Int J Pharm Bio Sci 2014 Jan; 5(1): (B) 765 - 772

Research Article **Microbiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

EVALUATION OF ANTAGONISTIC POTENTIALITY OF SOME NATURAL PLANT EXTRACTS AGAINST *FUSARIUM OXYSPORUM* F. SP. *LINI*

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ABSTRACT

The effectiveness of aqueous extract of seven locally available medicinal plants i.e. *Trachyspermum ammi* (seeds), *Zingiber officinale* (rhizome), *Tinospora cordifolia* (leaves), *Cymbopogon citratus* (leaves), *Moringa oleifera* (bark) were evaluated against the plant pathogenic fungus i.e. *Fusarium oxysporum* f. sp. *lini* (causing wilt in linseed). The plant extracts were screened for their antagonistic property against the mycelial growth of the *Fusarium oxysporum* f. sp. *lini* at four different concentrations i.e. 10, 25, 50 and 75 percent by using poisoned food technique. The results of this study have shown that all the five plant extracts significantly ($p \leq 0.01$) inhibited the growth of the test pathogen. It was observed that *Tinospora cordifolia* (leaves), *Cymbopogon citratus* (leaves) and *Moringa oleifera* (bark) completely inhibited the growth of pathogen at 75% concentration whereas *Zingiber officinale* (rhizome) caused 89.65% inhibition followed by *Trachyspermum ammi* (seeds) (82.50%) on the eighth day of inoculation.

KEY WORDS: **Antagonistic activity, *Fusarium oxysporum* f. sp. *lini*, Plant extracts**

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INTRODUCTION

At present due to the vast increase in the population, the agriculture is now focusing to increase the food production. However, as a result of pest infection, the yield is decreasing. In order to solve this problem synthetic chemicals are widely used to control the infectious diseases. But the frequent and indiscriminate use of chemical pesticides has posed serious threat to the environment. It has led to fungicide resistance (13,2) and are health hazardous (12). Synthetic chemicals are potentially toxic and have various side effects on host (6) due to which the demand of ecofriendly method has increased. The use of plant extract for disease control has been studied as they are environmental friendly (10). Medicinal plants are gifts of nature to humans and they are being used to control the various infectious diseases (9). The aim of the present study was to test the antifungal efficacy of some natural plant extracts against *Fusarium oxysporum* f. sp. *lini*.

MATERIALS AND METHODS

Sampling of wilted linseed plant

The diseased linseed plant (wilted) was obtained from the linseed agricultural field from the vicinity of Sitapur area, Sidhuali, Uttar Pradesh. The present experimental work was conducted in the Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow.

Isolation of Pathogen

The pathogenic fungus i.e., *Fusarium oxysporum* f. sp. *lini* was isolated from the infected root and shoot of the wilted linseed plant. The wilted linseed plant was washed under the running tap water to remove the adhered soil from the upper parts of the crop plants. The infected plant part was cut into small pieces and was surface sterilized with 2% sodium hypochlorite solution and then were washed thoroughly with distilled water for many times to remove sodium hypochlorite. The cut pieces were dried on sterilized filter paper. The pieces were inoculated onto sterilized and cooled down Czapek's-Dox agar plates. The inoculated plates were incubated at 25±2^{0}C. The identified fungal pathogen was purified and maintained on agar slants for the further studies.

Collection of plants and plant parts

The medicinal plant or plant part i.e. *Moringa oleifera* (bark), *Zingiber officinale* (rhizome) and *Trachyspermum ammi* (seeds) were collected from the south city areas of Lucknow. *Tinospora cordifolia* leaves were collected from the Babasaheb Bhimrao Ambedkar University, Lucknow campus area whereas *Cymbopogon citratus* leaves were collected from the CIMAP, Lucknow.

Preparation of aqueous extract

About 200g of each sample (*Trachyspermum ammi* (seeds), *Zingiber officinale* (rhizome), *Tinospora cordifolia* (leaves), *Cymbopogon citratus* (leaves) and *Moringa oleifera* (bark)) were washed under running tap water followed by distilled water to remove dirt from the upper surface of the samples. The samples were then surface sterilized with 1% sodium hypochlorite solution to avoid any type of contamination and then washed with distilled water (about 3 to 5 times) to remove the sodium hypochlorite remains. The washed samples were dried on sterilized tissue papers. The samples were ground with the help of pestle and mortar separately in sterilized distilled water (1:1, w/v). The crushed samples were transferred in sterilized beakers and were covered with sterile aluminium foil and kept in the oven (maintained at 40^{0}C) for 1 hour to allow the residual part to settle down. The sample extracts were then filtered through fine sterilized muslin cloth and then through Vacuum Seitz filter. The required concentration i.e. 10, 25, 50 and 75% were prepared with Czapek's-Dox agar medium separately just before pouring in sterilized Petri plates.

