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Research Article
Effect of Soil Properties and Chemotherapeutants on Pokkah Boeng Disease of Sugarcane

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Abstract

Background and Objective: Sugarcane (*Saccharum* spp.) is a commercially important cash crop of India utilized for the production of sucrose, ethanol, biofuel and fiber-related commodities. Amongst the foliar diseases, the pokkah boeng disease is becoming the major disease of sugarcane. In view of importance of this disease in sugarcane cultivation in India, a plan has been outlined for its study on different parameters of soil properties and their management by using some chemotherapeutants. **Materials and Methods:** Study on the different inoculation techniques on suitable host variety of sugarcane was done by inoculating with *Fusarium moniliforme* under suitable temperature (25-30°C), humidity (80-85%) and rainfall (300-400 mm.) under natural conditions. Sugarcane is grown on varieties of soil from sandy loam to heavy clay but is grown best on well drained sandy loam soil. **Results:** Data revealed that sandy clay loam soil and ph 6.5-7.5 exhibited highest incidence of disease among all type of soils. It was also observed that the percentage pokkah boeng incidence increased with the increase in moisture content of soil. In the soil temperature study, results revealed that 24-29°C temperature was favourable for disease development but maximum disease incidence was recorded at 27.5°C. Copper oxychloride was found more effective against pathogen out of all the chemical fungicides used during the experiment. **Conclusion:** Sandy clay loam exhibited highest incidence of disease and copper oxychloride was found more effective fungicide against *Fusarium moniliforme*.

Key words: *Saccharum* spp., pokkah boeng, *Fusarium moniliforme*, cash crop, foilian diseases, sugar-cane cultivation.

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sugarcane (*Saccharum* spp. complex) is the 3rd most grown crop in the word having more than 27 million ha under its cultivation with 1884 million t of cane production[1]. It is the most important cash cum industrial crop of the tropical and sub-tropical areas of the world and sugar industry is the second largest agro-based industry in India. Sugarcane is also considered to be one of the most energy efficient crops for conversion of solar energy into bio-mass[2]. Sugarcane is grown on varieties of soil from sandy loam to heavy clay but is grown best on well drained sandy loam soil[3].

Among the 180 disease reported in India over a period of time, only eight diseases viz., pokkah boeng, red rot, smut, wilt, pine apple, grassy shoot, ratoon stunting, leaf scald mosaic are of major concern. The economic importance and distribution of all the diseases of sugarcane on the varieties on which they occur and the agro climatic conditions under which varieties are cultivated were already described[4]. Pokkah boeng disease has been recorded in almost all countries where sugarcane is grown. Pokkah Boeng is a foliar disease caused by the fungus *Fusarium moniliforme* Sheldon. Pokkah boeng of sugarcane was first reported in India during 1983 to 1984 in month of July-November from Maharashtra in two cane varieties[5] viz., Co7219, CoC671. Amongst various sugarcane diseases, Pokkah boeng is becoming a major disease over the period in India[6].

Sugarcane production is hampered by diseases largely due to its vegetative propagation through stem sections (setts) and cultivation in large adjacent fields, resulting in easy spread of disease. *Fusarium* is one of the important pathogenic fungi that infect sugarcane and causes *Fusarium* pokkah boeng and stem rot. *Fusarium* is also associated with infestation of sugarcane by the inset-pest (Lepidoptera: Pyralidae) as the borings made by the stem borer allow the fungus access to the stem tissue[7]. Infection by *Fusarium* species, especially in lower internodes may have variable effects on resistance to the borer[8]. So, in view of importance of this disease in sugarcane cultivation in India, a plan has been outlined for its study on different parameters of soil properties and their management by using some chemotherapeutants.

MATERIALS AND METHODS

The present investigations were carried out in the Department of Plant Pathology, Lovely Professional University, Phagwara during the year 2017-2018. The soil used during the study was mixture of black and red sandy soil in the ratio of 1:1. The inoculum in the form of spore suspension (10^6 mL^{-1}) was added to the sterilized top soil, by frequent inversion so as to ensure uniform distribution.

