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EVALUATION OF SOME HIGHER PLANTS FOR THEIR ANTIFUNGAL ACTIVITY AGAINST RED ROT OF SUGARCANE

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ABSTRACT

Two hundred forty eight samples, comprising various parts of 170 plant species of 149 genera belonging to 66 familes, were screened against the test fungus *Collectotrichum falcatum*. Only the fruit extract of *Xanthium strumarium* Linn exhibited 100% fungitoxicity. Other plant extracts showed either poor fungitoxicity or some accelerated the mycelial growth of text fungus. The hydroethanolic extract of fruit was fractionated by different solubility method and only the petroleum ether extract was found to have fungitoxicity. MIC of the active fraction was worked out (1200 PPM) and at this concentration it was fungicidal in Nature. The active fraction, at MIC was able to withstand heavy inaculum density; killed the test pathogen in 1:30 hrs of exposure; could be more effectively used at any pH between 4 and 9; had a broad antifungal spectrum and proved to be superior than other commercial fungicides. The active fraction proved to be nonphytotoxic and it stimulated the rate of sprouting of setts and seedling growth of *Saccharum officinarum*.

KEYWORDS: Antifungal*, Collectotrichum falcatum, Xanthium strumarium*.

Use of chemicals (synthetic compounds) to control plant diseases and pests, is not preferred now days because these chemicals reach in biosystems through food chain and cause several disorders, even carcinogenicity and teratogeniciyty in humen beings. In comparison to synthetic compounds, the chemical of Plant origin are less phytotoxic, more systemic and easily biodegradable and have little or no side effects. This fact inspired to find out the antifungal activity in various available parts of some angiospermic plants against test fungus *Colletotirchum falcatum* ,the causal organism; Red rot of sugarcane.

MATERIALS AND METHODS

The pathogen was collected from the diseased sugarcane plants which were brought in the lab. and symptoms of *Colletotrichum falcatum* were identified by examining the morphology of the spots. The pieces of leaves (1^2cm.) were cut in square shape keeping the spot of disease in the middle of the square. These pieces were dipped in .01% $HgCl_2$ solution (w/v) for one minute to sterilize the surface of the piece. The surface sterilised pieces were washed thoroughly and repeatedly with sterile water to remove toxic and poisonous $Hgcl_2$(mercuric chloride). Ruptured cells on the border of square pieces of leaves absorbed mercuric chloride solution during the process of surface sterilization which could not be removed just by washing with sterile water and might affect the results of the experiment. Therefore the cells on the borders of the surface sterilized square pieces of the leaves were cut again from a sizable distance of the disease spot, with a sterilized blade, to be sure that even remains of mercuric chloride were not there to cause problem in the experiments afterwards. These pieces (explants), carrying the disease spot, were transferred on agar plates. Czapek dox agar medium (Thom and Raper, 1945) was chosen to isolate the test pathogen as this medium is very much suitable for mycelial growth. The compositon of the medium was as follows:

Sr. No.	Components	Amount
1	$NaNO_3$	3.00 g.
2	K_2HPO_4	1.00 g.
3	$MgSo_4.7H_2O$	0.50 g.
4	KCL	0.50 g.
5	$FeSO_4.7H_2O$	0.01 g.
6	Sucrose	30.00 g.
7	Agar Powder	15.00 g.
8	Distilled Water	1000 ml.

To prepare the medium above mentioned components (No.1 to 7) were weighed and dissolved in 1000 ml. of distilled water. The medium was sterilized for 20 minutes in an autoclave at 15 lbs/inch^2 pressure and 121^{o}C temperature. Thereafter it was cooled to about 50^{o}C. In order to prevent bacterial contamination, the antibiotic Ciprofloxacine was added to the medium at the rate of 30 mg./litre and mixed thoroughly, following the prescription of Gupta and Banerjee (1970), who used Streptopenicillin in his experiments. This medium was poured aseptically in presterilized petriplates and these agar-plates were left at room temperature for 48 hrs. to watch the contamination, if any. During this period, those plates were discarded in which any fungal contamination took place. The agar plates which were devoid of contamination(s) were selected and the explants (having spots of the disease) were aseptically transferred to the surface of this medium and incubated for 7 days at 37^{o}C (+1^{o}C). The fungus, hidden in the spot, grew during this

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period. It was reincoculated on fresh sterilized agar plate for identification and to get its pure culture.

