Agriways 3 (2) : 95-102 (2015)

ISSN: 2321-8614 (Print)
ISSN: 2454-2318 (Online)

EFFICACY OF FUNGICIDES AND ANTIBIOTICS AGAINST SPORE GERMINATION AND SPORULATION OF *FUSARIUM MONILIFORME* SHELDON; CAUSING POKKAH BOENG DISEASE OF SUGARCANE

D.D.K. Sharma and Arun Kumar[1]

Mewar University, Gangrar Chitourgarh-312901 (Rajasthan)
[1]Department of Plant Pathology, Chaudhary Chhotu Ram (PG) College, Muzaffarnagar-251001 (UP)
E-mail : ddksharma@gmail.com

ABSTRACT

The experiment was carrying out at CCR (PG) Collage, Muzaffernagar with the collaboration of Indian Institute of Sugarcane Research, Lucknow during our investigation an antibiotic and fungicides were tested for control of sugarcane pokkah boeng disease causing, *Fusarium moniliforme* Sheldon. A comparative study on chemotherapy test has been found most effective to several antibiotic and fungicides. The maximum inhibition were found copper oxichloride 98% followed by Bavistin 91% inhibition and minimum inhibition were found an aureofungin 90% followed by streptomycin 77% 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. Copper oxichloride was found most effective in checking the mycelia growth at 50 ppm concentration, copper oxichloride not only check the growth of mycelium of *F. moniliforme* but sporulation also at 50ppm concentration. Among remaining chemicals as aureofungin, streptomycin both of the growth and sporulation was checked considerable degree even at 50 to 100 ppm conc. while bavistin complete check of sporulation at 100 ppm conc. whereas, aureofungin could check the sporulation only at 200 ppm conc. In second test of *in-vitro* condition after showing cane 3-bud setts treated with 0.2% solution of copper oxichloride gave best result as there was more than 98% reduction of incidence in all the treatment. None of other fungicides completely inhibited fungus growth of *F .moniliforme*. The results showed a significant increase in the inhibition of mycelia growth with an increase in fungicidal concentration.

Key Words **: Sugarcane, Pokkah boeng, *F. moniliforme*, 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 pokkah boeng caused by *F .moniliforme* is one of the major constraints in the profitable cultivation

(Govender *et al*., 2010 and Karapaiyan *et al.,* 2015). Among various factors responsible for low yield, fungal diseases are the major cause gaining international importance an 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 (Sharma and Kumar, 2015). Only pokkah boeng 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 1–90 per cent annual loss depending upon the locality and variety of sugarcane (Vishwakarma *et al*., 2013). Pokkah boeng is responsible for failure of many popular varieties in different countries (Karapaiyan *et al.,* 2015). 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; Patil and Hapase 1987). 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 pokkah boeng 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 *F.moniliforme* to obtain economical control of this disease. The most satisfactory measure for pokkah boeng is the use of resistant varieties by Bailley and Bechet (1982).

MATERIALS AND METHODS

The efficacy of the Antibiotics, Systemic and Non–Systemic fungicides were assayed under *in-vitro* and *in-vivo* condition. Twelve isolates of *F .moniliforme* out of these two isolates were found most effective in our experiment (Paper has been accepted in Acta phytopathologica). Which isolates of *F.moniliforme* were obtained disease of sugarcane following routine mycological techniques with maintained on PDA slants. Five Systemic and Non–Systemic fungicides and two antibiotics each at 50, 100, 150, 200 and 250 ppm concentration were selected. These fungicides and antibiotics were mixed separately in the sterilized PDA 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 PDA medium was placed in the center of the Petriplate. After 7 days incubation at 28±2^{0}C the redial growth of the pathogen was measured. The PDA medium without having any antibiotic and fungicide served as control. All the experiments were performed in triplicates and percentage inhibition of *F.moniliforme* Sheldon colonies in each treatment was recorded over the control and calculated by using following formula :

$$Inhibition\ \%\ of\ F.moniliforme = \frac{Colony\ growth\ in\ control - Colony\ growth\ in\ treatment}{Colony\ growth\ in\ control} \times 100$$

