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Research Article

APPRAISEMENT OF CHEMOTHERAPY, PLANT DEFENSE ACTIVATORS, AND GENETIC RESISTANCE AGAINST EYESPOT DISEASE IN SUGARCANE

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ABSTRACT

Sugarcane (*Saccharum officinarum*) is a crucial agro-industrial crop that faces numerous biotic and abiotic challenges. One of the most severe biotic stresses affecting sugarcane is eyespot disease, caused by *Bipolaris sacchari*. This study focused on managing eyespot disease using resistant varieties, chemicals, and plant defense activators. Disease samples were collected from Lalian, Chiniot, and Faisalabad, and the pathogen was isolated and identified from the infected leaves. In the screening experiment, 10 sugarcane varieties were evaluated. CPF-246 exhibited high resistance, while CPF-247 and CPF-77400 showed resistance to eyespot disease. The poisoned food technique was used to assess fungicides at different concentrations (50, 100, and 150 ppm) under *in vitro* conditions. Tilt (Propiconazole) exhibited the maximum growth inhibition (4.645 mm) under these conditions. Plant activators were tested at different concentrations (0.25%, 0.5%, and 0.75%) in greenhouse conditions. Salicylic acid resulted in the minimum disease incidence (13.236%, 7.483%, and 6.473%) after 15, 30, and 45 days, respectively. Under field conditions, the combination of Salicylic acid and Tilt (12.395%) showed the lowest disease incidence. By employing these newly investigated management strategies, the farming community can effectively combat the significant threat posed by eyespot disease to the sugarcane industry.

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INTRODUCTION

Sugarcane (*Saccharum officinarum*) is a perennial crop belonging to the family Poaceae and the genus *Saccharum*. It is an important agro-industrial crop and leads in global production (Verma et al., 2022). Sugarcane is cultivated on 26.4 million hectares, with an annual production of 1,850 million tons worldwide (FAOSTAT, 2021). In Pakistan, it is grown on 1.16 million hectares, with an annual production of 81.009 million tons (GoKP, 2019). For Pakistan, sugarcane is the second-largest cash crop after wheat, contributing 2.9% to agriculture's value addition and 0.6% to the overall gross domestic product (GDP) of the country (Raza et al., 2021). Sugarcane requires a warm climate with temperatures between 20-35°C, high humidity (80-85 %), and annual precipitation of 75 cm (Cheng, 2017). The production of sugarcane is affected by several biotic (eyespot, smut, red stripe, rust, red rot, mosaic virus, and pokka boeing) (Majumdar and Prakash, 2020) and abiotic (light, temperature, water, and humidity) factors (Viswanathan, 2020).

Eyespot caused by *Bipolaris sacchari* results in 40% losses in sugarcane production (Wu et al., 2020) under favorable environmental conditions. This disease is common in areas with more than 70% relative humidity, high rainfall (500 mm), an optimum temperature of 25-30°C, and an optimum pH of 5 (Huang et al., 2018). It is an airborne disease (Raharjo et al., 2024) and its dispersion occurs primarily through wind and rain. Conidia of the fungus land on the sugarcane and germinate when they encounter water or dew. Asexual spores are dark brown conidia that are cylindrical (38-153 × 3-6 µm) and septate (1-6 septa) (Raharjo et al., 2024). It produces ascospores during its sexual stage. Reddish-brown elliptical lesions with yellow-brown margins and brown to yellow streaks (commonly known as runners) are typical symptoms of eyespot disease (Magarey, 2019).