Out of 248 samples comprising various parts of 170 plant species of 149 genera belonging to 66 families screened, the fruit extract of *Xanthium strumarium* Linn. Exhibited 100% fungitoxicity. Other tested plant part extracts showed either poor fungitoxicity or some accelerated the mycelial growth of the test pathogen. *Xanthium strumarium* Linn. is widely distributed in this locality and has not yet been evaluated for its fungitoxic properties against the fungal pathogen *Colletotrichum falcatum.*

The fractionation was done by differential solubility method. 20.0g. of fruits of *Xanthium strumarium* Linn. was crushed to pulp in a warring blender; 100ml. of 50% ethanol (v/v) was added, stirred with a glass rod and left overnight for the extraction of the contents in the pulp. Now the pulp in ethanol was filtered through a filter paper to get the extract containing compounds of the fruits. This extract was taken in a separating funnel and to it 20ml. of petroleum ether was added and mixed thoroughly. The compounds of ethanolic extract, which were soluble in petroleum ether, gradually diffused into the solvent and formed a distinct layer on the top of the ethanolic layer. This layer of petroleum ether was separated from the separating funnel and collected in a flask. Repeatedly petroleum ether was poured, mixed and separated till the formation of coloured layer of petroleum ether was stopped. Thereafter the remainder of ethanolic extract was treated with 20ml. of benzene. The compounds soluble in this solvent gradually diffused in and the layer of benzene was separated from the separating funnel. Benzene was poured, mixed and separated repeatedly till the coming off of colour into benzene layer was stopped. The benzene extract was also collected in another flask. In this fashion the remainder of the previous fractionation was treated with particular organic solvents i.e. carbon tetrachloride, chloroform, acetone and methanol, one by one, in the sequence from non-polar to polar ones to get the compounds in respective fractions. Then all the fractions were tested for antifungal activity against the test pathogen by the usual modified paper disk technique.

RESULTS AND DISCUSSION

The results indicated that the active fraction was contained in petroleum ether which inhibited the test pathogen completely. Different concentrations of the constituents of the active fraction, the solvent petroleum ether was evaporated at room temperature; the remainder was weighed on a chemical balance and again dissolved in a known volume of the solvent (10% methanol) to get a concentrate solution of 10,000 ppm. This was further diluted, as per requirement i.e. 500 ppm. to 5,000 ppm., and subjected to antifungal bioassay by the poisoned food technique adopted by Grover and Moore (1962). The data on percent mycelial inhibition were recorded in table 1.

Table 1: Effect of various concentrations of the active fraction on percent mycelial inhibition of the test fungus.

Concentrations (ppm.)	% Mycelial inhibition
500	90.00
1000	94.46
2000	100.00
3000	100.00
4000	100.00
5000	100.00

The fraction at 2000 ppm. onward completely inhibited the mycelial growth of the test fungus indicating its MIC between 1000 and 2000 ppm. Again different concentrations of the active fraction were made between 1000 and 2000 ppm and assayed against the test fungus.

The efficacy of the active fraction at its MIC on increased inoculam density was determined by increasing the number of fungal disks in the multiples of 5. Different sets, each with 1200 ppm. of the constituents of active fraction with respect to czapek dox medium (without agar) were prepared. Different desired numbers of mycelial disks were separately inoculated to different sets. The controls contained a single mycelial disk in the liquid czapek dox medium. The treatments as wll as controls were incubated at 37^{o}C (+1^{o}C) for six days and then observations were recorded on the seventh day. The active fraction at its MIC completely inhibited the mycelial growth of the test fungus in all the sets indicating its efficacy to withstand heavy inoculum density.

