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RESEARCH ARTICLE

EFFICACY OF FUNGICIDES AND ANTIBIOTICS AGAINST SPORE GERMINATION AND SPORULATION OF *COLLETOTRICHUM FALCATUM* WENT; CAUSING RED ROT DISEASE OF SUGARCANE *IN-VITRO* AND *IN-VIVO* CONDITION.

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ABSTRACT

The experiment was carrying out an antibiotic and fungicides were tested for control of sugarcane red rot disease causing, *Colletotrichum falcatum* Went. A comparative study on chemotherapy test has been found most effective to several antibiotic and fungicides. The maximum inhibition were found bavisten 98% followed by vitavax 91% inhibition and minimum inhibition were found an aureafungin 87% followed by streptomycin 83% spore germination of fungicides, at 100 ppm concentration. In aspects of minimum mycelia growth inhibition was observed in case of fungicides and it was found that gradual growth increase in inhibition with increasing concentration. Bavistin was found most effective in checking the mycelia growth at 50 ppm concentration, Bavistin not only check the growth of mycelium of *C. falcatum* but sporulation also at 50ppm concentration. Among remaining chemicals as carbendazim, streptomycin and brasical both of the growth and sporulation was checked considerable degree even at 50 to 100 ppm conc. while brasical complete check of sporulation at 100 ppm conc. whereas, carbendazim & streptomycin could check the sporulation only at 400 ppm conc. In second test of *in-vitro* condition after showing cane 3-bud setts treated with 0.2% solution of bavistin gave best result as there was more than 80% reduction of incidence in all the treatment. None of other fungicides completely inhibited fungus growth of *C. falcatum*. The results showed a significant increase in the inhibition of mycelia growth with an increase in fungicidal concentration.

Key Words **: Sugarcane, red rot, *Colletotrichum falcatum,* chemical control.**

Sugarcane (*Saccahrum officinarum* L.), is one of the most important cash crop of Indian, plays enormous role in the economy of India. It is grown in the tropical and sub tropical regions of world. Sugarcane disease red rot caused by *C. falcatum* Went is one of the major constraints in the profitable cultivation (Martin *et al*., 1961 and Chona, 1980). Among various factors responsible for low yield, fungal diseases are the major cause gaining international importance. Over 100 fungi, 10 bacteria, and 10 viruses and about 50 species of nematodes are pest of sugarcane in different part of the world (Subhani, *et al*., 2008). In fungal disease, red rot has become a major problem for the sugarcane growing countries (Tiwari *et al*., 2010). Only red rot is causing economic losses to the crop and major incidence in sugarcane growing areas of India as well as Uttar Pradesh. The incidence of the disease varies from 5–100 per cent annual loss depending upon the locality and variety of sugarcane. Red rot is responsible for failure of many popular varieties in different countries (Satyavir, 2003). Disease management is based not only on the principles of eradication of the pathogens, but mainly on the principle of maintaining the damage or loss below an economic injury level or at least minimizing occurrence of a disease above that level. Management suggests need for continuous adjustment in the cropping system (Apple, 1977). The various control methods can be classified as regulatory, cultural, biological, physical and chemical depending on the nature of the agents employed. Certain antibiotics and fungicides including systemic canes were used to find out some effective chemicals which may control the infection of red rot pathogens. Various chemotherapeutant were first screened *in vitro* against the pathogen with a view to select the most effective ones for the *in-vitro* experiments. The main objective of present studies was to find out comparative efficacy and specificity of the fungicides and antibiotics against the *C. falcatum* to obtain economical control of this disease.