Several approaches have been used for the management of eyespot disease, such as resistant varieties, chemicals, and plant activators. However, the use of resistant varieties is the most effective method for controlling the disease. The use of resistant varieties is a favorable method as it reduces the need for pesticides and helps maintain soil fertility. Twenty-seven sugarcane varieties were screened, and it was observed that 14 were susceptible and 13 were resistant (Viswanathan et al., 2021). In the current research, different sugarcane varieties were screened against eyespot disease. Although resistant varieties are the most appropriate way to manage eyespot disease, if the disease appears in epidemic form, farmers have no option except to use chemicals. In this contemporary study, different chemicals at various concentrations were evaluated against *B. sacchari*, the cause of eyespot. The use of chemicals is effective in managing eyespot disease because they are readily available and provide rapid results. Mane et al. (2018) tested fungicides and recorded 100% mycelial inhibition of *B. sacchari* with Propiconazole (0.1%) and Propiconazole + Difenoconazole (0.1%). Ramesh et al. (2021) evaluated seventeen new molecules of fungicides against *Bipolaris* spp. and found that Propiconazole showed 100% inhibition of mycelial growth at different concentrations. Poudel et al. (2019) investigated various chemicals (Propiconazole, Carbendazim, Mancozeb, Tebuconazole, and Hexaconazole) and concluded that Propiconazole can be used to reduce disease severity.

Apparently, chemicals are the most effective way to manage eyespot disease, but they have certain long-term side effects on human and animal health as well as the environment. Therefore, it is pressing to adopt alternative approaches for managing eyespot disease in sugarcane. Plant activators are chemicals that induce plant defense responses to a broad spectrum of pathogens (Bian et al., 2020). Proteins induced by pathogenesis are involved in plant defense, leading to pathogen death or inducing other plant defense responses (Zhang et al., 2020). Singh et al. (2019) evaluated the antifungal activity of signaling molecules of salicylic acid alone and in combination with Tricyclazole. It significantly increased the activation and accumulation of phenylalanine ammonia-lyase, peroxidase, 1-3 glucanase, and chitinase activity in *Bipolaris* spp. Shabana et al. (2008) evaluated the antifungal activities of plant activators against *Bipolaris* spp. at different concentrations *in vitro* as well as under field conditions. Benzoic acid and salicylic acid completely inhibited the growth of *Bipolaris* spp. at 9 mM *in vitro*. Spraying benzoic acid resulted in a remarkable reduction in disease severity and incidence on the leaves at 20 mM under field conditions. It was concluded that plant activators are an effective management strategy to control eyespot disease compared to commercial fungicides, which are hazardous to the environment. In the current study, different plant activators at various concentrations were evaluated against eyespot in sugarcane.

MATERIALS AND METHODS

Isolation, purification, and identification of *Bipolaris sacchari*

Leaves of sugarcane showing eyespot symptoms were collected from an area of Tehsil Lalian, district Chiniot. The collected samples were kept in a polythene bag and brought to the Plant Pathology Lab at the University of Agriculture Faisalabad (UAF). The diseased samples were cut into small pieces (3-5 mm long) along with a healthy portion. The samples were washed with tap water and surface sterilized with 1% sodium hypochlorite (NaOCl) for 30 sec. followed by three rinses with distilled water. PDA media was prepared using an autoclave at 121°C and 15 Psi for 15 min.. The PDA medium was poured into Petri plates, and after solidification, samples were placed on these plates and wrapped with paraffin tape. After labeling, the plates were incubated at 25 ± 5°C for mycelial growth, which was observed after 24 h. For the purification of fungus, the single hyphal tip method was used. A small mycelium was picked from the culture, placed into prepared PDA media, and incubated at 27°C. The pure culture of the isolated pathogen was observed and studied under a microscope for morphological characteristics (shape, size, and colony) and compared with relevant literature for identification (Senanayake et al., 2020).

Pathogenicity test

For the confirmation of the pathogen, a pathogenicity test was performed following Koch's postulates. For this purpose, sugarcane buds were grown in pots. When the plants attained five leaves, the inoculum was sprayed on healthy plants while one plant was kept as a control. After 14-15 days of inoculation, symptoms were observed. The pathogen was re-isolated from the artificially inoculated plants for further studies (Costa et al., 2021).