Two budded chip bud setts as seed materials were normally used for planting of sugarcane. Apparently healthy setts (seed material) of six varieties including standards viz. CoS 8436, CoS 8432, CoS 98259, CoLk 8102, CoSe 01434, Baragua were used. Study on the different inoculation techniques on suitable host variety of sugarcane was done by inoculating with *Fusarium moniliforme* under suitable temperature (25-30°C), humidity (80-85%) and rainfall (300-400 mm) under natural conditions.

Type of soils: Sugarcane is grown on varieties of soil from sandy loam to heavy clay but is grown best on well drained sandy loam soil. To study the effect of soil types on the incidence of sugarcane pokkah boeng four types of soil viz., sandy loam, sandy, sandy clay loam, clay loam were collected. Before use, these soils were sterilized (autoclaved for two consecutive days at 1.1 kg cm^{-2} pressure for 2 h) and mixed with inoculums of the pathogen, *F. moniliforme* Sheldon 5% weight of soil in pot. For all the treatments moisture was maintained at 35% by adding required quantity of distilled water as and when required. Control without inoculums of the pathogens was also maintained. After germination plants were regularly observed for the appearance of the pokkah boeng.

Effect of soil pH: Influence of pH on the growth and sporulation of fungal pathogen was assessed by growing the pathogen in three media viz., sugarcane leaf extract agar, PDA and OMA as suggested by Kiryu[9]. However, before making up the volume after adding all the ingredients, the pH of each of the three media was adjusted using N/10 HCl and N/10 NaOH. The volume was later made upto mark using sterilized distilled water. About 20 mL of the prepared medium was thus transferred to sterilize Petri plates. These Petri plates were incubated at 26±1°C for 72 h. Radial growth was measured and recorded.

Soil moisture: Soil moisture is an important regulator of temperature because more heat is needed to raise the temperature of wet soil. It would be difficult to define the effect of soil moisture without accounting for other factors as it is an extremely complex interaction in which a change in one factor leads to change in other factor[10]. A soil moisture level of 70% moisture holding capacity (MHC) favored survival of *F. moniliforme* in loam but not in sandy or clay soil[11]. At 40% (MHC) no difference in the survival were observed among the different soil types.

In present study sterilized sandy clay loam soil was filled in pots and inoculated with three pathotypes at 5% of weight of soil in pot. These pots were irrigated at the intervals of 7, 14, 21 and 28 days to maintain different moisture regimes. Four replications in each set were maintained.

Soil temperature: Propagation of pathogen in nature like all living organisms, fungi also requires an optimum temperature for best growth and development. Most fungi are able to grow at temperatures ranging from 5-35°C and the optimum being at 25-30°C. Usually fungi do not grow below 0°C or about 40°C but exceptions are not infrequent. In number of cases it has been observed that fungi are more resistant to lower temperatures than to higher temperatures because at lower temperatures their living activities ceases, while at higher temperatures they are killed or destroyed.

Soil temperature was observed by several investigators that *F. moniliforme* causing pokkah boeng is a warm weather disease. The disease developed severely and rapidly during the period of highest average temperature of 29°C in case of pokkah boeng of sugarcane[12]. An optimum of 28°C for disease development but no disease was seen in infested soil above 35°C and below[13] 20°C. In case of pathotypes the pokkah boeng due to *F. moniliforme* was severe[14] at 25-29°C. However, the fungus caused abundant injuries at all temperature above 33°C. However, the disease is destructive at any temperature below 27°C at which cane will grow[15].

Chemical control: The efficacy of a fungicide was expressed as inhibition (%) of mycelial growth over control that was calculated by using the formula suggested by Vincent[16]:

$$I = \frac{C - T}{C} \times 100$$

Where:

I = Inhibition (%)
C = Radial growth in control
T = Radial growth in treatment

Hanging drop techniques: The studies were made at room temperature employing at different concentrations of fungicide and antibiotics. The spores of pathogen were allowed to germinate in the hanging drop technique of the respective concentrations of the chemicals with sterilized distilled water. Germination (%) of spores was observed after 24 h. The average germinate (%) of the spore in three replications was calculated and is given in the Table 6.

