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SCREENING OF SUGARCANE CULTIVARS AGAINST *COLLETOTRICHUM FALCATUM* CAUSING RED ROT DISEASE AND ITS CONTROL WITH DIFFERENT FUNGICIDES UNDER LABORATORY CONDITIONS

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A B S T R A C T

Ten sugarcane (*Saccharum officinarum* L.) cultivars were screened against red rot disease caused by *Colletotrichum falcatum* under laboratory conditions by artificial inoculation technique. These cultivars were graded under various levels of resistance as well as susceptibility using a standard disease rating scale. Two cultivars i.e. NSG-59 and SPF-244 showed resistant reaction to red rot of sugarcane. Three cultivars i.e. CPF-246, CPF-247 and BF-138 showed moderately resistant (MR) reaction against red rot. Remaining five cultivars showed moderate, susceptible to highly susceptible reaction. The results of *in vitro* evaluation of seven fungicides at four concentrations (10 ppm, 15 ppm, 20 ppm and 25 ppm) showed that all tested fungicides with all concentrations significantly inhibited the mycelial growth of the pathogen as compared with control. However, the inhibition percentage was increased by increasing the concentrations of tested fungicides. Among fungicides Tilt proved the most effective fungicide by inhibiting linear mycelial growth at all concentrations against of *C. falcatum* followed Nativo while Metaxyl&Mencozeb was the least effective in terms of retarding fungal growth. The findings of the present study suggested that resistant cultivars may be utilized as a source of resistance and may be more useful as donors in breeding programme aimed at red rot disease resistance and the growth of the pathogen is effected by different concentrations of fungicides may play an important role to manage this disease.

Keywords: Sugarcane, *Colletotrichum falcatum,* red rot, management, fungicides

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is an important agro industrial and leading crop by production in the world (Kinkema *et al*., 2014). Pakistan ranks 4th in cane acreage and 5th in sugar production globally (Shahina *et al*., 2007). The main limitations in the ideal sugarcane production in Pakistan comprise abiotic and biotic stresses which are responsible for its low yield (Kumar *et al*., 2014). Sugarcane crop prone to almost 100 different diseases caused by fungi, bacteria, nematodes, among them, red rot of sugarcane is the most destructive disease of cane which are caused by fungus, called as Cancer of sugarcane. (Khan *et al*., 2011; Bharti *et al*., 2012). In the recent years 2003 to 2006, it caused maximum losses in cane industry. (Hussnain and Afghan, 2006). Therefore, an extensive range of cane cultivars have become prone to red rot (Viswanathan *et al*., 2003).

For the first time, it has been reported in Indonesia in 1893 which was later on named as red rot of sugarcane (Butler,1906). In Pakistan, this pathogen first reported in 1986 by Ahmed *et al*., 1986. The major reason of common of red rot disease in Pakistan is due to cultivation of susceptible Sugarcane varieties that difficult to incorporate the new sugarcane cultivars with developed agronomic features and resistance to biotic and abiotic stresses (Zamir *et al*., 2012).

Red rot of sugarcane may infect developed stems of cane, leaf mid ribs which results in considerable damages in sugar quality (Rao *et al*., 2008). The pathogen is setts-borne and inactive mycelia existing in the bud scales are responsible for post-germination

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and contaminate the freshly developing shoots of cane (Viswanathan and Rao, 2011).

Managing of red rot of sugarcane has been considered as thought-infuriating part of work for the scientists. The Pathogenicity of the pathogen depends upon environmental factors as well as its genotypes and existence of virulent pathogen. Furthermost the suggested management tools are aimed at agronomical practices to decrease the spread of pathogen in the field (Viswanathan and Rao, 2011). Integrated Disease Management should be adopted in the field of sugarcane because single method would not be useful to reduce the losses with respect to diversity in the types of pathogen (Agnihotri, 1996). Among all management strategies Fungicides show a vibrant role in disease management since they control many other plant diseases adequately. Several Fungicides used for the management of the disease but limited success was attained in field conditions (Singh and Singh, 1989).Keeping in the mind regarding efficacy of fungicides in the field the alternative way to combat with the disease is screening of sugarcane varieties for identification of commercially superior (high sugar and high yielding) varieties with resistance/tolerance against pathogen of red rot of sugarcane (Gupta *et al*., 1982; Viswanathan *et al*., 1996; Viswanathan and Samiyappan, 2002; Malathi *et al*., 2008). The objectives behind this work were (i) Screening of sugarcane varieties against *C. falcatum* causing red rot of sugarcane (ii) *In vitro* evaluation of different fungicide at different concentration against red rot through poisoned food technique