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

RESULTS AND DISCUSSION

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 200 ppm concentration all the two antibiotics gave 96 to 100 % inhibition in spore germination whereas

only two out of five fungicides proved to be equally efficient with increasing concentration of the chemicals inhabitation in spore germination gradually increased reaching to 82 - 100 % at 50 to 250 ppm concentration, however, there was recorded per cent inhibition in all the cases. Copper oxichloride was giving 100% and bavistin, 90% inhibition at 100ppm concentration among the fungicides and of the two antibiotics aureofungin and strepotcycline with 65% and 80% inhibition at 100 ppm concentration respectively.

Food poison technique :

In general % inhibition increased with the increase in the concentration of fungicides evaluation of selected chemicals was done by poisoned food technique following (Schmitz, 1930; Carpenter, 1942; Grover and Moore, 1962) followed by Raju and Lal (1977). The desired amount was added to the PDA medium before autoclaving so as to have different concentration of the chemicals. Corresponding controls were also maintained. The plates after inoculation were incubated at 28±2^{0}C for seven days. The diameter of the colony was measured and mean observed of three replications.

The results recorded in Table 2 reveal that Copper oxichloride was the most effective in checking the mycelial growth at 50 ppm concentration, the growth was completely arrested. This was followed by areofungin and streptomycin which gave 40 and 42% reduction respectively at the same concentration. At 200 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). Fifteen mm assay discs were cut from what man 45 filter paper and each disc was impregnated separately with 1 ml solution of the

Table 1: Efficacy of antibiotics and fungicides against spore germination *in-vitro* of *F. moniliforme* control

Sr. No.	Chemicals	Spore germination percentage				
A- Antibiotics		50 ppm	100 ppm	150 ppm	200 ppm	250 ppm
1.	Aureofungin	60	44	28	16	10
2.	Streptocycline	52	32	21	08	00
B- Fungicide						
1.	Bayleton	50	30	12	04	00
2.	Bavistin	50	26	12	02	00
3.	Blitox-50	48	28	14	06	00
4.	Copper Oxichloride	38	24	10	00	00
5.	Dithane m-45	52	32	14	06	00
6.	SE	5.32	2.77	1.90	2.14	00
7.	Control- 55	-	-	-	-	-

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

Sr. No.	Chemical 0.2%	Chemical in ppm concentration							
		50.0ppm		100.0ppm		150.0ppm		200.0ppm	
	Antibiotics &Fungicides	Colony (diam)	% Inhibition	Colony (diam)	% Inhibition	Colony (diam)	% Inhibition	Colony (diam)	% Inhibition
1	Aureofungin	2.6	20.0	1.8	76.6	0.4	90.8	0.0	100.0
2	Streptocycline	3.2	52.4	2.6	39.4	1.4	77.4	0.0	100.0
3	Copper Oxichloride	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
4	Bavistin	2.4	36.4	1.6	68.0	1.6	76.2	0.0	100.0
5	Control	4.5	--	--	--	--	--	--	--

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 the (Table 3). It is clear for the Copper oxichloride alone checked the mycelial growth completely even at 100 ppm concentration. The chemicals remaining three were also effective but to a much lesser degree.

Food poison technique :

The ascertain to effect of the both antibiotics and fungicides *viz.* Aureofungin, Streptomycin and Copper oxichloride, Bavistin on the growth and sporulation of the pathogen studies were carried out at different concentration *viz*. 50, 100, 150 and 200 ppm by the usual poisoned food technique along with control. Petri plates were incubated at 28±2^{0}C for seven days. The fungal growth as well as sporulation was observed and information recorded is presented in the (Table 4). Copper oxichloride not only checked the growth of the causal organism (*F.moniliforme*) 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 Bavistin, the observation was complete check of sporulation at 100ppm concentration where as aureofungin and streptomycin could check the sporulation only at 200 ppm concentration respectively.

