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## Effect of ethanol and chloroform extracts of selected plants against *Fusarium oxysporum* f. sp. *pisi*

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**Abstract-** Effect of ethanol and chloroform extract of leaf, stem, bark and seed of selected plants *Millettia pinnata* L., *Cassia tora* L., *Cymbopogon citratus* Stapf. and *Moringa oleifera* Lam. was studied against *Fusarium oxysporum* f. sp. *pisi* (ITCC No.-4814), the causal organism of *Fusarium* wilt in pea. Almost all the extracts inhibited mycelial growth of test pathogen. Among them the maximum inhibition was found in 10 % concentration of chloroform extract of *Millettia pinnata* L. seed with 70 mm inhibition zone and *Cassia tora* L. seed or bark with 65.7 mm against *Fusarium oxysporum* f. sp. *pisi*. Significant inhibition was also occurred in ethanol extract of *Millettia pinnata* L. seed with 65 mm inhibition zone. Ethanol and chloroform extracts were highly significant on inhibition of mycelial growth in different concentration.

**Key words:** Plant extracts, ethanol, chloroform, *Fusarium oxysporum* f. sp. *pisi*

### INTRODUCTION

Pea (*Pisum sativum* L.) is the most important and popular legume crop. It is grown in garden or field of temperate regions of the world. It is the most important Rabi pulse crop in India. It is consumed as pulse and vegetable. It is rich source of protein, also good source of carbohydrates, vitamins A and vitamins C, phosphorus and calcium, and less amount of iron.<sup>1</sup> Cultivation of pea is hindered by several diseases caused by plant pathogens. *Fusarium* wilt of pea caused by *Fusarium oxysporum* f. sp. *pisi* is the destructive disease and losses to farmers. Chemical fungicides are used for controlling the wilt disease which may cause adverse effects on environment and health of human being. Now a day management of *Fusarium* wilt disease has been done through plant

extracts.<sup>2-10</sup> To search plant extracts as an alternative source for control of plant diseases is challenge of time. The antifungal activity of some medicinal plant extracts has been recognized and reported by some researchers.<sup>11-13</sup> Antimicrobial activity of plant extracts of *Terminalia chebula*, *Mangifera indica* and *Eucalyptus citriodora* have been reported against plant pathogens. The chloroform, aqueous or other extracts of some plants like *Piper betle* L. was found to be effective in controlling *Fusarium* spp.<sup>14</sup>

In present study, effects of extracts of selected plants such as *Millettia pinnata* L., *Cassia tora* L., *Cymbopogon citratus* Stapf. and *Moringa oleifera* Lam. were studied.

*Millettia pinnata* L. is common medicinal plant, belongs to Fabaceae family. The common name of plant is "Karanja". It is grown all over the India especially near the coast and extending from the central to Eastern Himalayas to Ceylon. All parts of *Millettia pinnata* L. are

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used in Ayurveda as remedies for different diseases such as fever, piles, ulcer, bronchitis, whooping cough, skin diseases, rheumatic arthritis, liver pain and leprosy. It contains flavonoid constituents, alkaloids, phenolic compounds, phytosterol, saponins and terpenoids.

*Cassia tora* L. belongs to Fabaceae family. It is found in different parts of India. All parts of plant such as leaf, root, flower, fruit and seed possess medicinal properties and used in medicine. *Cassia tora* L. are used in simple cough, ringworm, leprosy, dyspepsia, constipation, bronchitis and cardiac disorders. *Cassia tora* L. contains chemicals constituents such as palmitate, stearate, oleate, Emodin, tricontan-1-0l, stigmaterol, succinic and d-tartaric acids uridine, quercitrin and isoquercitrin.

*Cymbopogon citratus* Stapf. (Family - Poaceae), is commonly known as lemon grass or oil grass, it is widely found in tropical countries. Various compounds mainly alcohols, ketones, terpenes, aldehyde, phenolic compounds and esters are present in lemon grass. It contains essential oils that contain Citral  $\alpha$ , Citral  $\beta$ , Terpinolene, Geranyl acetate, Nerol Geraniol, Citronellal, Myrcene and Terpinol Methylheptenone. It is commonly used in teas, soups and curries. It is also used for poultry, fish and seafood.