Antifungal activity

The antagonistic potential of aqueous extract of locally available medicinal plants was tested

against *Fusarium oxysporum* f. sp. *lini* by using Poisoned food technique given by Dennis and Webster (3). About 15ml of the agar medium was poured in each Petri plate and allowed to solidify. After solidification about a 5 mm block of fresh culture of the pathogen was placed in the center of each Petri plate and incubated at 25 ± 20C. For each treatment three replicates were maintained. The growth of the pathogen was recorded on 2,4,6 and 8th day of inoculation and compared with control.

The percent inhibition of fungal mycelial growth was calculated by using the formula given below

I=C-T/C×100

Where, **I** = Percent growth inhibition,
C=Colony diameter/ radial growth of pathogen in control, and
T=Colony diameter/ radial growth of pathogen in treatment.

Data analysis

The data were expressed as Mean ± SD and were analyzed statistically in completely randomized design (factorial) by using analysis of variance technique. Duncan's Multiple Range test and Least significant test were applied to compare the means.

RESULTS

All the tested aqueous plant extract showed antifungal activity against the test pathogen. The result of the present study showed that *Tinospora cordifolia* (leaves), *Cymbopogon citratus* (leaves) and *Moringa oleifera* (bark) showed antagonistic effect against *Fusarium oxysporum* f. sp. *lini.*

Percent inhibition

It was observed that mycelial growth decreases with the increase in concentration of the extract. Maximum inhibition (100%) of *Fusarium oxysporum* f. sp. *lini* was recorded under the treatment of aqueous extract of *Tinospora cordifolia* (leaves), *Cymbopogon citratus* (leaves) and *Moringa oleifera* (bark). The antifungal potential of aqueous extract was compared according to their zone of inhibition against the test pathogen. On the second day of inoculation (Table 1,Fig 1), all the plant extracts were significantly (p≤0.01) effective. Among all the five plant extracts *Cymbopogon citratus* (leaves) (83.67% inhibition) were highly antagonistic against the test pathogen at 50% concentration which was followed by *Tinospora cordifolia* (leaves) (82.09%), *Zingiber officinale* (rhizome) (73.20%), *Moringa oleifera* (bark) (73.02%) and *Trachyspermum ammi* (seeds) (55.21%) respectively. Mean radial growth was significantly (p≤0.01) different from control (Table 1).

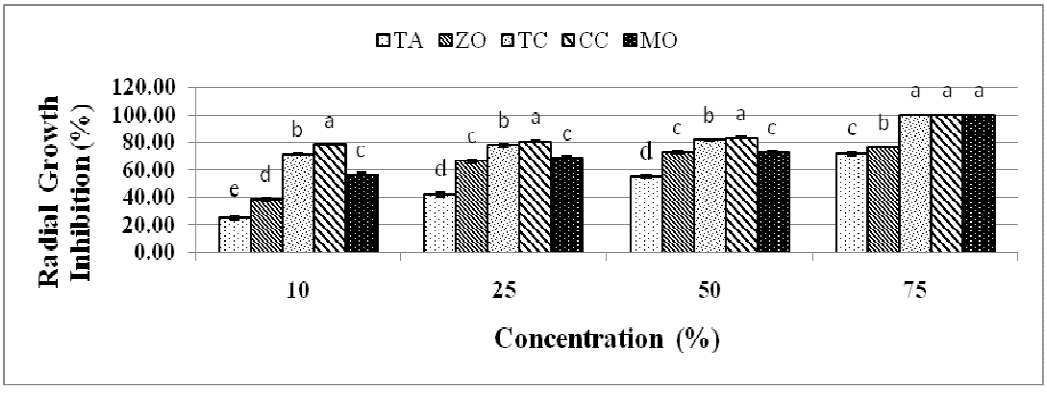

Figure 1

Bar graph showing the percent radial growth inhibition of Fusarium oxysporum f.sp. lini in the presence of aqueous plant extract at four different concentrations i.e. 10, 25, 50 and 75% on the second day. Vertical bars showing standard deviation of three replicates Mean separation at each concentration by DMRT at 5% level. Where TA (Trachyspermum ammi (seeds)), ZO (Zingiber officinale (rhizome)), TC Tinospora cordifolia (leaves), CC (Cymbopogon citratus(leaves)) and MO (Moringa oleifera (bark))

