectively, followed by eucalyptus (72.07), lemon grass (70.27) and clove oil (63.06) at 300 ppm concentration. The citronella oil was found least effective at all concentration.

Effect of different essential oils against Alternaria triticina

Total five essential oils were tested against A. triticina isolated from wheat seed samples. The results were presented in the table 3, showed that at all three

Table 3. Effect of different essential oils against *Alternaria triticina.*

Essential oils	Average growth of *Alternaria triticinain* mm at different concentration of essential oils					
	100 ppm	I	200 ppm	I	300 ppm	I
Citronella	29.00	9.37	24.33	23.95	20.00	37.5
Eucalyptus	13.33	58.33	11.33	64.58	10.00	68.75
Lemongrass	19.00	40.62	16.33	48.95	14.66	54.16
Clove	15.33	52.08	13.66	57.29	11.33	64.58
Neem	16.66	47.91	13.33	58.33	11.00	65.62
Control	32.00	0.00	32.00	0.00	32.00	0.00

Factors	CD at 5%	SEm±
Essential oils (B)	1.36	0.47
Concentrations (A)	0.96	0.33
Interaction (A X B)	2.36	0.82

I = Percentage inhibition over control.

concentrations100, 200 and 300 ppm, Eucalyptus oil showed highest GI(%) as 58.33%, 64.58% and 68.75% respectively, followed by neem (65.62%), clove (64.58%) and lemon grass (54.16%). The citronella oil was found least effective at all concentrations.

***Effect of different essential oils against Penicillium* sp.**

Total five essential oils were tested against *Penicillium* sp., isolated from wheat seed samples. The results were presented in the Table 4, showed that at all three concentrations 100, 200 and 300 ppm, Neem oil recorded highest GI (%) i.e. 38.33%, 41.66% and 50.0% respectively, followed by clove (46.66%) and citronella (46.66%) and lemon grass oil (45.00%). The eucalyptus oil was found least effective at all concentrations.

Table 4. Effect of different essential oils against *Penicillium sp.*

Essential oils	Average growth of *Penicillium sp*. in mm at different concentration of essential oils					
	100 ppm	I	200 ppm	I	300 ppm	I
Citronella	13.33	33.33	12.33	38.33	10.66	46.66
Eucalyptus	15.33	23.33	13.33	33.33	11.33	43.33
Lemongrass	14.00	30.00	13.00	35.00	11.00	45.00
Clove	14.33	28.33	13.00	35.00	10.66	46.66
Neem	12.33	38.33	11.66	41.66	10.00	50.00
Control	20.00	0.00	20.00	0.00	20.00	0.00

Factors	C.D. at 5%	SEm±
Essential oils (B)	0.72	0.25
Concentrations (A)	0.51	0.17
Interaction (A X B)	1.26	0.43

I = Percentage inhibition over control.

Table 5. Effect of different essential oils against *Aspergillus sp.*

Essential oils	Average growth of *Aspergillus sp*.in mm at different concentration of essential oils					
	100 ppm	I	200 ppm	I	300 ppm	I
Citronella	28.66	13.13	27.00	18.18	23.66	28.28
Eucalyptus	26.33	20.2	19.66	40.40	13.66	58.58
Lemongrass	24.33	26.26	23.33	29.29	21.33	35.35
Clove	18.33	44.44	17.33	47.47	17.33	47.47
Neem	13.00	60.6	10.66	67.67	10.66	67.67
Control	33.00	0.00	33.00	0.00	33.00	0.00

Factors	CD at 5%	SEm±
Essential oils (B)	1.11	0.38
Concentrations (A)	0.78	0.27
Interaction (A X B)	1.93	0.67

I = Percentage inhibition over control.

***Effect of different essential oils against Aspergillus* sp.**

Total five essential oils were tested against *Aspergillus* sp., isolated from wheat seed samples. The results were presented in the Table 5, showed that at all three concentrations 100, 200 and 300 ppm, Neem oil recorded highest GI (%) i.e. 60.60%, 67.67%, 67.67% respectively, followed by eucalyptus (58.58%), clove (47.47%) and lemon grass oil (35.35%) at 300 ppm concentration. The citronella oil was found least effective at all concentrations.

DISCUSSION

Seed plays an important role in the healthy and disease free crops production. Many important seed borne diseases are transmitted by seeds and it serves the carrier of many diseases, which causes considerable yield losses of many crops. Wheat (*Triticum aestivum*

L.) is one of the most important cereal food crops. Wheat seeds carry number of fungal pathogens, they causes many seed borne diseases in field and also causes adverse effect on seed germination, seedling vigour, plant growth and yield.

In the present investigations, total 70 seed samples of different variety were collected from the farmers of different villages of Samastipur and Muzaffarpur districts of Bihar state to study the health and quality of wheat seeds. Also to isolate and identify the seed mycoflora associated with seeds by different methods and their management by using essential oils.

In the present investigation different essential oils (Citronella oil, Eucalyptus oil, Lemon grass oil, Clove oil and Neem oil) were tested in *in-vitro* against isolated pathogens at different concentrations at 100ppm, 200 ppm and 300 ppm by "poisoned food technique". Lemongrass oil has shown highest percent inhibition of growth among different essential oils against *Bipolaris sorokiniana* at all three concentrations 100, 200 and 300 ppm. Neem oil has shown highest percent inhibition of growth among different essential oils against *Fusarium moniliforme, Aspergillus* sp. and *Penicillium* sp. at all three concentrations 100, 200 and 300 ppm. Eucalyptus oil has shown highest percent inhibition of growth among different essential oils against *Alternaria triticina* at all three concentrations 100, 200 and 300 ppm.

Sitara *et al*. (2008), also studied the antifungal activity of asafoetida (*Ferula asafoetida*), black cumin seed (*Nigella sativa*), neem (*Azadirachta indica*) and mustard (*Brassica campestris*) extracted essential oils against isolated eight fungi viz., *Aspergillus niger, A. flavus, Fusarium moniliforme*, *F. oxysporum*, *F. nivale*, *F. semitectum, Alternaria alternata, Drechslera hawiensis*. All the tested oils extract found effective, showed fungicidal activity of varying degree against tested species of fungus except mustard oil extract, which is similar to our findings.

In the present study, five fungus were recorded, which is found to associated with seeds i.e., *Bipolaris sorokiniana, Fusarium moniliforme, Alternaria triticina, Penicillium* sp., and *Aspergillus* sp. Tonu *et al.* (2017) in studied the health and quality of farmer saved wheat seeds. They recorded thirteen fungi in farmers' saved seed sample. The 5 major pathogenic fungi identified were *B. sorokiniana, A. tenuis*,*C. lunata*, *F. oxysporum* and *A. flavus*. This is also confirming to our findings.

It was found that seed-borne fungi from the seeds may be destructive during germination of seeds or may be bringing about mortality soon after the emergence of seedlings, so it is desirable that seeds should be tested for seed health before planting. Management of seed-borne fungi was done to test the efficacy of essential oil *in vitro*. It was found that colony growth of this fungus inhibited significantly. The application of essential oil to the seed is a common and effective means of controlling the majority of seed associated mycoflora of wheat seeds.

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