Screening of sugarcane varieties against eyespot disease under field condition

To achieve resistance against eyespot disease in sugarcane, 10 varieties (HSF-240, CPF-246, CPF-247, CPF-248, CPF-249, CPF-250, CPF-251, CPF-252, CPF-253, and CP-77400) were grown with a row-to-row distance of 60-75 cm and a plant-to-plant distance of 20-22 cm in the Research Area of Plant Pathology at UAF. All recommended agronomic practices were followed to ensure healthy crop growth. Inoculation was performed by mechanically injuring the leaf surface using carborundum powder. A spore suspension (10^4 spores/ml) (Zhang et al., 2015) was then sprayed using a hand sprayer in the morning. After 15 days of inoculation, disease symptoms appeared, and data regarding disease incidence was recorded at 15-day intervals using the disease rating scale of Lipps and Herr (1982). Disease was rated on a scale from 0 to 5. A rating of 0 indicated no detectable disease development, 1 indicated browning of the outer leaf sheath, 2 indicated a definite eyespot lesion on the outer leaf sheath less than 5 mm in length, 3 indicated one to several eyespot lesions on the outer leaf sheath greater than 5 mm in length, 4 indicated lesions with penetration to the inner leaf sheath, and 5 indicated a dead or severely affected plant (Lipps and Herr, 1982). These data were transformed to the uniform disease rating scale (0-9).

The disease incidence was measured by the following formula.

$$\text{Disease incidence (\%)} = \frac{\text{No.of infected plants}}{\text{Total no.of healthy plants}} \times 100$$

***In vitro* management of *B. sacchari* using chemicals and poisoned food technique**

Five chemicals (Tilt, Cabrio Top, Amister Top, Nativo, and Champion) (Table 1) were evaluated against eyespot disease of sugarcane at three different concentrations (50, 100, and 150 ppm) using poisoned food technique under completely randomized design (CRD). To prepare a stock solution, the percentage of the active ingredient of each chemical was divided by 100, and that quantity of the chemical was added to 100 ml of distilled water. To prepare 50, 100, and 150 ppm concentrations from the stock solution, 0.5, 1, and 1.5 ml were taken and mixed in 100 ml of PDA media respectively. PDA media containing chemical concentrations were poured into 90 mm petri plates. After solidification of the media, a disc (5 mm) of 8-day-old culture was placed in the center of Petri plates. These plates were incubated at 25 ± 5°C. The readings were taken after 24 h, 48 h, and 72 h of incubation. A volumetric scale was used to measure the radial growth of fungi.

Evaluation of plant activators against eyespot disease of sugarcane caused by *B. sacchari* under greenhouse conditions

A pot trial was carried out to select a plant activator in a greenhouse under CRD. Chip buds of a moderately susceptible variety of sugarcane were treated with hot water at 54°C for 20 m, along with plant activators (salicylic acid, benzoic acid, di-potassium hydrogen phosphate, potassium hydrogen phosphate, and citric acid) (Table 2). These plants were grown in pots under greenhouse conditions. When the plants reached the age of 60-65 days, they were sprayed with different concentrations (0.25 %, 0.5 %, and 0.75 %) of plant activators. One set of plants was treated as a control. Data regarding disease incidence was recorded at 15-day intervals.

Evaluation of chemicals and plant defense activators against eyespot disease of sugarcane caused by *B. sacchari* under field conditions

The most effective chemical (Tilt) and plant activator (Salicylic acid), with three replications at three concentrations (1%, 1.5%, and 2%), were applied against the pathogen on two different sets of plants separately in the field, and data were recorded at 15-day intervals after treatment. The combined effect of the most effective chemical (Tilt) and plant activator (Salicylic acid) was also evaluated against the pathogen. One set of plants was treated with these chemicals and plant activators with three replications at three different concentrations (1%, 1.5%, and 2%), while the other set was treated as a control (treated with distilled water). Data regarding disease incidence were recorded at 15-day intervals.