Table 1

Radial growth of Fusarium oxysporum f.sp. lini after 2 days of inoculation in the presence of aqueous plant extract at 10, 25, 50 and 75% concentrations

	Concentration[a]						
Treatment	**Control**	**10%**	**25%**	**50%**	**75%**	**F[b] Value**	**CV%**
Trachyspermum ammi (seeds)	21.77 ± 0.25	16.20 ± 0.26**	12.53 ± 0.31**	9.75 ± 0.25**	6.07 ± 0.21**	**	1.94
Zingiber officinale (rhizome)	21.77 ± 0.25	13.30 ± 0.30**	7.20 ± 0.26**	5.83 ± 0.35**	5.10 ± 0.40**	**	2.13
Tinospora cordifolia (leaves)	28.67 ± 0.31	8.10 ± 0.10**	6.33 ± 0.35**	5.13 ± 0.21**	0.00 ± 0.00**	**	2.32
Cymbopogon citratus (leaves)	31.63 ± 0.25	6.77 ± 0.25**	6.03 ± 0.06**	5.17 ± 0.06**	0.00 ± 0.00**	**	1.77
Moringa oleifera (bark)	20.18 ± 0.22	8.63 ± 0.35**	6.27 ± 0.28**	5.45 ± 0.25**	0.00 ± 0.00**	**	3.05

Mean ± SD, n = 3.
a** = significantly different from control at 1% level using LSD test.
b** = significant at 1% level.
CV% = Coefficient of variation.

At 25% concentration percent inhibition of *Tinospora cordifolia* (leaves), *Cymbopogon citratus* (leaves) and *Trachyspermum ammi* (seeds) treatment were significantly ($p \leq 0.05$) different from each other whereas percent inhibition of *Zingiber officinale* (rhizome) and *Moringa oleifera* (bark) were significantly ($p \leq 0.05$) similar.*Cymbopogon citratus* (leaves) (80.93%) ranked first in case of percent inhibition;*Tinospora cordifolia* (leaves) ranked second having percent inhibition 77.90%. At a concentration of 10% the minimum percent inhibition was recorded with *Trachyspermum ammi* (seeds) (25.56%). All the treatments were significantly ($p \leq 0.05$) different from each other. On the fourth day (Table 2, Fig. 2), at 75% concentration percent inhibition of *Tinospora cordifolia* (leaves), *Cymbopogon citratus* (leaves) and *Moringa oleifera* (bark) treatments were significantly ($p \leq 0.05$) similar. It was observed that all the treatments were significantly ($p \leq 0.01$) different from control. It was noticed that *Tinospora cordifolia* (leaves), *Cymbopogon citratus* (leaves) and *Moringa oleifera* (bark) were highly antagonistic and they completely inhibited the mycelial growth of the *Fusarium oxysporum* f. sp. *lini.*