*Moringa oleifera* Lam. belongs to the family Moringaceae, found in the tropical and subtropical regions worldwide. It is commonly known as miracle tree, drumstick or horseradish tree. It contains zeatin, quercetin, kaempferol and other phytochemicals. It is good source of vitamins A, C, B and minerals. It contains niazirin, niazirin, Benzoic acid, gallic acid, beta benzaldehyde. It regulates the metabolism of thyroid hormone, central nervous system, digestive system, genito-urinary system. It is used in the treatment of gastric ulcers, scurvy and asthma.

In this study, we observed the effectiveness of different concentration of ethanol and chloroform extracts of selected plants in reducing the mycelial growth of *Fusarium oxysporum* f. sp. *pisi*.

## **MATERIALS & METHODS**

### **Preparation of plant Extracts**

Fresh leaves, bark, stem and seeds of selected medicinal plants *Millettia pinnata* L., *Cassia tora* L., *Cymbopogon citratus* Stapf. and *Moringa oleifera* Lam. were collected from the surrounding areas of Ranchi, Jharkhand and washed thoroughly under running tap water and then with sterilized distilled water.

All collected parts of plants kept for shade dry at room temperature for 20 - 25 days. After making fine powder by pestle & mortar and electric grinder, 10 g of each fine powder was used for making plant extract in 100 ml of solvent such as ethanol and chloroform. The solution was filtered through Whatman filter paper.

### **Test Pathogen**

The test pathogen *Fusarium oxysporum* f. sp. *pisi* (ITCC No.-4814) was collected from Department of Mycology and Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi.

### **Antifungal activity of plant extracts**

Effect of ethanol and chloroform extracts of selected medicinal plants was evaluated by Poisoned food technique against Fungal Pathogen *Fusarium oxysporum* f. sp. *pisi*.<sup>14</sup> Antifungal activity of plant extracts was recorded at different concentration like 1.0 %, 2.5 %, 5.0% and 10.0% against test pathogen. Each experiment was repeated three times.

Inhibition zone and Inhibition percentage of *Fusarium oxysporum* f. sp. *pisi* was calculated by using formula (Vincent, 1947):  $I = \frac{C-T}{C} \times 100$ , where, I = Percent growth inhibition, C = Growth in control plates, T = Treated plates growth.

## **RESULT & OBSERVATION**

Effects of extracts of different parts of selected medicinal plants – *Millettia pinnata* L., *Cassia tora* L., *Cymbopogon citratus* Stapf. and *Moringa oleifera* Lam. in different solvents like ethanol and chloroform were recorded in Table 1 and Table 2. Mycelial growth of test pathogen *Fusarium oxysporum* f. sp. *pisi*. was inhibited by plant extracts.

For controlling of *Fusarium* wilt disease of pea crop, the antifungal activity of ethanol and chloroform extracts of selected medicinal plants at different concentration was tested. It was recorded that at 10% concentration of ethanol extracts of selected plants, almost all plant extracts were effective in reducing the mycelial growth of *Fusarium oxysporum* f. sp. *pisi* (Table 1).

Inhibition zone of *Fusarium oxysporum* f. sp. *pisi* in different extracts of plants was recorded in Table. Maximum inhibition zone of test pathogen was observed at 10% concentration of seed extract of *Millettia pinnata* L. with 65 mm, followed by bark of *Millettia pinnata* L. with 61.7 mm. Ethanol extracts of *Cassia tora* L. showed significant inhibition of *Fusarium oxysporum* f. sp. *pisi* with the range

of 55 mm to 64 mm. Selected plant extracts of *Cymbopogon citratus* Stapf. and *Moringa oleifera* Lam. also inhibited the mycelial growth of test pathogen. Bark extract of *Moringa oleifera* Lam. showed 59.3 mm inhibition zone. Root extract of *Cymbopogon citratus* was least effective in compare to other plant extracts. However, leaf of *Cymbopogon citratus* Stapf. showed 57.7 mm inhibition zone.

Effects of chloroform extracts of *Millettia pinnata* L., *Cassia tora* L., *Cymbopogon citratus* Stapf. and *Moringa oleifera* Lam. on inhibition of plant pathogenic fungi *Fusarium oxysporum* f. sp. *pisi*. were recorded in Table 2. It was observed that seed extract of *Millettia pinnata* L. at 10% effectively suppressed the mycelial growth of the *Fusarium oxysporum* f. sp. *pisi* with 70 mm

**Table 1- Effect of ethanol extracts of *Millettia pinnata* L., *Cassia tora* L., *Cymbopogon citratus* Stapf. and *Moringa oleifera* Lam. against *Fusarium oxysporum* f. sp. *pisi*.**