Food poison technique: The evaluation of selected chemicals was done by poisoned food technique[17-21]. The selected chemicals were tested by poisoned food technique. The desired amount was added to the PDA medium before autoclaving so as to have different concentration of the chemicals. Corresponding control was also maintained. The plates after inoculation were incubated at 26±1°C for 7 days. The diameter of the colony was measured and mean of three replications as calculated is given in the Table 7.

Modified paper disc technique: The selected antibiotics and fungicides were also tested for their efficacy against *F. moniliforme* by modified paper disc method 15 mm assay discs were cut from whatman-45 filter paper and each disc was impregnated separately with 1 mL solution of the respective chemical solution installments by repeated soaking and air drying. The assay discs were aseptically placed in the center, one in each of the Petri plate containing sterilized medium corresponding controls (with discs soaked in distilled water) were also maintained a 2 mm mycelial disc. From periphery of 7 days old culture, the test fungus was aseptically placed in the center of assay disc in the inverted position so that the fungus comes in direct contact with the disc.

***In vivo* experiment:** *In vivo,* experiment was laid out in small plots in the field. About 22 bud diseased setts were soaked for 30 min in 0.2% solution of the selected antibiotics and fungicides viz. Aureofungin, Streptomycin, Carbendazim and Copper oxychloride. The treated as well as corresponding control setts both (healthy and disease) canes were planted separately following the usual cultural practices.

Statistical analysis: The analysis of variance technique was applied for drawing conclusions from the data. The calculated value was compared with tabulated value at 5% level of probability for the appropriate degree of freedom[22].

RESULTS

The result (Table 1) revealed that sandy clay loam soil exhibited highest pokkah boeng incidence of 76.4 (Fm122) 72.8 (Fm126) and 69.1% (Fm129). The least incidence was found in clay loam soil.

Soil texture and pH are inseparable parameters as both are interdependent. In the studies on effect of pH, sandy loam soil with a pH of 7.8 was adjusted to the required pH by using calcium hydroxide or commercial sulphuric acid. After

Table 1: Influence of soil types on pokkah boeng incidence

	Pokkah boeng incidence (%)		
Soil types	Fm122	Fm126	Fm129
Sandy	42.60	41.8	45.8
	32.24	36.6	34.0
Sandy loan	53.90	41.0	61.2
	48.40	30.2	40.4
Sandy clay loam	76.40	72.8	69.1
	52.10	56.4	49.5
Clay loam	28.40	26.2	26.4
	25.00	20.2	20.0

Table 2: Influence of soil pH on pokkah boeng incidence

	Pokkah boeng incidence (%)		
Soil pH	Fm122	Fm126	Fm129
5.5	48.4	52.6	46.7
	42.0	42.8	42.1
6.5	90.0	86.4	88.9
	74.5	66.1	65.4
7.5	70.4	78.6	80.0
	66.0	64.3	62.4
8.5	35.4	32.1	28.0
	36.2	33.4	31.6
9.0	20.0	20.0	24.0
	26.5	26.2	28.2

Table 3: Influence of soil moisture on pokkah boeng incidence

	Pokkah boeng incidence (%)		
Interval of irrigation days	Fm122	Fm126	Fm129
7.0	30.0	30.0	27.0
	33.2	33.1	31.6
14.0	35.0	35.0	30.0
	36.1	33.2	33.1
21.0	50.0	45.0	45.0
	44.0	42.1	42.1
28.0	55.0	50.0	50.0
	46.8	45.0	45.0
CD CP = 0.05	(3.2)	(2.6)	(2.2)

adjustment of the pH soil was allowed to weather for 7 days and readjusted to the level 5,6,7,8 and 9 before sowing for each pH level three replications were kept (Table 2).

These results (Table 2) showed that the effect of soil pH on all isolates of pokkah boeng infection was significantly highest at 6.5 pH followed 7.5 and 5.5 pH and least infection was recorded at pH 9.