MATERIALS AND METHODS

The present study was conducted in the Laboratory of Plant Pathology, University College of Agriculture, University of Sargodha (Sargodha, Pakistan) during 2014-2015, in order to evaluate the efficacy of different fungicides against *C. falcatum* causal agent of red rot of sugarcane. Different varieties of sugarcane were also screened against red rot of sugarcane under field conditions at experimental area of University College of Agriculture, Sargodha. Sugarcane stalks were cut longitudinally with sterilized knife and part of stalk showing red color, typical symptoms of disease were cut into small pieces along with growing margins of about 1.5-2cm. Surface sterilized with 0.1% bleach for approximately 2 minutes then washed three times with distilled water and placed on petri plates having potato dextrose agar (PDA). Petri plates were incubated at 28 ±1°C for one week to check the sporulation for further studies. Single spore technique used for obtaining pure culture by incubating at 28°C for one week and observed it daily to get rid of contamination. (Hansen, 1926; Choi *et al.*, 1999).

Pathogenicity Test: Sugarcane stalks were cut into small pieces in such a way that each piece has 3-4 internodes of sugarcane. These small pieces were washed with water for removal of contamination i.e. dust etc. on their surface and then apply 1 % sodium hypochlorite for 2-3 minutes followed by washing with distilled water and then subjected to natural air drying. After drying, these pieces were split into two parts longitudinally with help of knife. Single bit of fungus was placed in center of each piece, with help of inoculating needle from petri plates having fungal colony, covered all sugarcane pieces so that moisture may not loss and placed in incubator at 28°C for the development of symptoms (Figure 1). After 5 days of inoculation, pieces were again cut longitudinally and watched for symptoms expression of disease. Re-isolations were made from artificially infected sugarcane pieces and compared with pure cultures to confirm pathogenicity of pathogen.

Varietal screening against red rot of sugarcane: Ten sugarcane varieties were screened against red rot disease of sugarcane in the field of Plant Pathology, University College of Agriculture, Sargodha during 2014-2015. Disease was rated by disease rating scale used by Srinivasan and Bhat, 1987 (Table 1). Sugarcane varieties used for screening were jhnag-59, CO-1148, CPF 247.CPF 248, CPF 246, NSG-59, S-2002-US-162, BF-138, SPF-244 and HS-12.

Table 1. Reaction to variety on 0-9 scale:

0.0 – 02	Resistant (R)
2.1 – 4.0	Moderately resistant (MR)
4.1 – 6.0	Moderately susceptible (MS)
6.1 – 8.0	Susceptible (S)
8.1 and above	Highly Susceptible (HS)

a b C

Figure 1: a=Symptoms after 5 days of inoculation b=Fungus colony after re-isolation c=Wrapped canes

Evaluation of fungicides: Seven different fungicides at different tested concentrations (Table 2) i.e. 10, 15, 20 & 25 ppm and each replicated thrice through use of poisoned food technique (Sharvelle, 1961) against *C. falcatum* causing red rot. Fungicides were obtained from registered pesticide dealers located in the market of Faisalabad (Punjab, Pakistan).

Whereas percent inhibition calculated by Percent inhibition = X−Y/X×100

Where, X = Colony diameter in check, Y = Colony diameter.

Table 2. Fungicides description.

Sr. No.	Trade Name	Active ingredient	Manufacturers
1	CabrioTop 600WDG	Metriam+Pyraclostrobin	Arysta Life Sciences
2	Dew 250%EC	Difenconazole	Four Brothers
3	Secex	Metaxyl	United Distribution
4	Metaxyl+MencozebWP	Metaxyl+Mencozeb	Bayer Crop Sciences
5	Tilt®250 EC	Propeconazole	Syngenta
6	Rally®40WSP	Myclobutanil	Dow AgroSciences
7	Nativo®WG 75	Trifloxystrobin+Tebuconazole	Bayer Crop Sciences

Preparation of fungicides concentration: Potato dextrose agar was used and requisite concentration of each fungicide was added to get a required concentration. The fungicides were carefully mixed by stirring and about 15 ml poisoned medium was poured to each of the 90 mm petri dishes and allowed for solidification. The actively growing margins of the seven days old culture of the pathogen was carefully cut using cork borer and transferred aseptically to the center of each petri dish. Suitable control was maintained on PDA having no fungicide. The petri plates were incubated at 28 ± 1°C for one week and the colony diameter was recorded after seven days growth of pathogen according to the description (Benicio *et al*., 2003).