Data Analysis

All statistical analyses were performed using SAS/STAT statistical software (SAS Institute, 2009). All the *in vitro* and *in vivo* experiments were conducted under CRD and RCBD respectively. Means were separated using Fisher's protected least significant difference (LSD) procedure at a P ≥ 0.05 probability level (Steel et al., 1997). Analysis of variance (ANOVA) identified significant treatments and their combinations for the management of red rot disease of sugarcane.

Table 1. Chemicals used in the experiment along with active ingredient and mode of action.

Chemicals	Active ingredients	Mode of action	References
Tilt	Propiconazole (25 %)	It stops the development of fungi by interfering with the biosynthesis of sterols in cell membranes	Uppala and Zhou (2018).
Cabrio Top	Pyraclostrobin + Metiram (50 % + 550 %)	It blocks the energy supply of the fungus	Younas et al. (2021)
Amistar Top	Azoxystrobin+ Difenoconazole (18.2 % + 11.4 %)	It inhibits spore germination at the early stages of fungal development	El-Enany et al. (2019)
Nativo	Trifloxystrobin + Tebuconazole (25 % + 50 %)	it inhibits the reproduction and further growth of fungus and also interferes with respiration process	Zhang et al. (2018)
Champion	Copper hydroxide (46.1 %)	Copper ions kill by denaturing proteins and enzymes in cells of pathogens	Majumdar et al. (2021)

Table 2: Plant activators used in the experiment along with mode of action.

Plant Activator	Mode of Action	References
Salicylic acid	SA may also increase the accumulation of Cd in the cell wall and prevent its translocation into other cell organelles.	Janda et al. (2020)
Benzoic acid	benzoic acid inhibited growth by acidification of the cytoplasm, leading to the inhibition of glycolysis because of a specific inhibition of phosphofructokinase	Krebs et al. (1983)
K_2HPO_4	It inhibits the mycelial growth of pathogen	Arslan (2015)
KH_2PO_4	It inhibits the mycelial growth of pathogen	Arslan (2015)
Citric acid	Citric acid helps support respiration demands and improves cellular energy	Roosterman and Cottrell, 2021

RESULTS

Screening of sugarcane varieties to identify resistance to eyespot disease

Ten sugarcane varieties were screened for resistance to eyespot disease. Among these, CPF-246 exhibited a highly resistant reaction, followed by CPF-247 and CPF-77400, which showed a resistant reaction. One variety, CPF-252, demonstrated moderate resistance. CPF-249 and CPF-251 were moderately susceptible, while HSF-240, CPF-248, and CPF-250 were susceptible. CPF-253 showed a highly susceptible response (Table 3 and Figure 1).

Table 3: Screening of sugarcane varieties against eyespot disease of sugarcane

Sr. No.	Varieties	Disease rating scale	Disease incidence (%)	Response
1	CPF-250	4	46.43 c	Suceptible (S)
2	CPF-247	1	18.65 g	Resistant (R)
3	CPF-251	3	36.71 e	Moderately Suceptible (MS)
4	CPF-253	5	51.81 a	Highly Suceptible (HS)
5	CPF-252	2	22.51 f	Moderately Resistant (MR)
6	CPF-246	0	9.10 i	Highly Resistant (HR)
7	CPF-248	4	43.28 d	Suceptible (S)
8	CPF-249	3	34.30 e	Moderately Suceptible (MS)
9	CPF-7740	1	14.72 h	Resistant (R)
10	HSF-240	4	48.72 b	Susceptible(S)
	LSD		0.0611	