Soil moisture was recorded by drying 100 g soil of each interval regimes filled in flask in oven at 160°C for 4 h. Data has been shown (Table 3) that in pot having irrigation of every 7 days, the percentage of pokkah boeng incidence increased with the increase in moisture content of soil.

It is noted from Table 4 that disease incidence varies under different soil temperature conditions 24-29°C temperature was favourable for disease development.

Table 4: Influence of soil temperature on pokkah boeng incidence

	Pokkah boeng incidence (%)		
Temperature (°C)	Fm122	Fm126	Fm129
February (18.3)	20.0	18.0	18.0
	26.5	26.4	26.8
March (20.9)	39.2	38.4	39.2
	40.0	40.0	40.0
April (28.4)	65.0	64.0	64.0
	53.7	54.0	54.0
May (31.1)	60.0	60.0	60.0
	50.8	50.7	50.0
June (32.5)	42.1	42.2	42.2
	50.0	52.0	52.0
July (30.3)	76.0	74.0	76.5
	62.4	62.4	62.4
August (28.5)	76.8	77.0	77.0
	66.8	65.0	65.0
September (25.3)	80.0	85.0	85.0
	44.2	45.0	45.2
October (24.7)	75.0	70.0	75.0
	42.1	39.2	36.2
November (21.1)	55.0	55.0	55.0
	46.6	46.1	46.4
December (19.8)	30.4	30.1	26.4
	35.8	32.4	34.6
January (14.1)	25.8	25.9	26.8
	33.6	30.4	32.6

Table 5: Influence of sowing dates on pokkah boeng incidence (Autumn and Spring planting)

	Pokkah boeng incidence (%)		
Sowing date	Fm122	Fm126	Fm129
8 February	60.5	60.3	55.4
	50.83	50.7	47.8
15 February	65.1	65.4	60.6
	52.0	53.7	60.76
28 February	75.0	75.3	70.4
	60.0	60.0	56.7
8 October	45.0	40.1	46.2
	4.24	29.20	39.0
15 October	50.4	45.2	40.6
	45.0	42.1	39.2
30 October	55.6	50.0	45.4
	46.0	47.1	42.1

Autumn and spring plants: An experiment was conducted to find out whether there was any variation in the incidence of the disease with crop sown on different dates starting autumn planting in October and spring planting in February. Sowing was done in sick plots. The observation on disease incidence was recorded throughout the crop period.

In spring season, sets sown in the February was found maximum infection followed by autumn season planting in October. The incidence of the disease also decreased due to the variation in the temperature as shown in Table 5.

Data presented in Table 6 showing effect of different chemical antibiotics and fungicides on spore germination *F. moniliforme* reveals that all fungicides exhibited better inhibition of spore germination. It is quite understandable that antibiotics do not have direct effect to inhibit the germination of spore of fungi. However, because of their toxic effects some reduction in the germination (%) of spore was observed. Among the chemical fungicides, copper oxychloride inhibited 100% spore germination even at the concentration of 200 ppm while others exhibited at some degree of inhibition at 250 ppm. Interestingly antibiotics viz. streptomycin also exhibited some degree of inhibition at 250 ppm concentration as the fungicides.

The results recorded in Table 7 revealed that copper oxychloride was the most effective in checking the mycelial growth even at 50 ppm concentration, where the growth was completely arrested. This was followed by streptomycin and carbendazim which gave 52.4 and 36.4% reduction, respectively at the same concentration. At 200 ppm, however, all the four chemicals checked the growth completely.

The observations were recorded in terms of colony diameter in centimeter and the data so obtained have been given in the Table 8. It is clear that the copper oxychloride alone checked the mycelial growth completely even at 100 ppm concentration. The remaining three chemicals were also effective but to a much lesser degree.

