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The inhibitory action of plant extracts on fungi associated with roots of pea (*Pisum Sativuim* L.)

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Abstract
The presence of antifungal compounds in higher plants has recocgnised as an important factor of disease resistance. The tropical and sub-tropical flora, like Indian flora, is rich source of biologically active compounds which exhibit antimicrobial and other biological properties. Plant parts and their constituents have proved their fruitifulness in providing less phytotoxic, more systemic,easily biodegradable and host metabolism stimulatory pesticides. In the present study, 28 fungi were isolated from roots of *Pisum sativum* and the *Fusarium oxysporum* found to be most dominant. Aqueous extract of different of 37 plant species belonging to different families have been tested for antifungal activity against doninant fungal species by "Poisoned food technique" of Grover and Moore., the leaf extract of *Lantana indica* showed highest mycelial growth inhibition against different fungi (100%) and *Cassia tora* showed lowest(13.25%) mycelial growth inhibition.

Keywords: Pea, fungi, plant parts, poisoned food technique

Introduction

Peas grow best in regions where there is a slow transition from cold to warm weather. Seeds can germinate at a minimum of 5°C. The young green seeds of peas are eaten raw or after cooking. The seeds are also used as pulse and they are good source of proteins, vitamins (B_1, B_{12} and Vit. K), Potassium and Phosphorus. Peas are consumed both in the fresh form as vegetable and in the dried form as pulse. The major obstacles in the way of increased pulse production are the diseases which are responsible for the reduction and uncertainty in pulse. So it is essential that we understand the relative importance of diseases and device (ways) to tackle them in effective manner (Grewal, 1988) [21]. The yield in pea, besides other factors is greatly affected by the diseases caused by fungi such as wilt, stem rot, root rot and mildew.

Minz *et al.* (2012) [39] studied on the effect of plant extracts on the growth of wilt-causing fungi *Fusarium oxysporum* has been a subject of significant research interest due to its potential application in the management of devastating plant diseases. Plant extracts, derived from various botanical sources, contain a wide array of secondary metabolites with antimicrobial properties, and these compounds have shown promise in inhibiting the growth and development of *Fusarium oxysporum*. Many plant extracts contain phytochemicals such as alkaloids, flavonoids, phenolics, and terpenoids, which possess fungicidal properties. These compounds can disrupt critical fungal processes, including spore germination, mycelial growth, and the formation of infection structures. As a result, *Fusarium oxysporum*'s ability to colonize and infect plant roots is compromised, leading to reduced disease severity. Some plant extracts have been found to stimulate the production of reactive oxygen species (ROS) in fungal cells, causing oxidative stress and further hindering their growth. Other extracts may interfere with key enzymatic activities essential for fungal survival. The effect of plant extracts on *Fusarium oxysporum* growth varies depending on the specific plant source, extraction method, and concentration used. Consequently, researchers are continually exploring different plant extracts to identify those with the most potent antifungal properties. Utilizing plant extracts as a natural fungicide offers potential benefits for sustainable agriculture, as they are biodegradable and less likely to lead to the development of fungicide-resistant strains. However, optimizing extraction methods, determining the most effective plant sources, and establishing appropriate application protocols are crucial steps to harness the full potential of plant extracts in managing Fusarium wilt and other fungal diseases in agriculture.

Few recent studies on the antifungal action of plants, especially medicinal and aromatic plants, have been carried out as a natural remedy in *Fusarium* wilt management. Secondary metabolites such as alkaloids, flavonoids, terpenoids and phenolic compounds produced by many plants have been shown to possess strong antifungal activity against diverse plant pathogens. These bioactive compounds can break fungal cell structure, stop growth and promote plant resistance, providing a non chemical, environmentally friendly, cost effective alternative fungicide.

Materials and Methods

Screening of angiospermic plant extracts

Twenty eight fungal species were isolated from root of pea. Plants belonging to different families of angiosperms were collected from different parts of Ghazipur and these were identified with the help of floras (Willis, 1957; Hutchinson, 1959; Talbot, 1976; Fyson, 1977; and Hooker, 1985) [20, 26, 28, 58].