Plant Extracts	1%	2.5%	5%	10%
<b><i>Millettia pinnata</i> L.</b>	<b>Inhibition Zone (mm)</b>	<b>Inhibition Zone (mm)</b>	<b>Inhibition Zone (mm)</b>	<b>Inhibition Zone (mm)</b>
a) Seed	51.7	59.3	61.7	65
b) Bark	42.3	49.7	54.7	61.7
c) Leaf	25.7	39.3	50	58
d) Stem	25.7	31.7	41.7	55.3
<b><i>Cassia tora</i> L.</b>				
a) Seed	49.3	51.3	57.7	60.7
b) Bark	44.3	48.7	60	64
c) Leaf	33	39.7	56	60.3
d) Stem	22.7	35.3	51	55.7
<b><i>Cymbopogon citrates</i></b>				
a) Leaf	31	37.7	49.3	57.7
b) Root	27	32.3	36.3	38.3
<b><i>Moringa oleifera</i> Lam.</b>				
a) Bark	47.7	50	55.3	59.3
b) Leaf	36	42.7	51.3	56
c) Stem	24.3	40.7	47.7	51.3
<b>Check</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>

**Table 2- Effect of chloroform extracts of *Millettia pinnata* L., *Cassia tora* L., *Cymbopogon citratus* Stapf. and *Moringa oleifera* Lam. against *Fusarium oxysporum* f. sp. *pisi*.**

Plant Extracts	1%	2.5%	5%	10%
<b><i>Millettia pinnata</i> L.</b>	<b>Inhibition Zone (mm)</b>	<b>Inhibition Zone (mm)</b>	<b>Inhibition Zone (mm)</b>	<b>Inhibition Zone (mm)</b>
a) Seed	38.7	47.7	57	70
b) Bark	34.3	45.3	51.3	64.3
c) Leaf	31.7	41.7	46	61
d) Stem	24.3	34.7	40.3	49
<b><i>Cassia tora</i> L.</b>				
a) Seed	33.7	40.3	55	65.7
b) Bark	26.7	37.3	46	65.7
c) Leaf	24	28.7	40	62.7
d) Stem	22.7	20	35	51.7
<b><i>Cymbopogon citrates</i></b>				
a) Leaf	20	29	38.7	43.3
b) Root	16.3	29.7	34.3	38
<b><i>Moringa oleifera</i> Lam.</b>				
a) Bark	41	48	52.7	64
b) Leaf	31.7	40.3	52.7	59.3
c) Stem	23	32	35.3	47.7
<b>Check</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>

inhibition zone. The inhibitory effect of seed or bark extracts of *Cassia tora* L. was effective in 10% concentration using chloroform with 65.7 mm inhibition zone. Antifungal activity of *Millettia pinnata* L. bark and leaf with different concentrations was found to be effective even at 1% concentration against *Fusarium oxysporum* f. sp. *pisi*. Inhibition zone was observed at 10% concentration by bark or leaf extract of *Millettia pinnata* L. with 64.3 mm and 61mm respectively. Bark extract of *Moringa oleifera* Lam. exhibited 64 mm inhibition zone. Other extracts were also inhibited the mycelial growth of *Fusarium oxysporum* f. sp. *pisi*.

Aqueous extracts of 48 plants belonging to six different major groups of the plant kingdom, two commercially available botanicals and different fungicides were screened for antifungal activity against *Drechslera bicolor* causing leaf blight of bell pepper. Aqueous extracts of 48 plants belonging to six different major groups of the plant kingdom, two commercially available botanicals and different fungicides were screened for antifungal activity against *Drechslera bicolor* causing leaf blight of bell pepper. Aqueous extracts of 48 plants belonging to six different major groups of the plant kingdom, two commercially available botanicals and different fungicides were screened for antifungal activity against *Drechslera bicolor* causing leaf blight of bell pepper.

#### CONCLUSION

Antifungal activity of ethanol and chloroform extracts of selected medicinal plants are due to the presence of minerals, vitamins, essential phytochemicals, alkaloids and secondary metabolites such as flavonoids, phenolic compounds, phytosterol, saponins, polysaccharides, terpenoids and resins. Seed extract of *Millettia pinnata* L. showed maximum antifungal activity using chloroform in compare to other treatments. Ethanol and chloroform extracts of *Millettia pinnata* L., *Cassia tora* L., *Cymbopogon citratus* Stapf. and *Moringa oleifera* Lam. can be used as an alternative source for diseases management of pea crop.

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