STATISTICAL ANALYSIS

Statistical analysis was performed using R Software. Three factor factorial analysis were used for the evaluation of synthetic fungicides results. Fisher's LSD test was used to compare treatment means (Fisher, 1948).

RESULTS

Pathogenicity Test: After 5 days of inoculation, sugarcane pieces were split longitudinally and observed for symptoms expression of disease. Red color symptoms were seen as reddening of vascular area and also bad odour (Figure 1).

Screening: After two months of inoculation, inoculated canes were split longitudinally and disease incidence was noticed on basis of lesions breadth, spots color i.e. white or black, number of nodes transgressed by fungal development and condition of tops. Linear spread of disease in stalk was measured in centimeters in all varieties. Characteristic symptoms of red rot disease i.e. yellowing, drying of leaves, red discoloration throughout the length of the stalk and production of white spots within

the internal portion of cane appeared on some inoculated plants.

Among ten varieties (Table 3), two varieties such as NSG-59 and SPF-244 scored 2 on disease rating scale and proved to be resistant against the attack of red rot disease. Three varieties such as BF-138, CPF 246and CPF 247 score 4, 3 and 4 respectively on disease rating scale. These three varieties showed moderately resistant response to attack of pathogen. CPF 248 showed moderately susceptible response towards red rot disease, Whereas, all other varieties have susceptible and highly susceptible response on disease attack. Long lasting and more reliable method for controlling disease is to introduce such varieties that are resistant to disease by breeding programs (Sengar *et al*., 2009).

Table 3: Response of varieties towards red rot of sugarcane

Sr No.	Variety	Score on 0-9 scale	Response
1	CPF 247	4	Moderately resistant
2	CPF 248	5	Moderately susceptible
3	CPF 246	3	Moderately resistant
4	Jhnag-59	7	Susceptible
5	NSG-59	2	Resistant
6	SPF-244	2	Resistant
7	HS-12	9	Highly Susceptible
8	S-2002-US-162	7	Susceptible
9	BF-138	4	Moderately resistant
10	CO-1148(Check)	9	Highly Susceptible

Evaluation of synthetic fungicides: All tested fungicides with all concentrations significantly ($P \leq 0.01$) inhibited the mycelial growth of the pathogen when compared with control (Table 4).

The fungicides evaluated after seven days of colony growth by taken inhibition percentage of all tested concentrations. Data regarding inhibition percentage of fungus (Table 4, Figure 2) revealed that Tilt was the most effective fungicide inhibited mycelial growth at all concentrations (57.85% to 81.67%) against *C. Falcatum* followed by Nativo (50.37% to 75.18%), Secex (45.18% to 71.12%), Dew (39.63% to 64.63%), Cabrio top (25.74% to 42.59%), Rally (10.67% to 53.7%) and least inhibition was recorded with Metaxyl and Mancozeb (7.78% to 33.7%) when compared with control. The mycelial growth of pathogen was significantly different within the 4 concentrations of each fungicide. Similar results were found Bharti *et al*., (2014) by reporting Tilt effectiveness against *C. falcatum*. Metaxyl and Mencozeb and Cabrio Top don't show god effects on fungal mycelium. These both fungicides have very narrow effects on *C. falcatum* growth. Dew and Rally have also not significant effects on fungal growth at lower concentrations but inhabit fungal development at higher concentrations. Dew showed good effects on 25 ppm concentration as compare to Rally. Cabrio Top failed to inhabit fungal growth at highest concentration used (Figure 3).