The plant samples (dry leaves) were powdered with the help of sterilized pestle and mortar and sieved through 1 mm mesh. The powdered plant material dissolved in alcohol in 1:4 (w/v) ratio and kept for 24 hour and filtered through double layer muslin cloth. The extract was centrifuged at 5000 rpm for 10 minutes and the supernatant was used to assess the bioactivity against fungi by 'Poisoned food technique' of Grover and Moore (1962) [22]. Potato Dextrose Agar medium was prepared with the following composition: Peeled potato, 200.00 g, Dextrose, 20.00 g; Agar, 20.00 g and distilled water, 1000.00 ml. The medium was sterilized at 20 lb/square inch pressure for 30 minutes and cooled to about 40°C. The antibiotic strepto-penicillin was added to medium and mixed thoroughly for prevention of bacterial activity in it during course of experimentation as suggested by Gupta and Banerjee (1970) [23].

For treatment sets, 5 ml of prepared extract (supernatant) of each plant was mixed with 5 ml of molten Potato Dextrose Agar medium in a pre-sterilized Petri plate separately and agitated in round fashion in order to mix the extract homogeneously. In control sets, requisite amount of sterilized distilled water was added in place of the extract. Fungal disc (5 mm diam) cut from the periphery of 7 day old culture of each fungal species was inoculated aseptically in separate assay Petri plates of the treatment and control sets and all the plates were incubated for six days at 28±2°C.

Colony diameters in mutual perpendicular directions of treatment as well as control sets of fungal discs were measured on seventh day. Fungitoxicity was recorded in terms of per cent inhibition of mycelial growth and calculated on mean values of increase in colony diameters by the following formula :

$$\text{Per cent inhibition of mycelial growth} = \frac{dc - dt}{dc} \times 100$$

where,

dc = Average increase in diameter of fungal colony in control sets

dt = Average increase in diameter of fungal colony in treatment sets.

Result and Discussion

28 fungi were isolated from roots of *Pisum sativum* and the *Fusarium oxysporum* found to be most dominant. Aqueous extract of different of 37 plant species belonging to different families have been tested for antifungal activity against doninant fungal species by "Poisoned food technique" of Grover and Moore (1962) [22]., the leaf extract of *Lantana indica* showed absolute mycelial inhibition (100%) and *Cassia tora* showed lowest(13.25%) mycelial growth inhibition (Table-1).

A large number of pathogens are known for causing various diseases in plants and human beings. Major emphasis for control of diseases in plants remained through physical, chemical and biological methods. In recent years higher plants proved their antifungal activity and their antifungal compounds could be used for the control of various plant diseases (Table-2).

Table 1: Fungitoxicity of higher plants against *Fusarium oxysporum*

S.N.	Plant species	Families	Per cent mycelial inhibition of *Fusarium oxysporun*
	1	2	3
1	*Artabotrys hexapetalous* (L.f.)Bhandari	Annonaceae	79.00
2	*Artocarpus heterophyllus* Lamk	Moraceae	27.37
3	*A. lakoocha* Roxb.	Moraceae	40.92
4	*Azadirachta indica* A. Juss	Meliaceae	47.94
5	*Butea monosperma* (Lamk) Taub	Papilionaceae	33.75
6	*Cassia tora* L.	Caesalpiniaceae	13.25
7	*Cicer arietinum* L.	Papilionaceae	17.39
8	*Colocasia esculenta* L.	Araceae	62.76
9	*Coriandrum sativum* L.	Apiaceae	15.96
10	*Cynodon dactylon* L.	Poaceae	14.30
11	*Cyperus killingiai* Endl.	Cyperaceae	45.90
12	*C. rotundus* L.	Cyperaceae	22.90
13	*Desmodium triflorum* (L.) DC	Papilionaceae	22.90
14	*Lantana indica* Roxb.	Verbinaceae	100.00
15	*Jasminum arboreum* Roxb.	Rubiaceae	70.00
16	*Mangifera indica* L.	Anacardiaceae	66.49
17	*Momordica charantia* L.	Cucurbitaceae	35.22
18	*Moringa oleifera* Lam.	Papilionaceae	60.87
19	*Morus alba* L.	Moraceae	43.63
20	*Musa paradisica* L.	Musaceae	47.58
21	*Ocimum basilicum L.*	Lamiaceae	54.10
22	*Oxalis pescarpae* L.	Geraniaceae	46.20
23	*Phyllanthes asperulatus* Hutch.	Euphorbiaceae	17.10
24	*Pithocelobium dulce* (Roth) Bth	Mimosaceae	26.09
25	*Polyalthia longifolia* (Thw) Sonner	Annonaceae	47.64
26	*Polygonum barbatum* L	Polygonaceae	43.34
27	*Psidium guajava* L.	Myrtaceae	35.98
28	*Ricinus communis* L.	Euphorbiaceae	65.68
29	*Rosa chinensis* Jacq.	Rosaceae	22.39
30	*Sida cardifolia* L.	Malvaceae	37.80
31	*Smilex prolifera* Roxb.	Smilaceae	35.10
32	*Spinacea oleracea* L.	Chenopodiaceae	20.69
33	*Tabernaemontana divericata* (L.) R.Br.	Apocynaceae	34.05
34	*Tamarindus indica* L.	Caesalpiniaeae	59.68
35	*Tinospora cardifolia* (Willd) Miers	Menispermaceae	51.40
36	*Tradescantia virginiana*	Commelinaceae	34.3
37	*Vitis vinifera* L.	Vitaceae	64.24