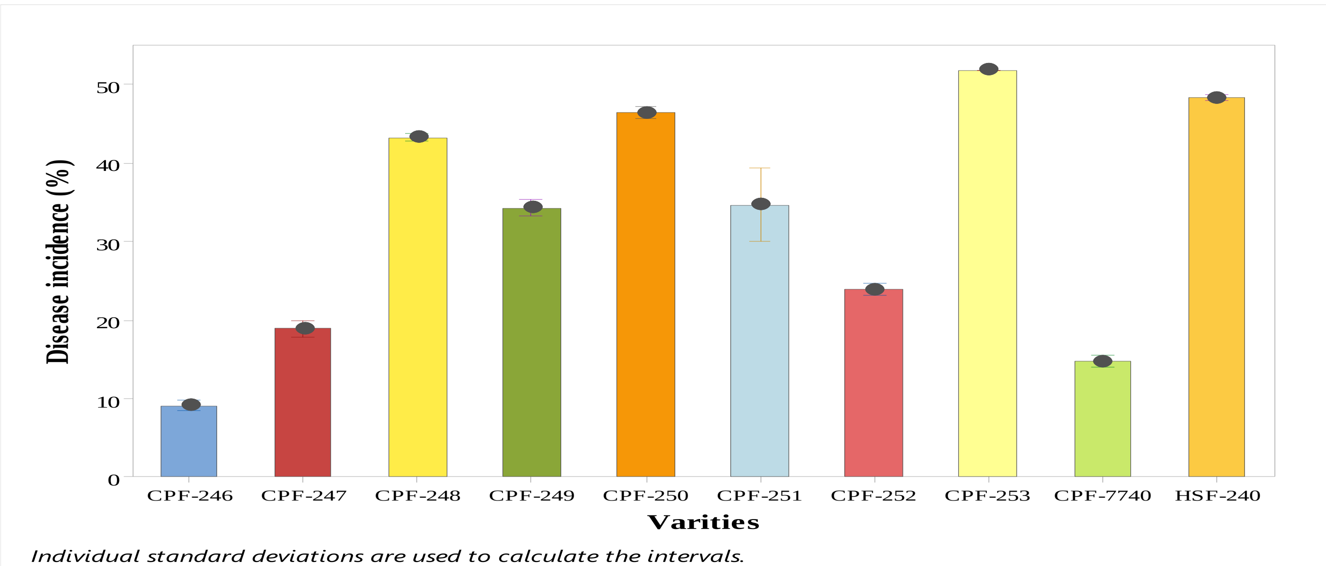

Figure 1. Screening of sugarcane varieties against eyespot disease of sugarcane under field conditions at (P ≥0.05). The bars in the figure express SD values.

Evaluation of fungicides against eyespot disease of sugarcane caused by *B. sacchari* under laboratory conditions

Among all the treatments, Tilt exhibited the minimum growth (4.645 mm), followed by Amistar Top (5.826 mm), Cabrio Top (8.448 mm), Nativo (10.132 mm), and Champion (12.470 mm) compared to the control (Table 4 and Figure 2). The interaction between treatments and concentrations (T×C) showed that minimum growth (3.193 mm) was expressed by Tilt at 150 ppm, 4.033 mm at 100 ppm, and 6.708 mm at 50 ppm, respectively. In contrast, Amistar Top exhibited minimum growth of 8.050 mm, 5.624 mm, and 3.802 mm; Cabrio Top showed 9.737 mm, 8.653 mm, and 6.953 mm; Nativo displayed 12.953 mm, 9.764 mm, and 7.679 mm; Champion demonstrated growth of 15.693 mm, 11.953 mm, and 9.764 mm at concentrations of 50 ppm, 100 ppm, and 150 ppm respectively compared to the control (Table 5 and Figure 3). Interaction between treatments and days (T×D) showed that Tilt exhibited minimum growth (5.709, 4.47, and 3.75 mm) after the 1st, 2nd, and 3rd days respectively, followed by Amistar Top (6.800, 5.733, and 4.943 mm), Cabrio Top (9.743, 8.289, and 7.311 mm), Nativo (11.361, 10.104, and 8.931 mm), and Champion (13.694, 12.452, and 11.254 mm) after the 1st, 2nd, and 3rd days respectively compared to the control (25.000, 25.000, 25.000) (Table 6 and Figure 4).

Table 4: Evaluation of fungicides against *B. sacchari* causing eyespot disease of sugarcane under laboratory conditions.