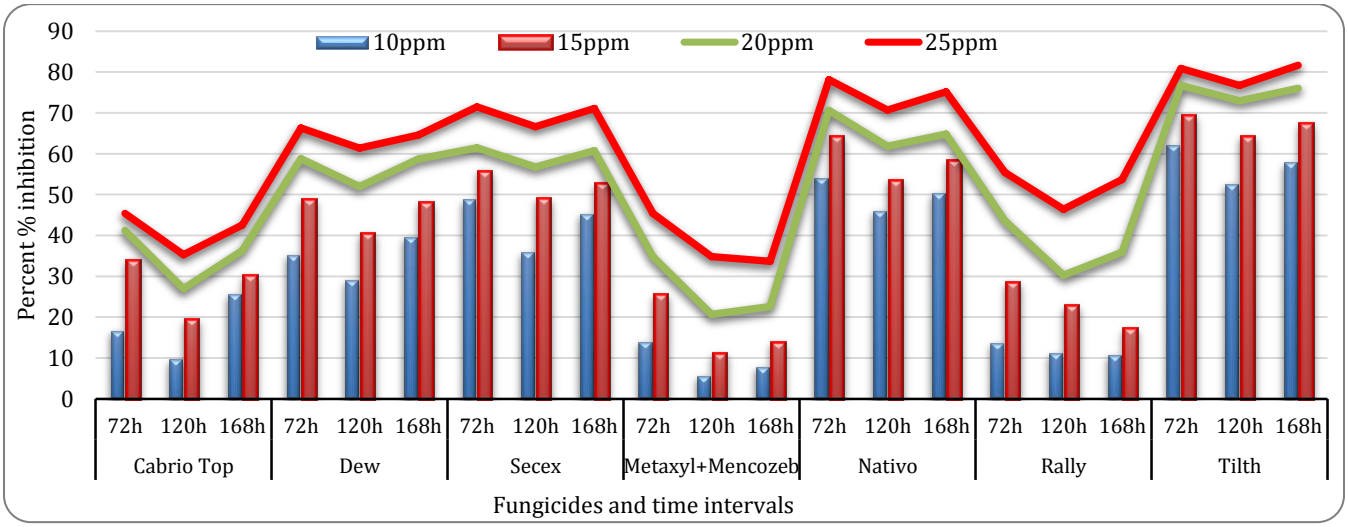

Figure 2. Growth inhibition percentage (%) of *C. falcatum* against different concentrations of fungicides, applied as food poison technique