Table 6: *In-vitro* efficacy of antibiotics and fungicides against spore germination of *Fusarium moniliforme* Sheldon

	Spore germination percentage (ppm)				
Chemicals	50	100	150	200	250
A-Antibiotics					
Aureofungin	60	44	28	16	10
Streptocycline	52	32	21	08	00
B-Fungicides					
Bayleton	50	30	12	04	00
Carbendazim	50	26	12	02	00
Copper oxychloride	38	24	10	00	00
Mancozeb	52	32	14	06	00

DISCUSSION

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 suggested need for continuous adjustment in the cropping system[5,23]. 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 ones were used to find out some effective chemicals which may control the infection of pokkah boeng pathogens. Various chemotherapeutants were first screened *in vitro* against the pathogen with a view to select the most effective ones for *in vivo* experiments. The main objective of present studies was to find out comparative efficacy and specificity of the fungicides and antibiotics against the *Fusarium moniliforme* to obtain economical control of this disease. The most satisfactory measure for pokkah boeng is the use of

Table 7: Effect of selected chemicals at different concentrations on the conidial growth and inhibition (%) in *F. moniliforme* by food poisoning technique

	Concentration (ppm)							
	50.0		100.0		150.0		200.0	
Chemicals name	Colony (diam)	Inhibition (%)	Colony (diam)	Inhibition (%)	Colony (diam)	Inhibition (%)	Colony (diam)	Inhibition (%)
Aureofungin	2.6	20.0	1.8	76.6	0.4	80.5	0.0	100.0
Streptocycline	3.2	52.4	2.6	39.4	1.4	77.8	0.0	100.0
Copper oxychloride	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
Carbendazim	2.4	36.4	1.6	68.0	1.6	76.2	0.0	100.0
Control	4.5	--	--	--	--	--	--	--

Table 8: Effect of chemicals by modified paper disc technique

	Concentration (ppm)							
	50.0		100.0		150.0		200.0	
Chemicals name	Colony (diam)	Inhibition (%)	Colony (diam)	Inhibition (%)	Colony (diam)	Inhibition (%)	Colony (diam)	Inhibition (%)
Aureofungin	3.2	30.0	1.8	50.0	0.4	96.4	0.0	100.0
Streptocycline	3.8	36.4	2.4	60.2	0.8	88.2	0.0	94.0
Copper oxychloride	1.6	100.0	0.0	100.0	0.0	100.0	0.0	100.0
Carbendazim	3.6	34.6	1.6	76.4	0.6	80.4	0.0	100.0
Control 4.5	--	--	--	--	--	--	--	--

resistant varieties[24]. However, the best practice to control this disease may be the integration all available resources and methods. Optimum temperature is also one of the pre-requisite for the growth and sporulation of the fungus. In the present study, fungus grew well and sporulated in abundance in the temperature range of 25-30°C. In case of pokkah boeng due to *F. moniliforme* was severe at 25-29°C. However, the disease is destructive at any temperature below 27°C at which cane will grow[15]. High range of fungal growth and more susceptibility of cane plants was found between[25] 25-30°C. Similar results were obtained in the present investigation. At higher temperature 30°C, the plant is infected without showing symptoms. Soil temperature was recorded daily at 5 cm depth both in the morning at 8.30 am and afternoon at 5.30 pm throughout the crop period.

In effect of growth regulator hormone for lower concentration of IAA and IBA proved stimulatory to the growth of selected isolates, while higher concentration produced adverse effect on the growth of *F. Moniliforme*[26].

The fungus generally grows maximally over a certain range of initial pH of the medium and will fail to grow at high and low extremes under given conditions. Many factors like temperature, time of harvest, gross changes in medium and nitrogen supply may change the shape of the pH growth curve. In the present study involving three media with different pH, high growth was revealed in PDA[9,27] at pH 6.5. It also reinstates the superiority of PDA medium for growth of the fungus even at different pH.

In spring season sets sown in February was found maximum infestation followed by autumn. In autumn planting, the disease incidence ranged 4.82% while spring planting, the incidence ranged 9.29%. Spring planting manifested higher pokkah boeng incidence than the autumn planting[6]. Disease incidence was highest at both extremes of relative humidity (80-85%), indicating the maximum growth and sporulation of pathogen under stress conditions. The maximum incidence of *F. Moniliforme* was found when relative humidity[28] was 90%. The proportion of soil particles which decided texture would also be a deciding factor for pH[29]. The highest at 76 and 90% relative humidity with pH of 6.5 and temperature of 28°C is suitable for *F. moniliforme*[30].