Table 2: Fungitoxic activity of Crudes (extracts) of higher plants

Plant(s) tested	Fungi	Results	Investi-gator(s)
1	2	3	4
Rhus hirta (leaf and stem)	7 fungi	Extracts having fungitoxic activity against all the fungi	Carlson *et al.* (1948) [9]
Musa sapientum (dried leaf, stem and fruit skin)	*Fusarium lycopersici* and *F. oxysporum*	Inhibited the mycelial growth of both fungi	Scott *et al.* (1949) [53]
Several plant spp.	*Aspergillus niger*	Extract of *Cinnamomum zeylanicum* and *Eugenia caryophyllata* were most effective against test fungus	Anand and Johar (1957)
Lupinus lutens	*Pythium meyalaceuthum*	Extract completely inhibited the growth of test fungus	Anselme (1959)
1281 plant spp.	Some fungi	22 plant species belonging to 11 families showed fungicidal activity	Petrushova (1960) [49]
Agrostis alba, Anthoxanthum odoratum, clove and *Phelum pratense*	*Fusarium nivale*	All the plant extracts showed strong antifungal activity against test fungus	Hajak (1961)
Several plant spp.	*Fusarium moniliforme*	*Artocarpus rigida* and *Brunfelsia americana* extracts showed fungitoxic activity	Masilungan *et al.* (1963)
Citrus spp. (Epicarp and mesocarp extract)	*Phomopsis citri*	Both extracts of plant showed antifungal activity in alone or in combination against test fungus	El-Tobshy and Sinclair (1964)
Areca catechu	Several fungi	Alcohlic extract showed wider antifungal spectrum than the aqueous extaract	Lalithakumari *et al.*(1965) [35]
Allium sativum (root extract)	*Cylindrocarpon radicicola, Fusarium culmorum* and *Gliocladium roseum*	Adventitious root extracts were active only against *G. roseum* and autoclaved extracts showed no antifungal activity	Clarke (1966)
4 plant spp.	*Verticillium dahliae*	Extracts of all plant species showed high fungitoxic activity	Bandarenko *et al.* (1967) [7]
88 plant spp.	*Helminthosporium turcicum*	The extract of 14 plant spp. Inhibited the growth of test fungus completely	Nene *et al.* (1968) [46]
21 higher plants	*Puccinia recondita*	11 plants species inhibited the germination of test fungus completely	Misra and Dixit (1976)
Clematis gouriana and *Ranunculus sceleratus*	26 fungi	Extract of both plants showed strong antifungal activity against all the fungi tested	Misra and Dixit (1977) [42]
50 plant spp. (flower)	*Helminthosporium oryzae*	Extract of 5 plants inhibited the myciloal growth of test fungus	Dikshit *et al.* (1978) [16]
34 higher plants	Three fungi	Extract of 7 plants showed antifungal activity against test fungi	Singh and Sharma (1978) [55]
40 plant spp. (root and flower)	*Curvularia lunata* and *Drechslera rostrata*	Root extract of *Lawsonia inermis, Prosopis juliflora* and flower of *Rosa chinensis* showed complete mycelial inhibition	Charya *et al.* (1979) [12]
60 plant spp.	*Helminthosporium oryzae*	Extract of *Adenocalyma allicea* was found to be fungitoxic against test fungus	Chaturvedi (1979) [13]
Some plant spp.	*Alternaria alternata, Crynospora coasseicda, Drechslera rostrata* and *Fusarium oxysporum*	Aqueous extract of *Allium cepa, A. sativum, Gossypium* sp., *Kalnchoe* sp., *Parhonium* sp. and *Phaseolus autropur-pureus* inhibited the spore germination	Kumar *et al.* (1979) [12]
14 plant spp.	*Alternaria tenuis, Curvularia lunata, Fusarium nivale* and *Helminthosponium gramenium*	Extracts of 4 plants showed fungitoxic activity against test fungi	Misra and Dixit (1979) [43]
Cyamopria tetragenolobus (bark and root)	Several fungi	Aqueous extract inhibited conidial germination and mycelial growth of several plant pathogenic fungi	Johnson and Reinosch (1980) [30]
Some plant spp. (leaf)	*Rhizoctonia solani*	Extract of *Cestrum diurnum,* showed fungitoxic activity	Renu *et al.* (1980) [52]
19 plant spp.	*Aspergillus flavus, A. versicolor* and *Fusarium monili- forme*	Extract of *Ocimum canum* fungitoxic to all the test fungi	Bhargava *et al.* (1981) [8]
110 plant spp. (leaf)	*Colletotrichum capsici* and *Penicillium italicum*	Only *Ageratum conyzoides* showed absolute activity	Chandra and Dikshit (1981) [11]
22 plant spp.	*Fusarium moniliforme*	Only extract os *Xanthium strumarium* was effective against test fungus	Kishore *et al.* (1982) [31]
Some plant spp.	*Fusarium lateritium*	Extract of *Aegle marmelos Citrus aurantifolia* and *Mentha arvensis* exhibited absolute toxicity	Pandey *et al.* (1983a)
45 plant spp. (pollen suspension)	*Alternaria alternata* and *Helminthosporium oryzae*	Pollen extract of *Lycopersicum esculentum* was most active against both the fungi	Pandey *et al.* (1983b)
Narvelia zeylanica (different parts)	*Curvularia eragrostidis* and *Helminthosporium oryzae*	Fresh parts of plant are toxic than the dried parts	Nath and Bordoloi (1984) [45]