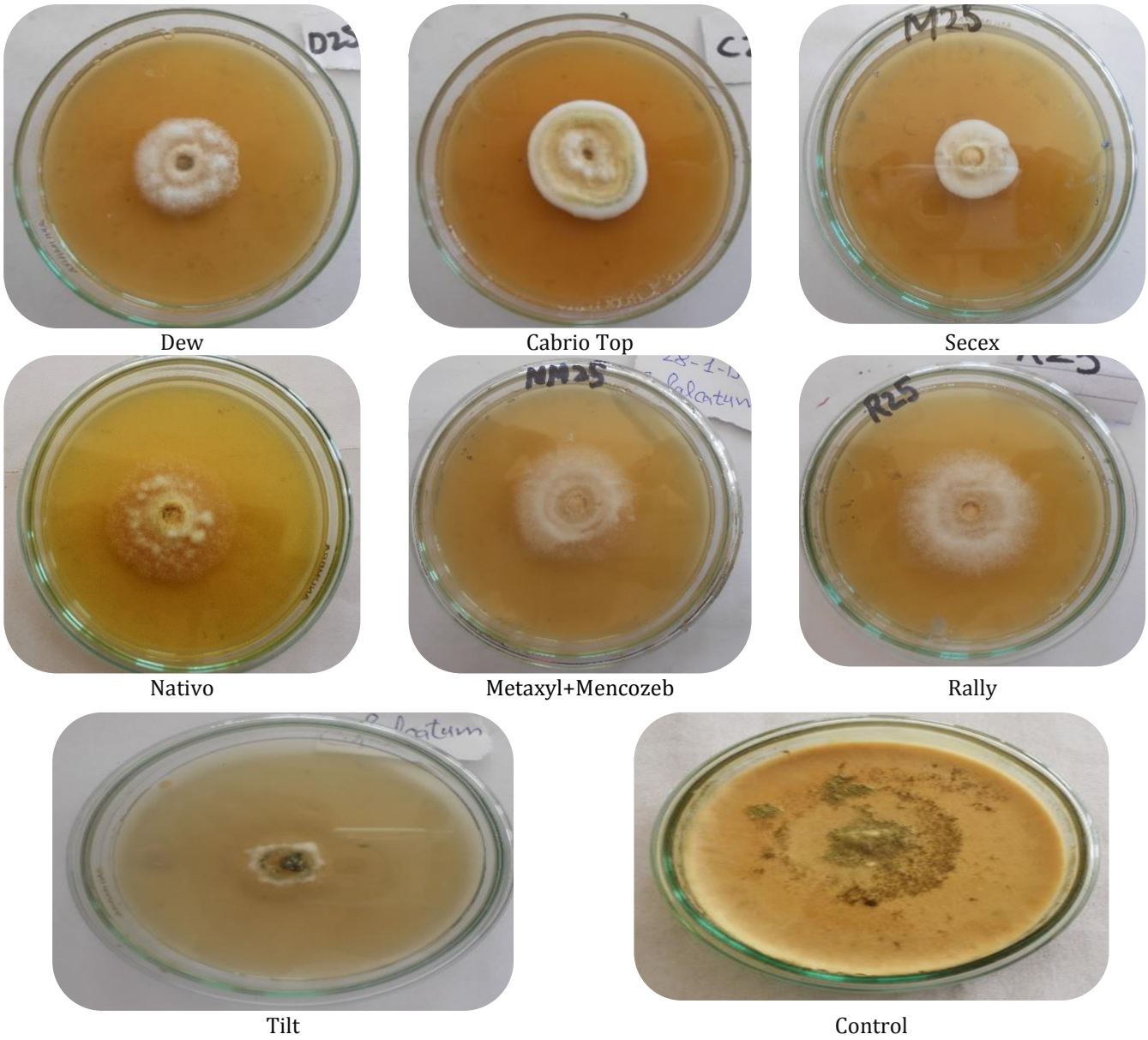

Figure 3. Effect of different fungicides on colony growth of *C. falcatum* at 25ppm concentration through food poison technique compared with control

DISCUSSION

Management of red rot through the use of resistant verities is the most reasonable tool but when red rot disease free seed is not accessible, farmers left with no choice other than use of synthetic fungicides (Viswanathan *et al*., 2009). The effect of seven fungicides were *in vitro* evaluated for their inhibitory effect on linear growth of *C. falcatum*, causal agent of red rot of sugarcane. Results of tested fungicides indicated that the inhibition percentage was increased by increasing the concentrations of fungicides (Tsakiris *et al*., 2002; Olanya *et al*., 2001). Horst, (2012) reported that fungicides have different kind of action on plants to retard fungal development such as action on unspecific site, on cell membranes, on energy production, on nuclear division, on metabolism of sterol and synthesis of chitin. The mycelial growth of *C. falcatum* showed a different trend in response to different tested fungicides. Among tested fungicides Tilt showed maximum inhibited mycelial growth of *C. falcatum*. Similar results were found by Bharti *et al.,* (2014) who reported that Tilt most effective fungicide for the management of *C. falcatum* (red rot of sugarcane).

Contrary to the results of Abbas *et al*., 2016; Shukla *et al*., 2013; Imtiaj *et al*., (2007) who observed that Dithane M-45 (Mancozeb) to be the best for percent inhibition colony growth of *C. falcatum* while our findings recorded similar to Shovan *et al*., 2008; Vijaya *et al*., 2010 studies that best inhibition was attain with Tilt for all tested concentrations while Mancozeb are almost completely fail to inhabit fungal growth. It may be due to sensitivity of isolates of *C. falcatum* to fungicides application and environmental factors.

Table 4: Mycelium growth (mm) of *C. falcatum* against different concentrations of fungicides after seven days of inoculation, applied as food poison technique