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

Sr. No.	Chemical 0.2%	Chemical in ppm concentration 50.0ppm Colony (diam) cm.	50.0ppm % Inhibition	100.0ppm Colony (diam) cm.	100.0ppm % Inhibition	150.0ppm Colony (diam)cm.	150.0ppm % Inhibition	200.0ppm Colony (diam)cm.	200.0ppm % Inhibition
1.	Aureofungin	3.2	30.0	1.8	50.0	0.4	96.4	0.0	100.0
2.	Streptocycline	3.8	36.4	2.4	60.2	0.8	88.2	0.0	94.0
3.	Copper oxichloride	1.6	100.0	0.0	100.0	0.0	100.0	0.0	100.0
4.	Bavistin	3.6	34.6	1.6	76.4	0.6	80.4	0.0	100.
5.	Contorl	4.5	--	--	--	--	--	--	--
6.	SE								

Table 4: Effect on chemicals of the growth and sporulation of *F .moniliforme* Sheldon observation based on 3-set of experiments

Chemicals, 0.2%		Concentration in ppm 50.0ppm	100.0ppm	150.0ppm	200.0ppm
1.	Aureofungin	++	++	+	0
2.	Streptocycline	+++	++	+	0
3.	Copper Oxichloride	0.0	0.0	0	0
4.	Bavistin	++	+	+	0
5.	Control +++	--	--	--	--
6.	SE				

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

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.* Aureofungin Streptomycin, Bavistin and Copper oxichloride. 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 Copper oxichloride gave best results as there was more than 96% reduction in the incidence of the disease and these are followed by aureofungin, streptomycin and bavistin. With all the treatments there was recorded some improvement in percentage germination of the care as compared to the diseased ones.

The most satisfactory measure for pokkah boeng is the use of resistant varieties. It is reported that Dithane

Table 5 : Effect of chemotherapeutants on the incidence of pokkah boeng disease of sugarcane varieties

Sr. No.			Observations based on 3-bud setts of experiments				
	Treatments 0.2%	Variety	% Germination	% incidence	No of internodes	No. of cane each clamp	Each cane wait
1.	Aureofungin	CoS8436	43.56	6.6	24.2	16	0.950
		CoS8432	38.0	6.1	25.1	14	0.840
		CoS98259	39.42	6.2	24.6	11	1.10
		CoLk8102	43.5	6.3	25.4	15	0.900
		CoSe01434	46.33	6.4	24.8	18	0.900
2.	Streptocycline	CoS8436	43.26	8.6	24.1	14	0.900
		CoS8432	38.4	8.1	25.1	14	0.800
		CoS98259	39.2	8.2	24.6	12	0.750
		CoLk8102	43.6	8.3	25.4	16	0800
		CoSe01434	46.13	8.4	24.8	17	0.850
3.	Copper	CoS8436	43.16	4.1	24.0	18	0.980
	Oxichloride	CoS8432	38.0	4.6	25.1	14	0.830
		CoS98259	39.22	4.2	24.6	11	1.200
		CoLk8102	42.5	4.4	25.8	15	0.830
		CoSe01434	46.31	4.0	24.1	19	0.990
4.	Bavistin	CoS8436	43.46	10.2	24.3	15	0.960
		CoS8432	38.2	10.6	25.1	13	0.810
		CoS98259	39.12	10.4	24.2	10	1.000
		CoLk8102	43.1	11.3	25.0	14	0.810
		CoSe01434	45.4	10.5	22.8	17	0.900
5.	Control	CoS8436	36.4	16.4	22.2	9.0	0.640
		CoS8432	33.8	16.2	23.0	10.0	0.550
		CoS98259	34.8	16.1	21.8	8.0	0.800
		CoLk8102	36.2	14.8	22.4	9.0	0.625
		CoSe01434	38.5	15.2	21.2	10.0	0.760
6.	SE	CoS8436	3.11	4.63	0.879	3.361	0.140
		CoS8432	1.95	4.55	0.924	1.732	0.121
		CoS98259	1.98	4.57	0.812	1.516	0.183
		CoLk8102	3.14	4.11	1.316	2.774	0.032
		CoSe01434	3.39	4.26	1.442	3.563	0.083