MATERIALS AND METHODS

The efficacy of the Antibiotics, Systemic and Non–Systemic fungicides were assayed under *in-vitro* and *in-vivo* condition. Thirty isolates of *C. falcatium* out of these three isolates were found most effective in our experiment (Paper has been accepted in Acta phytopathologica). Which isolates of *C. falcatum* were obtained disease of sugarcane following routine mycological techniques with maintained on OMA slants. Twelve Systemic and Non–Systemic fungicides and three antibiotics each at 10, 50, 100, 500, 1000, 1500 and 2000 ppm concentration were selected. These fungicides and antibiotics were mixed separately in the sterilized OMA medium in such a way to get the final desired concentrations. A disc (5 mm diam) obtained from the periphery of 7 days old fungal colony of the pathogens grown on OMA medium was placed in the center of the Petriplate. After 7 days incubation at 29±2^{0}C the redial growth of the pathogen was measured. The OMA medium without having any antibiotic and fungicide served as control. All the experiments were performed in triplicates and percentage inhibition of *C. falcatum* Went colonies in each treatment was recorded over the control and calculated by using following formula :

$$\text{Inhibition \% of } C.\ falcatum = \frac{\text{Colony growth in control} - \text{Colony growth in treatment}}{\text{Colony growth in control}} \times 100$$

The data were analyzed by Simple Factorial Design (Steel *et al*., 1996) with four replications was used to determine the difference between individual treatments i.e., antibiotics systemic and non-systemic fungicide and their doses.

RESULTS AND DISCUSSION

The Results obtained from the present investigation has been discussed below :

Hanging drop techniques:

The studies were made at room temperature employing at different concentration of fungicide and antibiotics. The pathogens of spores were allowed to germinate in the hanging drop technique of the respective concentrations of the chemicals with sterile distilled water was maintained. Percentage germination of spores was observed after 24 hours. The results incorporated in Table 1 represent the mean of three replicates in whole numbers.

The data indicates that the antibiotics gave comparatively better results than several fungicide tested. At 500 ppm concentration all the three antibiotics gave

94 % to 100 % inhibition in spore germination whereas only two out of twelve fungicides proved to be equally efficient with increasing concentration of the chemicals inhabitation in spore germination gradually increased reaching to 82-100 % at 100 to 2000 ppm concentration. However, there was recorded per cent inhibition in all the cases. Bavistin was giving 100% and Vitavax 91% inhibition at 100ppm concentration among the fungicides and of the three antibiotics Aureofungin, Agrimycin and Strepotcycline with 87%, 80% and 83% inhibition at 100 ppm concentration respectively.

Food poison technique :

Evaluation of selected chemicals was done by poisoned food technique following (Schmitz, 1930; Carpenter, 1942; Grover and Moore, 1962) followed by Gupta (1980). The desired amount was added to the oat meal agar medium after autoclaving so as to have different concentration of the chemicals. Corresponding controls were also maintained. The plates after inoculation were incubated at 28 ± 2^0C for seven days. The diameter of the colony was measured

Table 1 : Efficacy of fungicides against spore germination of *Colletotrichum falcatum* W. in vitro control

Sr. No.	Chemicals	Spore germination percentage					
		10 ppm	50 ppm	100 ppm	500 ppm	1000 ppm	1500 ppm
A- Antibiotics							
1.	Agrimycin-100	38	30	20	06	00	00
2.	Aureofungin	40	11	13	00	00	00
3.	Streptocycline	41	24	17	00	00	00
B- Fungicide							
1.	Agallal	52	50	30	24	15	05
2.	Aretan	51	50	50	26	20	04
3.	Benelate	23	14	10	07	04	00
4.	Bavistin	15	05	00	00	00	00
5.	Blitox-50	55	52	32	21	05	00
6.	Brassicol	52	51	32	30	14	04
7.	Cuman	49	28	26	12	00	00
8.	Discon-Z	52	37	28	20	03	00
9.	Thiram	56	54	39	20	18	04
10.	Vitavax	49	27	09	00	00	00
11.	Ceresan	52	50	37	28	16	02
12.	Dithane M-45	53	51	20	18	12	00
	Contorl	54	-	-	-	-	-

Table 2 : Effect on selected chemicals by food poison technique observation based on 3-set of experiments

Sr. No.	Chemical	Chemical in ppm concentration							
		50.0ppm		100.0ppm		400.0ppm		800.0ppm	
1.	Carbendazim	Colony (diam)	% Inhibition	Colony (diam)	% Inhibition	Colony (diam)	% Inhibition	Colony (diam)	% Inhibition
2.	Streptocycline	2.6	52.0	1.4	74.6	0.4	92.8	0.0	100.0
3.	Bavistin	4.2	20.4	2.8	39.4	1.2	78.9	0.0	100.0
4.	Brassicol (PCNP)	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
	Contorl 4.5	2.8	37.4	1.6	67.0	1.4	74.2	0.0	100.0

and mean observed of three replications.

