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Antifungal Potentiality of Leaf Extracts of *Saraca indica* Against *Fusarium* Species, An Approach towards Eco-friendly Management

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Abstract: ***Fusarium species are the most frequently soil borne fungal pathogen on vegetable crops, causing significant yield losses and made economical problems for growers. Fusarium control through chemical fungicides causes serious environmental problems and are toxic to non-target organisms as well. Plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumer. In our study, in-vitro antifungal potentiality of leaf extract (aqueous and ethanolic) of Saraca indica was tested against Fusarium solani and Fusarium moniliforme through poisoned food technique and was compared with leaf extracts of Azadirachta indica for five different concentrations(10%, 20%, 30%, 40% and 50%). Ethanolic extract of Saraca indica was found to be more efficient showing excellent inhibitory effect, 77.22% for Fusarium solani and 80% for Fusarium moniliforme at 50% concentration, in comparison to that of Azadirachta indica,70.83% and 72.77% respectively. The results indicate that some common plants could be exploited in developing a potent plant based fungicides which can be used in organic farming for the eco-friendly management of fusarium species.***

Keywords: Leaf extracts, *Fusarium solani, Fusarium moniliforme, Azadirachta indica*, Poisoned food technique

1. Introduction

Among the greatest hazards in crop-production, diseases and pests are the main problems. *Fusarium* species are the best known soil borne plant pathogens in terms of economical damage in agricultural productions all over the world[1] – [3] .Dry rot on potato, wilting and decline on bean or pea, crown rot and head blight on wheat, bakanae disease on rice caused by *Fusarium* species result to yield losses in most crop fields[4].*Fusarium solani* (Mart.) Sacc., a soil inhabiting pathogen, attacks a large number of host plants, including oilseeds pulses, vegetables and ornamentals [5] – [8].*Fusarium* dry rot of seed tuber can reduce crop establishment by killing developing potato, where crop losses can be up to 25%, while more than 60% of tubers can be infected in storage [9]. Many methods such as chemical, cultural and biological techniques have been developed forthe control of plant disease by soil borne pathogens [10]. The efforts were made in other regions to manage the *Fusarium* species through phytoextracts [11] – [15] in various crops.

Saraca indica, commonly known as Ashoka and one of the most common tree of India, is an evergreen tree with numerous spreading and drooping glabrous branches and has been extensively used in Ayurveda. This plant has shown many pharmacological properties such as antidiabetic [16], antiulcer [17], anthelmintic [18] and antimicrobial [19] – [20] activities.

The present study is designed to evaluate the antifungal properties of Ashoka tree against destructive soil borne plant pathogenic fungus *Fusarium* and also the percentage inhibition is compared with *Azadirachta indica*(Neem- a well known antibacterial and antifungal agent).

2. Literature Survey

Azadirachta indica, commonly known as a Neem, found throughout India is the most extensively used tree in the field of medicine. It is also used world-wide to control different plants diseases and pests. Study shows that growth of soil borne pathogens (*Fusarium oxysporumf*. sp. *cicero*, *Rhizoctonia solani*, *Sclerotium rolfsii* and *Sclerotinia sclerotiorum*) in liquid medium was inhibited by extracts of leaf, trunk, bark, fruit pulp and oil of neem[21]. Aqueous leaf extracts of neem exhibited considerable control of *Fusarium oxysporum*disease development in banana [22].

*Saraca indica*is also a massive tree found throughout India and used in Ayurveda. The bark of this plant is used as astringent, demulcent, emollient and stomachic. The leafis used to treat stomachalgia and flowers are used for treating of syphilis, inflammation, dysentery, haemorrhoids and scabies in children [23]. It is also screened to control microbes (fungi and bacteria) causing different plant diseases leading to a great crop loss. This paper screens the antifungal properties of leaf extracts *Saraca indica* against some soil borne pathogens escpecially *Fusarium* species to be used as fungicide next to *Azadirachta indica*.

3. Material and Methods

Plant materials

Fresh leaves of two plants, *Azadirachta indica* and *Saraca indica*were collected from local area of Patna. The plants were authenticated by taxonomist from Botany Department, P.U., Patna.

Extract preparation

Collected fresh leaves of the aforesaid plants were washed separately with tap water followed by sterile distilledwater and dried in shade. The dried materials were finally grinded

to powder, sealed in polythene bags and stored away from light and moisture until used for extraction[24] – [26].

Aqueous extract- 50gm of each material was soaked in 200ml of distilled water for 30 minutes and then boiled to half volume. After cooling, filtered with muslin cloth followed by Whatman filter paper no.-1 and kept in dark glass bottles at 5 °C in a refrigerator.