50 plant spp.	*Alternaria alternata* and *Curvularia lunata*	Extracts of several plants showed fungitoxic activity	Pravindra-chary *et al.* (1984)
40 plant spp.	*Botrytis cineria* and *Colletotrichum gleoesporiodes*	Extract of *Abrus prectorius, Brassica campestris, Carum capticum* and *Raphanus sativus* exhibited 100% toxicity against both fungi	Dixit *et al.* (1986) [17]
30 plant spp.	Species of *Aspergillus,* Mucor and *Penicillium*	Extracts exhibited strong fungitoxic activity	David Yuk *et al.* (1987) [15]
35 plant spp.	*Aspergillus sydowi*	Extracts of six plants showed absolute toxicity	Mishra *et al.* (1987)
30 plant spp.	*Aspergillus flavus* and *Penicillium oxalicum*	Extract of *Aegle marmelos* and *Eupatorium capillifolium* showed strong fungitoxcity against *P. oxalicum only*	Tiwari *et al.* (1987)
Codiaeum variegatum	*Alternaria alternata* and *Fusarium oxysporum*	Extract showed antifungal activity against both fungi	Naidu (1988) [44]
Acacia arabica, Azadirachta indica, Ipomea cornea and *Porosopsis juliflora*	*Fusarium oxyrporum* f. sp. *cepae* & *Sarocladium oryzae*	All the plants showed antifungal activity	Eswaramur-thy (1989)
50 taxa of higher plants	*Fusarium oxysporum* f. sp. *ciceri*	Extract of *Iberis amara* and *Xanthium strumarium* exhibited strong antifungal activity	Mishra and Tripathi (1989) [41]
18 plant spp.	*Fusarium wxysporum, Pythium debaryanum, Rhizoctonia solani* and *Sclerotium rolfsii*	Extract of *Eupatorium cannabinum* showed strong fungitoxicity against all the fungi	Kumar and Tripathi (1990) [32]
Allium sativum, Azadirachta indica and *Bougainvillea spectabiles*	*Thanatephorus cucumeris*	Singnigicantly inhibited mycelial growth and *Sclerotial* germination	Laxamanan *et al.* (1990) [38]
16 plant spp.	Some fungi	Extracts exhibited more than 50% antifungal activity	Miah *et al.* (1990) [38]
Cannabis sativa, Cymbopogon pendulus and *Lantana camara* (leaf)	*Alternaria solani Colletotrichum cammeliae, & Curvulaaria lunata*	The aqueous leaf extracts inhibited the mycelial growth of all the fungi	Chakraborty *et al.* (1990) [38]
Adhatoda vasica, Allium sativum, Cassia fistula and *Prosopis juliflora* (leaf)	Some fungi	Chloroform and ethanolic extracts showed fungitoxic effect against test fungi	Malik *et al.* (1991) [36]
Some wild plants (flower)	*Alternaria solani*	*Lantana camara* was most effective	Sundriyal (1991) [57]
Argemone maxicana	*Alternania* sp., *Aspergillus flavus, Curvularia lunata, Microphomina phaseoliva* and *Mucor* sp.	Extract showed antifungal activity against *A. flavus*	Hussain Shah *et al.* (1992) [27]