The results recorded in Table 2 reveal that Bavistin was the most effective in checking the mycelial growth at 50 ppm concentration, the growth was completely arrested. This was followed by Carbendazim, Brassicol (PCNP) and Streptomycin which gave 52.0, 37.4 and 20.4 percent reduction respectively at the same concentration. At 800 ppm concentration, however, the entire four chemical checked the growth completely.

Modified paper disc technique :

Evaluation of the selected antibiotics and fungicides was also done by modified paper disc method of (Lakshman *et al*., 1973 & Gupta 1980). Fifteen mm assay discs were cut from what man 45 filter paper and each disc was impregnated separately with one ml solution of the respective solution in installments by repeated soaking and air drying. The assay discs were aseptically placed in the centre, one in each of the Petriplate containing sterilized medium corresponding controls (with discs soaked in distilled water) were also maintained and 2 mm disc of mycelia out from periphery of seven days old culture of the test fungus was aseptically placed in the centre of assay disc in the inverted position so that the fungus comes in direct contact with the disc. The observations were recorded in terms of colony diameter and the data obtained have been given in (Table 3). It is clear for the Bavistin alone checked the mycelial growth completely even at 50 ppm concentration. The chemicals remaining three were also effective but to a much lesser degree.

Food poison technique :

The ascertain to effect of antibiotics and fungicides *viz.* Carbendazim, Streptomycin, Bavistin and Brassicol (PCNP) on the growth and sporulation of the pathogen studies were carried out at different concentration *viz.* 50, 100, 400 and 800 ppm by the usual poisoned food technique along with control. Petri plates were incubated at 29±2^{0}C for seven days. The fungal growth as well as sporulation was observed and information recorded is presented in the (Table 4). Bavistin not only checked the growth of the fungus (*C. falcatum*) but sporulation also at 50 ppm concentration with the remaining three chemicals both the growth and the sporulation was checked to a considerable degree even at 50 and 100 ppm concentrations. In case of Brassical, the observation was complete check of sporulation at 100ppm concentration where as Carbendazim and Streptomycin could check the sporulation only at 400 ppm concentration respectively.

***In- vivo* experiment :**

In-vivo experiment was laid out in small plots in the field. Twenty, 3-bud diseased setts were soaked for 30 minutes in 0.2% solution of the selected antibiotics and fungicides *viz.* Carbendazim, Streptomycin, Bavistin and Brassicol. The treated as well as corresponding control setts both (healthy and disease) came were planted separately following the usual cultural practices. The diseased plants as soon as observed, were uprooted and destroyed in order to avoid secondary spread of the disease observation was presented in (Table 5). It is clear that Bavistin gave best results as

Table 3 : Effect on chemical by modified paper disc techniques observation based on 3-set of experiments

Chemical		Chemical in ppm concentration							
		50.0ppm		100.0ppm		400.0ppm		800.0ppm	
		Colony (diam) cm.	% Inhibition	Colony (diam) cm.	% Inhibition	Colony (diam) cm.	% Inhibition	Colony (diam) cm.	% Inhibition
1.	Carbendazim	3.6	30.3	2.4	52.3	0.4	96.5	0.0	100.0
2.	Streptocycline	2.8	36.4	1.7	59.2	0.8	85.2	0.0	94.0
3.	Bavistin	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
4.	Brassicol (PCNP)	3.8	32.6	1.8	69.3	0.6	80.7	0.0	100.
	Contorl 4.5	--	--	--	--	--	--	--	--

there was more than 80% reduction in the incidence of the disease. These are followed by Streptomycin, Carbendazim and Brassicol (PCNB). With all the treatments there was recorded some improvement in percentage germination of the care as compared to the diseased ones.