Ethanolic extract-50gm of each material was homogenized in 200ml mixture ofethanol anddistilled water (50:50, v:v) and left in dark bottlesfor72hrs on shaker.Then extracts were filtered with muslin cloth, followed by Whatman filter paper no.-1in other dark bottles and exposed to 60 °C in water bath for 30 minutes for ethanol evaporation. Extracts were stored in dark bottles at 5°C.

Isolation of pathogen

Fusarium species were isolated through serial dilution culture technique of soil collected from vegetable crop fields. PDA(Potato Dextrose Agar) was used as growth medium. Further a pure culture of each colony type, growing in petri plates, was obtained and maintained by sub-culturing. The temperature was maintained at 25 ± 2 °C. Cultures were identified on the basis of macro and microscopic characteristics, reverse surface coloration of colonies, conidial morphology and slide culture technique[27] – [31].The technique of slide culture was also used to identify the species of *Fusarium*[32] which allow the direct microscopic observation of morphological structure of taxonomic value.

Identification of *Fusarium* species as *Fusarium solani* and *Fusarium moniliforme* was also confirmed by IARI, New Delhi.

Figure 1: Different fungal-colonies growing in PDA (serial dilution culture of soil)

Figure 2: Isolation of *Fusarium* species from petri plates of soil culture.

Antifungal screening

Different concentration viz-10%, 20%,30%, 40% and50% of leaf extracts (both aqueous and ethanolic) of aforesaid plants were screened for their antifungal activities through poisoned food technique [33].Proportionate amount of leaf extracts of two botanicals were added to separate flasks containing media to prepare media having 10% -50% concentration of leaf extracts. To avoid bacterial contamination an antibiotic (Chloramphenicol, 0.10 mg/l) was supplemented to the media.20 ml of media was poured into petri plates, allowed to solidify and inoculated individually with 5mm diameter discs of the tested *F. solani* and *F. moniliforme*. Plates with media, not supplemented with leaf extracts and inoculated with *Fusarium* species, served as negative control. After seven days of incubation at 25 ± 2°C, orthogonal measurements of colonies were taken using the control plates as a reference. The percentage inhibition of growth was calculated according to following formula-

% inhibition = (dc-dt /dc) 100

Where, dc=Average increase in mycelial growth in control.
dt= Average increase in mycelia growth in treatment [34].

The two botanicals were also screened for antifungal activities in combination of 10% and 20% each and the two combination with a fungicide in a very small percentage (Bebestin, 0.5% of mother solution-5gm/l).

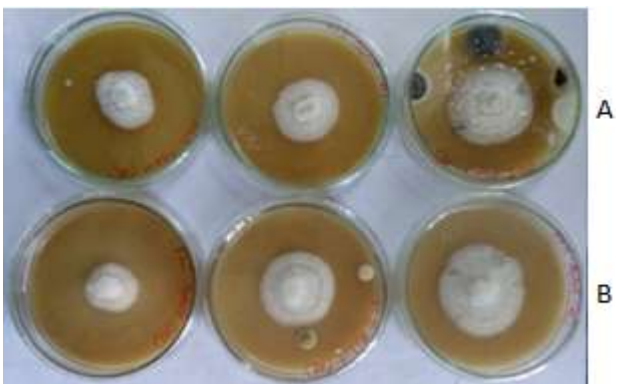

Figure 3: Comparative study of the inhibitory effect of leaf extracts of *A. indica (A)* and *S. indica (B)* on *Fusarium*

Figure 4a Figure 4b

Figure 4: Growth of *Fusarium* in 50% alcoholic extract of *A. indica* (4a) and *S. indica* (4b)

4. Results and Discussion

Results presented in Table 1, 2, 3, 4 and 5 show the inhibitory effect of aqueous (Table-1 and3) and ethanolic (Table-2, 4 and 5) leaf extracts of the two botanicals against *Fusarium* species. Effects of the leaf extracts of the two botanicals were shown in Table 1 and 2 on *Fusarium solani* and in Table 3 and 4 on *Fusarium moniliforme*. For both the plant extracts radial growth of tested fungi decreased significantly with increased concentration of added extracts. The interesting observation was that ethanolic extracts were found more effective then aqueous one and also *Saraca indica* was found more effective in both the conditions. Also the botanical control of *Fusarium moniliforme* was more than *Fusarium solani*, *as* 50% ethanolic leaf extract concentration of *Saraca indica*inhibited 80% of the mycelial growth of *Fusarium moniliforme* (upto 77% mycelial growth inhibition of *Fusarium solani*).The result could be used as an indicator to exploit *Saraca indica*, a common medicinally important evergreen tree, as a fungicide.