Fungal disks, cut from the periphery of the test fungus, were treated with 1200 ppm. (fungicidal concentration) of active fraction keeping them immersed for different periods of time. The treated disks were washed thoroughly with sterilized distilled water and inoculated in presterilized petriplates containing sterilized czapek dox agar medium and incubated for six days at 37^{o}C (+ 1^{o}C). The observations were recorded on the seventh day. The active fraction, at its MIC (fungicidal concentration), was found to kill the test fungus in 1.30 hour.

Studies were made to see if fungitoxicity of the active fraction could be increased by amending its original pH 7 to acidic or alkaline sides. Two lots of czapek dox agar medium were prepared in buffer solutions.The media thus prepared were separately mixed thoroughly with requisite amount of the constituents of active fraction to get the final concentrations of 1200ppm. (fungicidal). Controls with same pH were prepared by adding an equal amount of sterilized water in place of the active fraction. The antifungal bioassay was done by the usual poisoned food technique and the observations were recorded on the seventh day. At pH 4 & 9 there was complete inhibition of the mycelial growth of the test pathogen at MIC (fungicidal concentration) of the active fraction. Thus the active fraction can be more effectively used at any pH between 4 & 9.

The antifungal spectrum of the active fraction at its fungicidal concentration was determined against 10 pathogenic fungi, collected from different sources. The fungitoxic bioassay was undertaken by the usual poisoned food technique. The sensitivity of the pathogenic fungi to the active fraction was determined by calculating the toxicity index of each fungus on the basis of their percent mycelial inhibition at fungicidal concentration.

Table 2: Fungitoxic spectrum of the active fraction.

Fungi tested	% mycelial inhibition at MIC (1200 ppm.)
Alternaria brassicae	100.00
A. rumphi	100.00
A. solani	100.00
Aspergillus flavus	50.00
A. fumigates	66.67
A. niger	63.33
Cercospora personata	100.00
Colletotrichum falcatum	100.00
Helminthosporium oryzae	100.00
Pyricularia oryzae	100.00

The table,2 indicates that the active fraction at its MIC i.e. fungicidal concentration (1200 ppm.) completely inhibited all the tested fungal pathogens except the species of *Aspergillus*. However, the three tested species of *Aspergillus* were inhibited upto some extent and thus the active fraction shows a broad antifungal spectrum Tripathi and Tripathi(2005) also reported similar findings. Different concentrations of some commercial fungicides were prepared with respect to the czapek dox agar medium and subjected to antifungal bioassay against the test fungus by the usual poisoned food technique. The minimum inhibitory concentration (MIC) of Bavistin was found to be 15000 ppm.; that of Blitox-50 was 12000 ppm.; of Karathane was 5000 ppm.; Brassicol and Dithane Z -78 was 4000 ppm. and Hinosan - 50 was 3000 ppm.. The efficacy of the active fraction was compared with these fungicides on the basis of their respective MICs. Phytotoxicity of the active fraction of the fruits was observed on the basis of its effect on sprouting of the setts and growth of the test plant, *Saccharum officinarum*. 1200 ppm. concentration was used for experimentation.

Effect on sprouting of sugarcane setts

Setts of Saccharum officinarum were soaked in 1200 ppm. solution of the active fraction for 5 hours. For control sets the setts were soaked in sterilized distilled water instead of active fraction. One sett was placed in an earthen pot filled with sterilized sand and five replicates were made for each treatment and control sets. These sets were kept in dark at room temperature. On 16 th day number of sprouted setts were recorded. The observations were recorded in table . 3.

Table 3: Effect of active fraction on the percent sprouting of setts of Saccharum officinarum

	Concentration of the	% sprouting of setts
Experiment	soaking solution	
Treatment	1200 ppm.	96
Control	sterile water	92

Sprouting of setts was not affected by the active fraction at its MIC 1200 ppm., rather it was stimulatory.