M-45 proved to be the best fungicide for the control of *Fusarium oxysporium* isolated from post harvest decay of lemon. Raju and Lal (1977) reported that captain, thiram and duter were effective against *Fusarium moniliforme*. Chattopadhyay and Nandi (1977) reported Fytolan (Copper oxichloride) at 0.2% controlled malformation of mango caused by *Fusarium moniliforme w*ar. Subglutinans. Zafar Iqbal *et al.* (2011) and Wang (1984) have been reported the effect of fungicides on phyllophene fungi.

The preliminary screening of the chemicals was done following the slide germination technique of Riddick and Wallace (1910), and technique recommended by American Phytopathological Committee on standardization of fungicidal test 1944, followed by Zafar Iqbal *et al*. (2011). 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 (Patil, 1995). 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 Capton at both concentrations (0.1 to 0.2%), whereas, Mancozed and Copperoxychloride were list 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 top-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).

Bailley (1983) reported from *in-vitro* studies that Benomyl 100ppm was most effective while Aurefungin was effective only at higher concentration 2000ppm against *Fusarium moniliforme.* Anon (2009) tested nine fungicides against *Fusarium oxysporium* and proved that Benomyl and Dithane M-45 inhibited the growth of fungus. Efficacies of six fungicides were tested against *Fusarium moniliforme* Sheld for conidial germination and poison food technique *in-vitro* condition. Patil (1995) found that, Bavistin could inhibit conidial germination completely in all the concentration i.e. 50 to 500ppm. Bayleton also could inhibit conidial germination of conidia. In general, the inhibition increases with the concentration of fungicides except Bavistin total inhibition rewarded. Patil (1995) reported Bavistin as the most effective control against the *Fusarium moniliforme* Sheld causal organism of pokkah boeng disease. Pokkah boeng disease caused by *Fusarium moniliforme* was effectively controlled by foliar application of fungicides *viz*. 0.1 to 0.2% of Bavistin, 0.1 to 02% of Blitox-50 at interval of 15 to 21 days of four sprayings. The average losses in cane yield and commercial cane sugar due to disease were found from 15 to 25% respectively.

Acknowledgement :

Authors are thankful to the Dr. Radha Jain, Principal Scientist, Indian Institute of Sugarcane Research, Lucknow for providing necessary facilities.

REFERENCES

Anonymous (2009). Pest and disease control regulations. *Swazi. Sugar Assoc. Simunye, Swaziland*, pp 20.

Apple JL (1977). Theory of disease management. In J.G. Horsfall and F.B. Cowling (eds.) Plant disease-An advanced treaties. *Academic press* pp. 79 - 101.

Bailey RA (1983). The effect of soil and seedcane applications of triadimefon on the incidence of sugarcane smut. *Proc. S. Afr. Sugar Technol. Assoc*., **57**: 99 - 104.

Bailey RA and Bechet GR (1982). Progress in screening for resistance to sugarcane diseases in South Africa. *Proc. S. Afr. Sugar Technol. Assoc.*, **56:** 143-149.

Carpenter JB (1942). A taximetrics studies some eradicant fungicides. *Phytopathol.*, **32**: 845 - 856.

Chattopadhyay D and Nandi LB (1977). The elimination of leaf scald from infected planting material. *Proc. Int. Soc. Sugar Cane Technol*., **14:** 925 - 929.