The two botanicals leaf extracts were also tested for their inhibitory effects in a combination (Table-5). There were four formulations. In first, PDA medium had 10% leaf extracts of each plant and in the second one it had 20% each. The two botanicals showed a superior inhibitory effects on radial fungal growth in their mixed combination. In third and fourth formulations, 0.5% of Bebestin (a fungicide) was supplemented to aforesaid first and second formulations and these showed 100% control of *Fusarium* species in 20% leaf extract preparation with Bebestin (fourth formulation). Even in 10% each botanical leaf extracts + 0.5% Bebestin combination, there was a 100% inhibition for *Fusarium moniliforme* and 77.78% for *Fusarium solani* which was also a remarkable result.

Table 1: Inhibitory effect of aqueous leaf extracts of Azadirachta indica and Saraca indica on mycelial growth of Fusarim solani

	Azadirachta indica		*Saraca indica*	
Different concetration of aqueous leaf extracts	Mycelial growth in mm	% Inhibition	Mycelial growth in mm	% Inhibition
10%	82.5	8.33	81	10
20%	77.25	14.16	77	14.44
30%	66.5	26.11	63	30
40%	56	37.77	35.5	40.55
50%	44	51.11	38	57.77

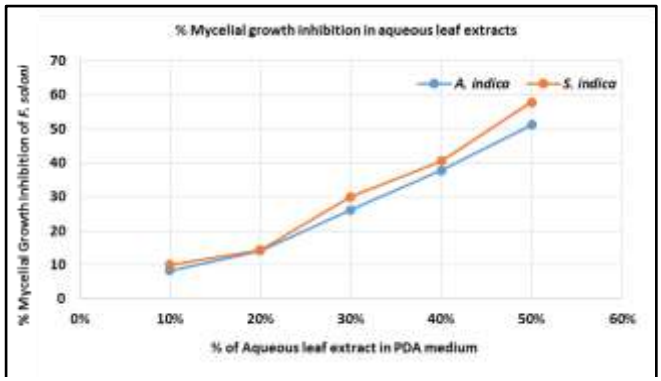

Figure 5: Graphical representation of Table-1

Table 2: Inhibitory effect of ethanolic leaf extracts of Azadirachta indica and Saraca indica on mycelial growth of Fusarim solani

	Azadirachta indca		*Saraca indica*	
Different concentration of ethanolic leaf extracts	Mycelial growth in mm	% Inhibition	Mycelial growth in mm	% Inhibition
10%	77.5	13.88	74	17.77
20%	68.0	24.44	64	28.88
30%	58.5	35.00	53.25	40.83
40%	37	58.88	34.5	61.66
50%	26.25	70.83	20.5	77.22

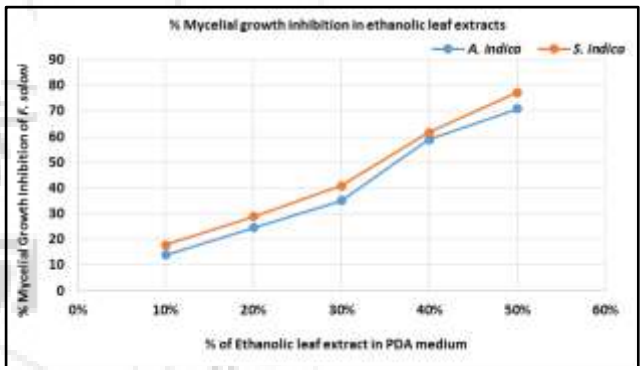

Figure 6: Graphical representation of Table-2

Table 3: Inhibitory effect of aqueous leaf extracts of Azadirachta indica and Saraca indica on mycelial growth of Fusarium moniliforme

	Azadirachta indica		Saraca indica	
Different concentration of aqueous leaf extracts	Mycelial growth in mm	% Inhibition	Mycelial growth in mm	% Inhibition
10%	81	10	78	13.33
20%	73.5	18.33	72	20
30%	63.5	29.72	62	31.11
40%	50	44.44	48.5	46.11
50%	41	54.44	36	60

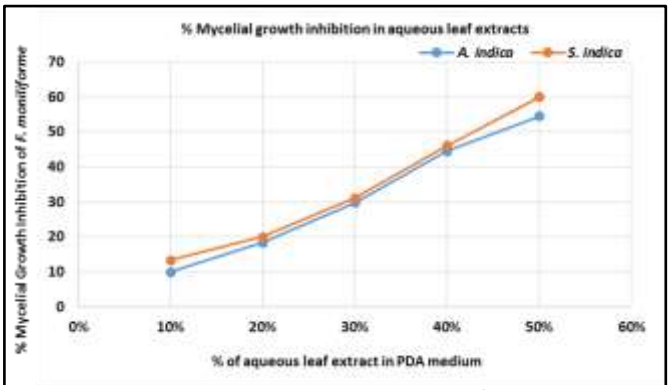

Figure 7: Graphical representation of Table-3

Table 4: Inhibitory effect of ethanolic leaf extracts of Azadirachta indica and Saraca indica on mycelial growth of Fusarium moniliforme.