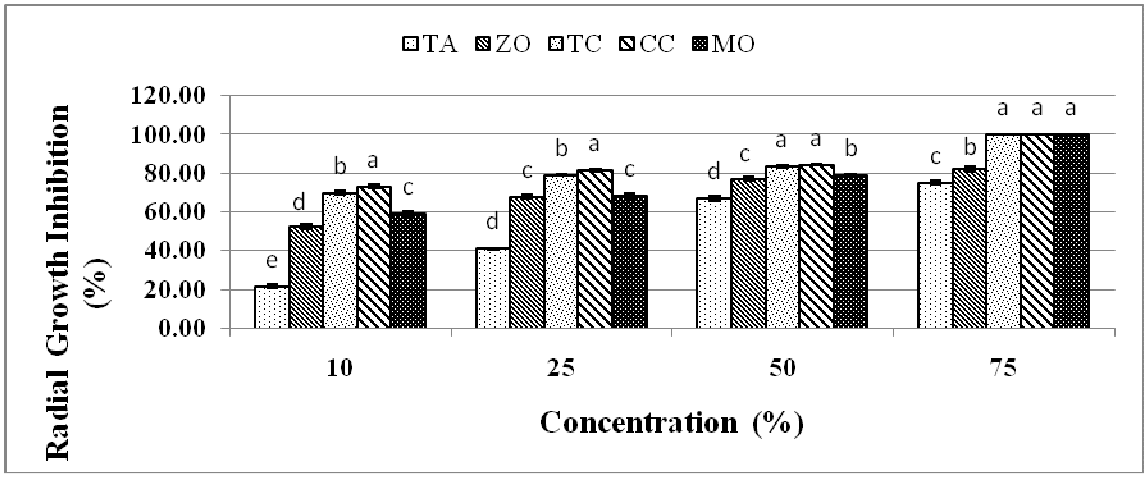

Figure 2

Bar graph showing the percent radial growth inhibition of Fusarium oxysporum f.sp. lini in the presence of aqueous plant extract at four different concentrations i.e. 10, 25, 50 and 75% on the fourth day. Vertical bars showing standard deviation of three replicates Mean separation at each concentration by DMRT at 5% level. Where TA (Trachyspermum ammi (seeds)), ZO (Zingiber officinale (rhizome)), TC Tinospora cordifolia (leaves), CC (Cymbopogon citratus (leaves)) and MO (Moringa oleifera (bark))

Table 2

Radial growth of Fusarium oxysporum f.sp. lini after 4 days of inoculation in the presence of aqueous plant extract at 10, 25, 50 and 75% concentrations

	Concentration[a]						
Treatment	**Control**	**10%**	**25%**	**50%**	**75%**	**F[b]Value**	**CV%**
Trachyspermum ammi (seeds)	30.23 ± 0.25	23.67 ± 0.35**	17.77 ± 0.25**	10 ± 0.30**	7.43 ± 0.25**	**	1.59
Zingiber officinale (rhizome)	30.23 ± 0.25	14.30 ± 0.30**	9.70 ± 0.30**	6.87 ± 0.32**	5.37 ± 0.30**	**	2.23
Tinospora cordifolia (leaves)	37.83 ± 0.31	11.43 ± 0.40**	7.93 ± 0.21**	6.23 ± 0.25**	0.00 ± 0.00**	**	2.12
Cymbopogon citratus (leaves)	38.23 ± 0.25	10.30 ± 0.36**	7.03 ± 0.15**	5.90 ± 0.06**	0.00 ± 0.00**	**	1.73
Moringa oleifera (bark)	29.97 ± 0.25	12.27 ± 0.25**	9.47 ± 0.35**	6.24 ± 0.14**	0.00 ± 0.00**	**	2.01

Mean ± SD, n = 3.
a** = significantly different from control at 1% level using LSD test.
b** = significant at 1% level.
CV% = Coefficient of variation.

At 50% concentration the percent inhibition of *Tinospora cordifolia* (leaves), *Cymbopogon citratus* (leaves) treatment were significantly ($p \leq 0.05$) similar whereas all other treatments were significantly ($p \leq 0.05$) different from each other. Least inhibition was recorded by *Trachyspermum ammi* (seeds) (66.92%).At 25% concentration percent inhibition of *Zingiber officinale* (rhizome) and *Moringa oleifera* (bark) were significantly ($p \leq 0.05$) similar. *Cymbopogon citratus* (leaves) (81.60%) showed the highest percent inhibition followed by *Tinospora cordifolia* (leaves) (79.03%), *Moringa oleifera* (bark) (68.40%), *Zingiber officinale* (rhizome) (67.92%) and *Trachyspermum ammi* (seeds) (41.24%) respectively. .At 10% concentration all the treatments were significantly ($p \leq 0.05$) different from each other. The percent of inhibition ranged between 21.72% to 73.06%. On sixth day of inoculation (Table 3, Fig. 3), all the treatments were significantly ($p \leq 0.01$) different from control at all the five concentrations. At 75% concentration all the treatments were significantly ($p \leq 0.01$) antagonistic against *Fusarium oxysporum* f. sp. *lini.* At 50%concentration the highest percent inhibition was recorded by *Cymbopogon citratus* (leaves) (86.67%) followed by *Tinospora cordifolia* (leaves) (84.06%), *Moringa oleifera* (bark) (82.49%), *Zingiber officinale* (rhizome) (81.25%) and *Trachyspermum ammi* (seeds) (70.13%) respectively. At 25% concentration the percent of inhibition ranges between 46.06% to 83.84%. At 10% concentration minimum percent of inhibition was noticed by *Trachyspermum ammi* (seeds) (31.89%).