Govender P, McFarland ESA, Rutherford RS (2010). Fusarium species causing pokkah boeng and their effect on eldana saccharina walker (lepidoptera: pyralidae). *Proc. S. Afr. Sug. Technol. Ass*., **83**: 267 – 270.

Grover RK and Moore JD (1962). Taximetrics studies of fungicides against brown rot organism. *Sclerotinia fructicola* and *Sclerotinia laxa*. *Phytopathol.,* **52:** 876 - 880.

Karuppaiyan R, Bakshi Ram, Ramdiya S, Masawwar Ali and Meena MR (2015). The incidence of pokkah boeng in indigenous and exotic sugarcane (*Saccharum officinarum*) clones. *Indian J. Agril. Sci.,* **85** (4): 596–601.

Lakshman M, Seth SDS and Srinvas (1973). Evaluation of filter paper and thickness of agar base in conducting antimicrobial sensitivity by disc method. *Indian J. Med. Res*., **61**: 8 -10.

Mehhta A, Thakar C, Shukla S, Mehta P and John J (2009). Fungicides: Inhibitory Agents of Cell Wall Degrading Enzymes of *Fusarium moniliforme* by Emblica officinalis Gaertn. *J. Basic Appl. Mycol.,* **8** (I & II): 94-95.

Patil AS (1995). Studies on Pokkah boeng and pine apple disease of sugarcane in Maharashtra with their economic losses in yield and quality of sugarcane. *Final Project Report, ICAR, VSI,* Pune. pp 162–170.

Patil AS, Hapase DG (1987). Studies on Pokkah boeng disease in Maharashtra. *Ind Phytopath*., **40**: 290.

Raju P and Lal A (1977). A guide to sugarcane diseases. *CIRAD/ISSCT, CIRAD Publications, Montpellier*, France, pp 339 - 342.

Reddick D and Wallace E (1910). On a laboratory method of determining the fungicidal value of spray mixture of or solution. *Science and Technol*., **31**: 798.

Schmitz H (1930). A suggested taximetrics method for preservatives. *Indust. Engg.Chem. Analyst.*, **2**: 361-362.

Sharma DDK and Kumar A (2015). Ascorbic acid content in sugarcane as affected by pokkah boeng disease caused by *Fusarium moniliforme* Sheldon. *Agrica J.,* **4**: 45–47.

Steel RGD, Torre JH and Dicky DA (1996). Principles and Procedures of Statistics: A Biometrical Approach, 3rd edition pp 204-27. *McGraw Hill Book Int. Co. Singapore*.

Subhani M N, Munir Ahmad Chaudhary, Abdul Khaliq and Faqir Muhammad (2008). Efficacy of Various fungicides against Sugarcane Red Rot (*Colletotrichum falcatum*). *Int. J. Agric. Biol*., **10**: 725–727.

Vijaya HK, Srikant Kulkarni and Yashoda R. Hegde (2007). Chemical Control of Sett Rot of Sugarcane Caused (*Ceratocystis paradoxa*). *Karnataka J. Agric. Sci*., **20** (1): 62 - 64.

Vishwakarma SK, Kumar P, Nigam A, Singh A and Kumar A (2013). Pokkah Boeng: An Emerging Disease of Sugarcane. *Journal Plant Pathology and Microbiol*. **4** (3):170-174.

Wang CA (1984). Pineapple disease: In Diseases of sugarcane – Major diseases. Ricaud C, Egan BT, Gillaspie AG Jour and Hughes CG (eds). *Elsevier, Amsterdam. The Netherlands*, pp 145-155.

Zafar Iqbal, Neem Akhtar, Muhammad Usman Ghazanfar, She Muhammad Shelzad, Salman Ahmad, Muhammad Asif, Muhammad Yasin, Muhamad Aslam Prevez, Altaf Ahmad Dasti and Ahmad Saleem (2011). Management of Mango malformation through physical alteration and Chemical spray. *African J. Agriculture Res.*, **6**(7): 1897-1901.

****