	Azadirachta indica		Saraca indica	
Different concentration of ethanolic leaf extracts	Mycelial growth in mm	% Inhibition	Mycelial growth in mm	% Inhibition
10%	70	22.2	63	30
20%	60	33.33	51.25	43.06
30%	48.5	46.11	44.25	50.83
40%	36	60	30.5	66.11
50%	24.5	72.77	18	80

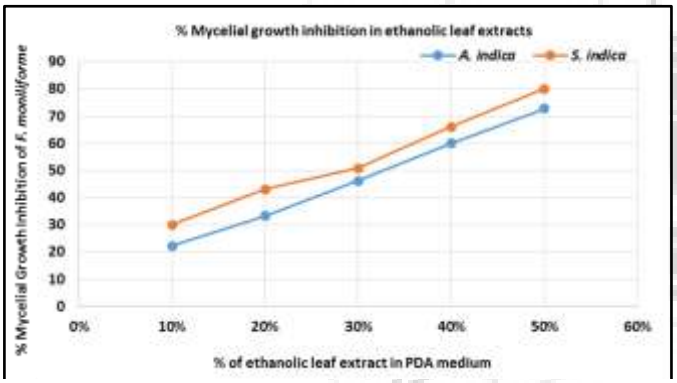

Figure 8: Graphical representation of Table-4

Table 5: Inhibitory effect of ethanolic leaf extracts of Azadirachta indica and Saraca indica on mycelial growth of Fusarium solani and Fusarium moniliforme

	Fusarium solani		*Fusarium moniliforme*	
Concentrati-on of leaf extracts of *A. indica* and *S. indica*	Mycelial growth in mm	% Inhibition	Mycelial growth in mm	% Inhibition
10% of each	63	30	53.25	40.83
20% of each	36	60	31.5	65
10% of each +0.5% Bebestin	20	77.78	00	100
20% of each +0.5% of Bebestin	00	100	00	100

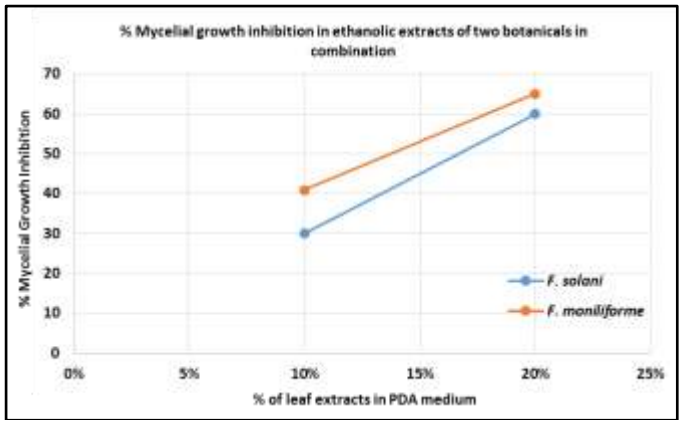

Figure 9: Graphical representation of Table-5 for leaf extract combination

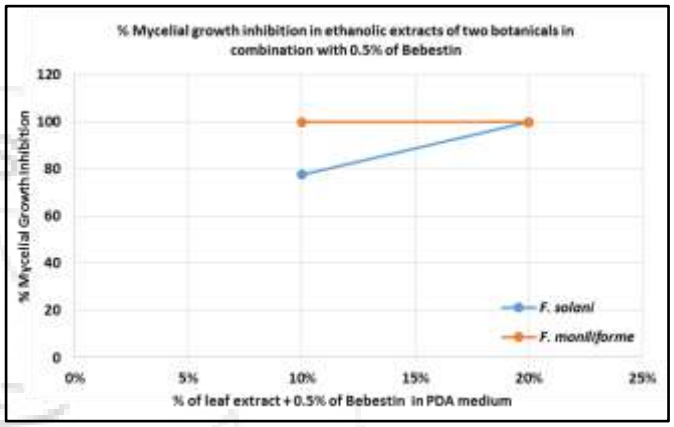

Figure 10: Graphical representation of Table-5 for leaf extract combination with 0.5% Bebestin

5. Conclusion

This study was focused on screening the antifungal activity in some common botanicals to use as natural, harmless fungicide, aiming at partial or total replacement of chemical synthetic fungicide to control soil borne diseases in vegetable crops. A chemical fungicide, Bebestin, at very low percentage (0.5% of mother solution 5gm/ l) show 100% control in combination with 20% ethanolic extracts of both plants (Table-5).

6. Future Scope

The results obtained in the present study reflect light on successful development of safe and cheap compound as antifungal which provide a potent tool to control not only *Fusarium* species but also for controlling other soil borne pathogens and getting success in the avoidance of environmental pollution and side effect of fungicide.

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