Fungicides	Time interval	Concentrations				
		10ppm	15ppm	20ppm	25ppm	Control
Cabrio Top	72h	45.83±0.76a	36.33±1.15b	32.34±0.57b	30±1a	55.66±2.08a
	120h	59.67±0.57ab	53.17±0.76b	48.16±1.04b	42.67±1.15a	67.67±0.57a
	168h	66.83±1.04c	62.83±0.76c	57.33±2.51b	51.67±1.04b	89.33±1.15a
Dew	72h	35.67±0.57b	28.17±1.25c	22.67±0.28c	18.5±0.86c	55.33±0.57a
	120h	46.83±0.28c	39.33±0.57c	31.67±1.52c	25.5±0.50c	65.66±2.08a
	168h	54.33±1.15d	46.83±0.76d	37.17±0.28c	31.83±0.76d	88.33±2.08a
Secex	72h	28.16±1.04c	24.33±0.57d	21.17±0.76c	15.67±0.57d	54.67±0.57a
	120h	42.33±0.57d	33.67±1.52d	28.5±1.32d	22±1d	66.6±1.15a
	168h	49.33±0.57e	42.5±0.50e	35.34±1.52c	26±1e	90±0.00a
Metaxyl+ Mencozeb	72h	47.33±1.15a	41.00±0.00a	35.83±0.29a	30±1a	55.33±1.15a
	120h	61.67±0.57a	58.67±0.57a	52.33±0.57a	43.00±1a	65.66±0.57a
	168h	83±1a	77.67±0.57a	69.67±0.57a	59.67±1.52a	89.67±0.57a
Nativo	72h	25.33±0.57d	19.67±0.28e	16.17±0.28d	12±1e	55.67±0.57a
	120h	35.67±1.52e	30.67±1.52e	25.17±0.28e	19.33±0.57e	66±1a
	168h	44.67±0.57f	37.63±0.55f	31.67±0.57d	22.33±0.57f	89.34±1.15a
Rally	72h	47.50±0.86a	39.33±0.57a	31±1b	24.50±0.50b	55±1a
	120h	58.67±1.52b	51.00±1b	46.00±1b	35.33±0.57b	65.33±0.57a
	168h	80.4±0.52b	74.53±0.50b	57.67±0.57b	41.67±0.57c	89±1a
Tilt	72h	20.87±0.23e	16.90±0.36f	12.8±0.72e	10.5±0.50e	56±1.7a
	120h	31.34±0.57f	23.67±0.57f	17.83±1.04f	15.33±0.57f	67±1a
	168h	37.93±0.90g	29.33±0.57g	21.57±0.51e	16.5±050g	89±1.73a

Our results are to some extent also coordinated with the results obtained by Subhani *et al*., (2008) who reported that *C. falcatum* inhibition (100%) was also found in case of Tilt at different concentrations. Similarly, Bhardwaj and Sahu, (2014) reported that Carbendazim was found to be most effective against *C. falcatum* followed by Folicur, Contaff and Tilt at different concentrations respectively. In the present study, it was found that all the chemicals worked in dose dependent manner (showed maximum inhibition activity at highest concentration).

Constant dependence on fungicides has proven incompatible leading towards severe environmental problems. Screening of sugarcane varieties against red rot of sugarcane helped to evade such problems because it is environmentally sound and appropriate approach to minimize the use of chemicals. Among ten varieties used, two varieties such as NSG-59 and SPF-244 scored 2 on disease rating scale and proved to be resistant on the attack of *C. falcatum* causing red rot of Sugarcane disease. These above-mentioned results are in accordance with those of Singh and Waraitch, (1977) and Gill and Saleem, (1983 a; b) who reported that eight varieties/lines were susceptible, one moderately resistant and four were resistant to red rot disease of sugarcane. These results are in conformity with those of Gupta *et al*., (1982) who found four varieties/lines resistant and three susceptible to red rot disease. This differential behavior may be attributed to pathogenic variability among *C. falcatum* isolates. The development of new strains in the pathogen due to environmental changes is possible which is not allowing complete resistance in all verities which is already observed by Chona (1980). Our Study also indicated that resistance in sugarcane may be polygenic which is controlled by many genes (Khokhar *et al*., 2002).

CONCLUSION

Conclusively, it is urged that fungicide application is one of a sharp tool against disease control in plants if use in integrated manner. Results in present study showed that by increasing concentration of fungicides more fungal inhibition can be achieved. Fungicides can inhabit *C.*

falcatum efficiently and can be used for treatment of red rot disease of sugarcane. Regular use of fungicides is neither economical nor valuable for the surroundings. On the other hand, varieties don't show significant resistance to red rot disease. Long period of time is required for development of new varieties, because with the emergence of new virulent pathotypes resistance may breaks down and it will become susceptible with the passage of time. That's why; other way of controlling disease such as use of synthetic fungicides for activation of plant defense system can be used.

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