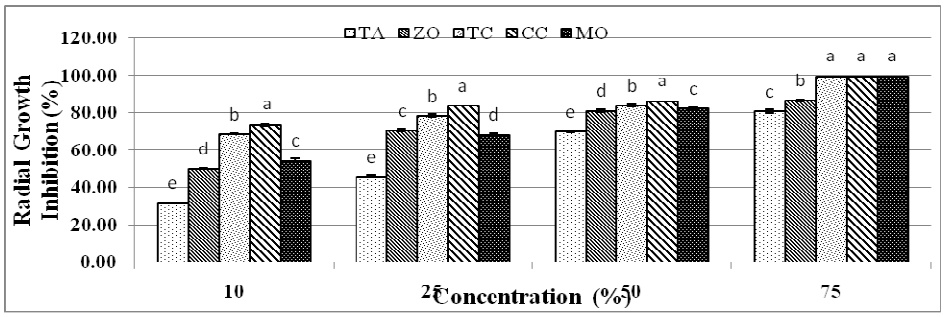

Figure 3

Bar graph showing the percent radial growth inhibition of Fusarium oxysporum f.sp. lini in the presence of aqueous plant extract at four different concentrations i.e. 10, 25, 50 and 75% on the sixth day. Vertical bars showing standard deviation of three replicates. Mean separation at each concentration by DMRT at 5% level. Where TA (Trachyspermum ammi (seeds)), ZO (Zingiber officinale (rhizome)), TC Tinospora cordifolia (leaves), CC (Cymbopogon citratus (leaves)) and MO (Moringa oleifera (bark))

Table 3

Radial growth of Fusarium oxysporum f. sp. lini after 6 days of inoculation in the presence of aqueous plant extract at 10, 25, 50 and 75% concentrations

	Concentration[a]						
Treatment	**Control**	**10%**	**25%**	**50%**	**75%**	**F[b]Value**	**CV%**
Trachyspermum ammi (seeds)	43.75 ± 0.25	29.80 ± 0.26**	23.60 ± 0.40**	13.07 ± 0.21**	8.40 ± 0.40**	**	1.33
Zingiber officinale (rhizome)	43.75 ± 0.25	21.67 ± 0.25**	12.77 ± 0.25**	8.20 ± 0.26**	5.80 ± 0.26**	**	1.39
Tinospora cordifolia (leaves)	48.53 ± 0.15	15.17 ± 0.21**	10.40 ± 0.36**	7.73 ± 0.38**	0.00 ± 0.00**	**	1.59
Cymbopogon citratus (leaves)	46.75 ± 0.25	12.27 ± 0.25**	7.53 ± 0.18**	6.23 ± 0.06**	0.00 ± 0.00**	**	1.11
Moringa oleifera (bark)	40.17 ± 0.21	18.23 ± 0.25**	12.70 ± 0.36**	7.03 ± 0.15**[c]	0.00 ± 0.00**	**	1.46

Mean ± SD, n = 3.
a** = significantly different from control at 1% level using LSD test.
b** = significant at 1% level.
CV% = Coefficient of variation.

On eighth day (Table 4, Fig. 4), all the aqueous plant extract was significantly (p≤0.01) effective against the test pathogen. At 75% concentration the percent of inhibition ranges between 82.50% to 100%. At 50% concentration maximum percent of inhibition was observed with *Cymbopogon citratus* (leaves) (89.88%) followed by *Tinospora cordifolia* (leaves) (87.26%), *Moringa oleifera* (bark) (87.18%), *Zingiber officinale* (rhizome) (84.37%) and *Trachyspermum ammi* (seeds) (74.13%) respectively. At 25% and 10% concentration all the treatments were significantly (p≤0.05) different from each other. At both concentrations *Cymbopogon citratus* (leaves) ranked first in the case of percent of inhibition whereas *Tinospora cordifolia* (leaves) ranked second.