The preliminary screening of the chemicals was done following the slide germination technique of Reddick and Wallace (1910), and technique recommended by American Phytopathological Committee on standardization of fungicidal test 1944, followed by Gupta (1980). The fungicides were tested for red rot disease of sugarcane in which three fungicides Benomyl 50WP, Folicar and Randomil 75WP completely inhibited to the growth of *C. falcatum* at 5, 10, 20, and 50 µm mL^{-1} and complete inhibition in case of Tilet 250EC at a 20 to 50 µm mL^{-1} followed by Nimrod 25EC 5 µm mL^{-1} and gradual increase inhibition has found with significant increase in the inhibition of mycelial growth with an increase in fungicidal concentration for all tested fungicides (Subhani *et al*., 2008). The germination of conidia have been found uniolar and bipolar with 1 to 3 germ tubes. Bavistin and bleaching powder at 1, 5, 10 & 15 ppm concentration has been found 2.5 to 4.3% conidial germination after 46 hours of infection. However, at higher concentrations (20, 25 and 50 ppm), both tested chemicals completely inhibition of conidial germination. Bleaching powder effectively reduced setting mortality over inoculated check and red rot disease in varieties Co1148 and 10.0 to 18.0% in CoJ64 as against 100.00- 93.0% respective checks (Agnihotri *et al*., 1997). Five systemic and non-systemic fungicides have test into concentration *in-vitro* against *Ceratocystis paradoxa* causing sett rot of sugarcane disease. Systemic fungicides were found more effective in controlling the pathogen growth than that of non-systemic fungicides (Vijaya *et al*., 2007). In which systemic fungicides Carbendazim and Propiconazole were found most effective in complete inhibition of the pathogen at both concentration (0.05 & 0.1%). While non-systemic fungicides had tested Thiram was found best followed by Captan at both concentrations (0.1 to 0.2%), whereas, Mancozed and Copper oxychloride were less

Table 4 : Effect on chemicals of the growth and sporulation of *C. falcatum* W. observation based on 3-set of experiments

Chemicals		Concentration in ppm			
		50ppm	100ppm	400ppm	800ppm
1.	Carbendazim	++	++	+	0
2.	Streptocycline	++	++	+	0
3.	Bavistin	0.0	0.0	0	0
4.	Brasical (PCNB)	++	+	+	0
	Control +++	--	--	--	--

Where:
+++ = Growth and good sporulaltion
++ = Poor growth and sporulation
+ = Fungal growth only
0 = Growth completely checked

Table 5 : Effect of chemotherapeutants on the incidence of red rot disease of sugarcane

Sr. No.	Treatments	% Germination	% Disease incidence
1.	Carbendazim	26.5	35.4
2.	Streptocycline	28.2	28.8
3.	Bavistin	24.0	7.9
4.	Brassicol (PCNB)	23.0	40.3
5.	Untreated (Control)	18.7	88.6
6.	Healthy (Control)	34.3	0.0

effective.

Three different concentrations of each fungicide which included as Bavistin, Captan, Sulfex, Dithene M-45 and Thiram at 50, 250 and 500 ppm concentrations has tested for the radial growth of pathogen (*Fusarium moniliforme*), and in rot development and production of cellulytic and pectolytic enzymes in fruit disease of Amla. The complete inhibition of mycelia growth and rot development has observed by Bavistin (50ppm) and Dithane M-45 (250ppm) concentration. Amongst all the fungicides tested, Bavistin (50ppm), Sulfex, Dithene M-45(250ppm), Captan and Thiram, (500ppm conc.) could completely control the secretion of cellulolytic and pectolytic enzyme by the test of pathogen (Mehta, *et al.*, 2009).

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