Effect on plantlet growth

The sprouted setts in the above experimental sets were planted in sterile soil and allowed to grow for a month (30 days) to observe the effect of the treatment of active fraction on shoot growth. The observations were recorded on the 31st day and the tentative growth in all the replicates was recorded in table , 4.

Table 4: Effect of the active fraction on plant growth

Experimental sets	Shoot growth (mm.)
1200 ppm (Treatment)	vigourous
Sterile water (Control)	Normal

The fungitoxic plant *Xanthium strumarium* Linn. Chosen for detailed investigation, is distributed through out the tropical parts of India. The plant is considered to be important from medicinal point of view but none report has been made, as yet, for its antifungal utility to control fungal pathogens causing diseases in our various

agricultural crops. Minimum inhibitory concentration (MIC) of some active fractions/compounds has been studied and found to vary considerably Shakil et al. (2004) found that the methanolic extract of Piper attenuatum inhibited *Agaricus bisporus, Penicillium* sp., *Aspergillus niger* and *Cladosporium dendroides* at 125 ppm concentration. Sadeghi-Nezad et al. (2007) reported that the ethanolic extract of the root of Ixora brachiata inhibited 14 isolates of pathogens at a concentration of 125 micro g/ml. In the present investigation, MIC of the active petroleum ether fraction was found 1200 ppm. The variation in the MICs of various fractions may either be due to the differences in the chemical compositions of the active constituents or due to differences in resistance of different test fungi.

Upadhyay (2008) found that the antifungal volatile oil of the leaves of *Ocimum basilicum*, at its MIC, was not affected by the increased inoculum density of the test fungus *Fusarium* sp. In the present study also, the increase of inoculum density exhibited no effect on the fungitoxicity of the active fraction of the fruits.

Saxena (1980) found that the fungitoxicity of oil of Putranjiva roxburghii seeds increased two folds when the pH of the oil was amended to alkaline side. Yadav (2002) found that the fungitoxicity of the active fraction of *Sarraca indica* increased at the amended pH 9 and the active fraction inhibited the test fungus (Cercospora personata) at its sublethal concentration i.e. 2000ppm.

Upadhyay (2008) found that the fungitoxicity of the volatile oil of *Ocimum basilicum* leaves at the amended pH 9 increased and it inhibited the test pathogen *Fusarium* sp. even at the sublethal concentration i.e. 625 ppm. In the present work also, studies were made to see if increased fungitoxic activity can be obtained by amending the pH of the active fraction. It was found that fungitoxicity of the active fraction was not affected at the amended pH 4 and 9 and the test pathogen was completely inhibited.In the present comparative study the active fraction of *Xanthium strumarium* Linn. fruits proved to be more potent- about 13 times more efficaceous than Bavistin; 10 times than Blitox - 50; about 4 times than Karathane; about 3 times than Brassicol and Dithane Z - 78 and more than 2 times than Hinosan - 50.

Rosca-Casian et al. (2007) investigated the fungicidal concentration of hydroethanolic extract of Aloe vera leaves against the mycelial growth of Botrytis gladiolorum, *Fusarium oxysporum* f.sp.*gladioli*, *Heterosporium pruneti* and *Penicillium gladioli* on czapek dox agar medium and found that this concentration varied between 80-100 micro l/ml, depending on fungal species.

Tripathi et al., (1978) After fractionation all the fractions were assayed against the test pathogen and only petroleum ether fraction was found to possess antifungal activity.

The findings thus suggest that the active fraction of the fruits of *Xanthium strumarium* Linn., having strong fungitoxic activity, efficacy to withstand heavy inoculum density, broad antifungal spectrum, superiority over several commercial fungicides and non phytotoxicity, can be exploited as a potent fungicide against plant pathogens.

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