Suitable inoculation techniques are very much necessary in the studies on life history of the pathogen and physiology of parasitism, maintenance of cultures, determination of the existence of physiologic specialization and selection of the disease resistant hosts in plant breeding programmes. In the present investigation, foliage injury method of conidia ensured incidence (%) of pokkah boeng. Germinated conidia of *F. moniliforme* as the best method of inoculation, which resulted in disease (%) development[31].

Soil type quantum of inoculums also produces varying degree of infection in different soil in planted sugarcane. The present study revealed that increase in the sandy clay loam soil highest an incidence was found in clay loam soil level of *F. moniliforme.* The decrease in incidence per cent was more pronounced in sterilized soil but increasing the inoculum level, caused increase in disease development[32]. Sandy soil supported highest wilt incidence of watermelon, tomato and marigold and the least incidence was found in silt clay soil[33].

Soil moisture is growth determinant for most of the soil dwelling organisms. It is a medium through which the soil fungi absorb their nutrients and carryout most of their metabolic and physiological functions. In the present study their percentage of pokkah boeng incidence increased with moisture content of soil was observed at 55% moisture holding capacity. A soil moisture level of 70% moisture holding capacity (MHC) favored survival of *F. moniliforme* in loam but not in sandy or clay soil[11]. About 40% (MHC) no differences in the survival were observed among the different soil types[34]. Soil texture and pH are inseparable parameters as both are interdependent. In the present study on effect of pH, sandy loam soil revealed that the pokkah boeng infection was highest at 6.5 pH least infection was recorded[35]. Increasing of particles which decided texture would also be a deciding factor for pH level, caused increase in disease development[35].

In the present study of hanging drop technique were results revealed that all fungicides exhibited better inhibition of spore germination. Copper oxychloride was the most effective in checking the mycelial growth and however, because of their toxic effects some reduction in the germination percentage of spore was observed. The copper oxychloride inhibited 100% spore germination even at the concentration of 200 ppm while others exhibited at some degree of inhibition at 250 ppm. Many pathogenic microorganisms produce toxins and anti-metabolites in culture or in the host or in both, which are involved in manifestation of disease symptoms[36]. Seed germination bioassay conducted to know such characteristics of the pathogen revealed drastic reduction in germination percentage in tomato seeds, while all others remained unaffected. This may be due to the host specificity of the toxin or loss of phytotoxicity on prolonged exposure of the culture filtrate to air[37-38]. Captain, thiram and duter were effective against *Fusarium moniliforme*[20]. Fytolan (Copper Oxychloride) at 0.02% controlled malformation of mango caused by *Fusarium moniliforme* war. *Subglutinans*[39]. In poisoned food technique, Bavistin could inhibit mycelia growth completely

at 100 ppm concentration followed by Bayleton and aureofungin where inhibition was 76.34%, respectively. Bavistin as the most effective control against the *Fusarium moniliforme* of pokkah boeng disease[40-41]. Bavistin, vitavax and benomyl to be the best fungicides for control of *Fusarium axysporum*[42-43].

CONCLUSION

In the study of soil type, result showed that sandy clay loam soil exhibited highest incidence among all type of soils. The study of influence of soil pH on pokkah boeng disease, highest disease incidence was recorded on 6.5 pH among all parameter. In the study of influence soil moisture on pokkah boeng disease, result showed that while interval of irrigation was increased percent of disease incidence is also increased. In the soil temperature study, results revealed that 24-29°C temperature was favourable for disease development.

Copper oxychloride was found more effective against pathogen out of all the chemical fungicides used during the experiment. So, according by using the above findings efforts should be made to keep the incidence of pokkah boeng disease below its injury level right from the beginning stage of sugarcane crop by adopting all available resources to integrate the practices as it affects the quality of original as well as byproducts of sugarcane followed by even macro and micro-constituents.

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