Azadirachta indica	*Alternaria alternata, Aspergillus fumigatus, A. niger, Fusarium oxysporum, F. solani* and *Pythium aphanodermatum*	Extract showed antifungal activity against test fungi	Arumuga-samy and Udaiyan (1995) [6]
40 plant spp. (leaves)	*Fusarium oxysporum* f. sp. *lentis*	*Impatiens balsamina* and *Lawsonia inermis* showed complete mycelial inhibition	Singh and Tripathi (1995) [54]
Azadirachta indica, Calotropis gigentea, Eucalyptus spp., *Parthenium hysterophorus* and *Pongamia pinnata* (leaves)	*Fusarium moniliforme* var. *intermedium, F. oxysporum and F. pallidoroseum*	Extract of all the plant effective against test fungi	Gupta *et al.* (1996)
Aegle marmelos, Azadirachta indica and *Eucalyptus* sp. (leaves)	*Pyricularia grisea* f. sp. *oryzae*	Extract of *A. indica* was found to be most fungi-toxic against both *Pyricularia* spp.	Jain and Tripathi (1998) [29]
Allium sativum, Callistemon lanceotatus, Curcuma aromatica, Euphorbia hirta and *Zingiber officinale* (bulbs, rhizome and leaf)	*Fusarium oxysporum* f. sp. *lycopersici* and *Macrophomina phaseolina*	The extract of *C. lanceolatus* and *E. hirta* totally inhibited the growth of *F. oxysporum* f. sp. *lycopersici*	Raja and Kurucheve (1999) [51]
Andnographis alata, A. elongata, A. paniculata A. macrobotrys and *A. neesiana* (leaves)	*Helminthosporium oryzae*	Extract of plants inhibited the growth of test fungus *A. macrobotrys* was found to most effective	Alagesaboo-pathi and Balu (2000) [2]
Some plant spp.	*Fusarium udum*	The extract was active against test fungus	Singh and Rai (2000) [56]
Adesmia aegiceras	Some fungi	Ethanolic extracts showed antifungal activity	Agnese *et al.* (2001) [3]
11 plant spp.	Some fungi	All the plant extracts showed antifungal activity	Ali *et al.* (2001) [3]

Conclusion

The conventional use of synthetic fungicides has led to the emergence of numerous challenges, including environmental pollution, residue accumulation in grains, and harm to non-target organisms. As a result, there is an urgent need for cost-effective and environmentally sustainable management practices to mitigate the losses incurred by Fusarium wilt. This necessitates a comprehensive understanding of the disease's various aspects, including symptomatology, and the pathogen's behavior.

Antifungal activity of plant products against pathogens can be explored as a promising alternative to synthetic fungicides for their selection in disease management including planting of resistant varieties, appropriate fungicide use and application time, and integration of plant products with fungicide for enhanced disease management.

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