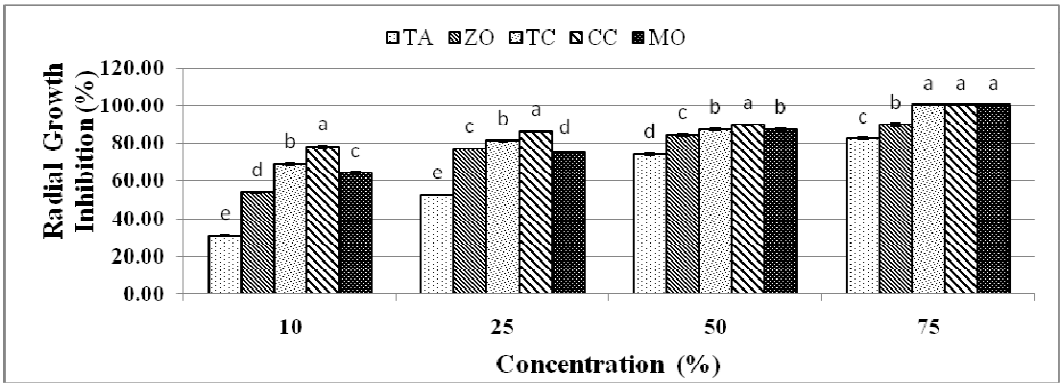

Figure 4

Bar graph showing the percent radial growth inhibition of Fusarium oxysporum f.sp. lini in the presence of aqueous plant extract at four different concentrations i.e. 10, 25, 50 and 75% on the eighth day. Vertical bars showing standard deviation of three replicates. Mean separation at each concentration by DMRT at 5% level. Where TA (Trachyspermum ammi (seeds)), ZO (Zingiber officinale (rhizome)), TC Tinospora cordifolia (leaves), CC (Cymbopogon citratus (leaves)) and MO (Moringa oleifera (bark))

Table 4

Radial growth of Fusarium oxysporum f.sp. lini after 8 days of inoculation in the presence of aqueous plant extract at 10, 25, 50 and 75% concentrations

	Concentration[a]						
Treatment	**Control**	**10%**	**25%**	**50%**	**75%**	**F[b]Value**	**CV%**
Trachyspermum ammi (seeds)	60.57 ± 0.45	41.70 ± 0.36**	28.73 ± 0.31**	15.67 ± 0.40**	10.60 ± 0.46**	**	1.27
Zingiber officinale (rhizome)	60.57 ± 0.45	27.87 ± 0.42**	13.93 ± 0.06**	9.47 ± 0.15**	6.27 ± 0.46**	**	1.49
Tinospora cordifolia (leaves)	65.23 ± 0.32	20.23 ± 0.32**	12.20 ± 0.20**	8.31 ± 0.30**	0.00 ± 0.00**	**	1.22
Cymbopogon citrates (leaves)	70.50 ± 0.20	15.33 ± 0.31**	9.87 ± 0.15**	7.13 ± 0.06**	0.00 ± 0.00**	**	0.92
Moringa oleifera (bark)	60.33 ± 0.35	21.63 ± 0.35**	14.87 ± 0.15**	7.73 ± 0.25**	0.00 ± 0.00**	**	1.23

Mean ± SD, n = 3.
a** = significantly different from control at 1% level using LSD test.
b** = significant at 1% level.
CV% = Coefficient of variation.

DISCUSSION

All the aqueous plant extracts had the antifungal activity against the test pathogen at all the five concentrations. The result showed that the inhibition zone increases with the increase in concentration. *Fusarium oxysporum* f.sp. *lini* was most sensitive to *Tinospora cordifolia* (leaves), *Cymbopogon citratus*(leaves) and *Moringa oleifera* (bark) at all four days of inoculation. Aqueous plant extracts were used as an alternative to synthetic chemical as they are less toxic and environmental friendly. From last many years large number of plants have been studied for their antifungal activity and positive results have been reported (16,17). Zingiber has been known for its medicinal property. It contains gigerdiol, gingerols and shagelol as main antifungal compound (5). *Moringa oleifera* contains pterygospermin and benzyl isocyanate which have strong antifungal activity (8). *Trachyspermum ammi* was reported to be inhibitory against *Helminthosporium oryzae* (15). *M. oleifera* was found to be antagonistic against *Mucar mucedo* and *Aspergillus niger* (7).*Cymbopogan citrates* are generally called lemon grass (1). The oil possesses bactericidal and antifungal property (11). It was reported that the lemongrass water extract contains alkaloids and flavonoids. These active compounds have bactericidal, pesticidal and fungicidal property (4, 14).

CONCLUSION

The main purpose of the present study was to explore the possibility of effectiveness of some medicinal plants against the selected pathogen in the present study as an alternative to the chemical pesticides. The aqueous plant extract proved to be a better alternative to the synthetic chemicals. It was observed that *Tinospora cordifolia* (leaves), *Cymbopogon citratus* (leaves) and *Moringa oleifera* (bark) were highly antagonistic against *Fusarium oxysporum* f.sp. *lini.* From the result of the present study it can be concluded that *Tinospora cordifolia* (leaves), *Cymbopogon citratus*(leaves) and *Moringa oleifera* (bark) can be used as natural pesticides.

ACKNOWLEDGEMENT

The authors are thankful to the Head, Department of Environmental Science, B.B.A.U., Lucknow for providing the necessary facilities. One of us (Sangeeta) is grateful to UGC, New Delhi for providing Rajiv Gandhi National Fellowship.

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