Sr. No.	Treatments	Active ingredients	Fungal growth (mm)
1	Tilt	Propiconazole	4.645 f
2	Champion	Copper hydroxide	12.470 b
3	Nativo	Tebuconazole and Trifloxystrobin	10.132 c
4	Amistar Top	Azoxystrobin and Difenoconazole	5.826 e
5	Cabrio Top	Pyroclostrobin and Metiram	8.448 d
6	Control (DW)	Distilled water (H_2O)	25.000 a
	LSD		0.2792

The mean value in a column sharing similar letters does not differ significantly as figured out by the LSD test ($P<0.05$).

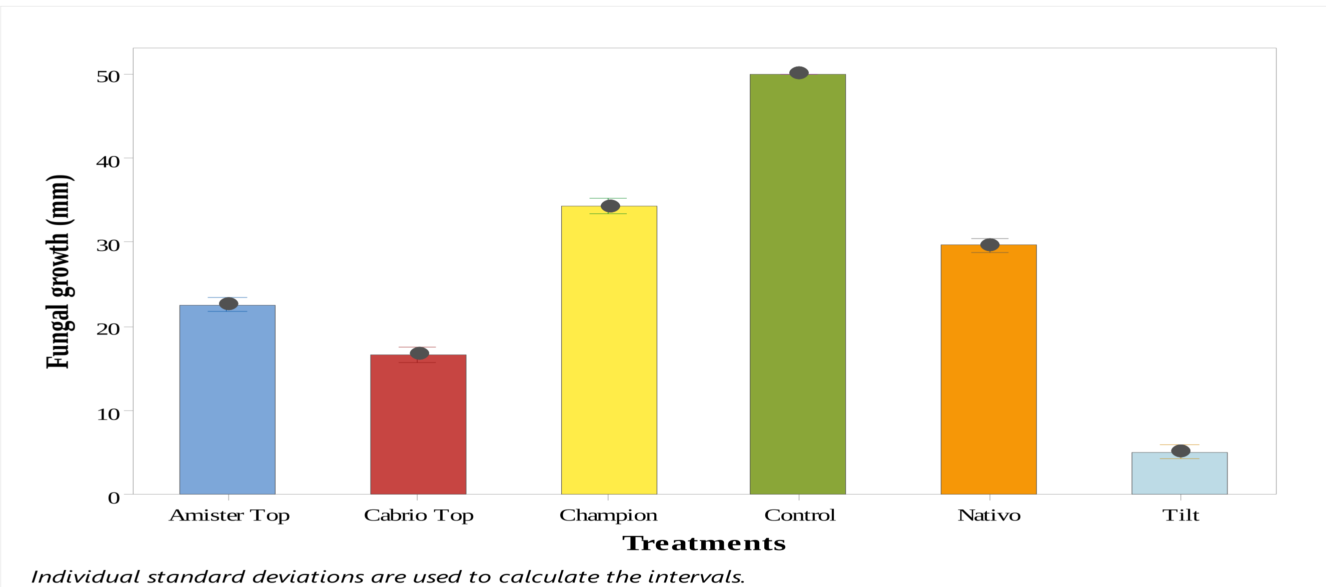

Figure 2. Evaluation of chemicals against eyespot disease of sugarcane under *in vitro* conditions at ($P \geq 0.05$). The bars in the figure express SD values.

Table 5: Impact of interaction between treatments and concentrations (T×C) against eyespot disease of sugarcane under *in vitro* conditions.

Treatment	Fungal growth (mm)		
	Concentrations		
	50 ppm	100 ppm	150 ppm
Tilt	6.708 fg	4.033 hi	3.193 i
Champion	15.693 b	11.953 c	9.764 d
Nativo	12.953 c	9.764 d	7.679 ef
Amistar Top	8.050d ef	5.624 gh	3.802 i
Cabrio Top	9.737 d	8.653 de	6.953 efg
Control (H_2O)	25.000 a	25.000 a	25.000 a
LDS	0.4836		

The mean value in a column sharing similar letters does not differ significantly as figured out by the LSD test ($P<0.05$).

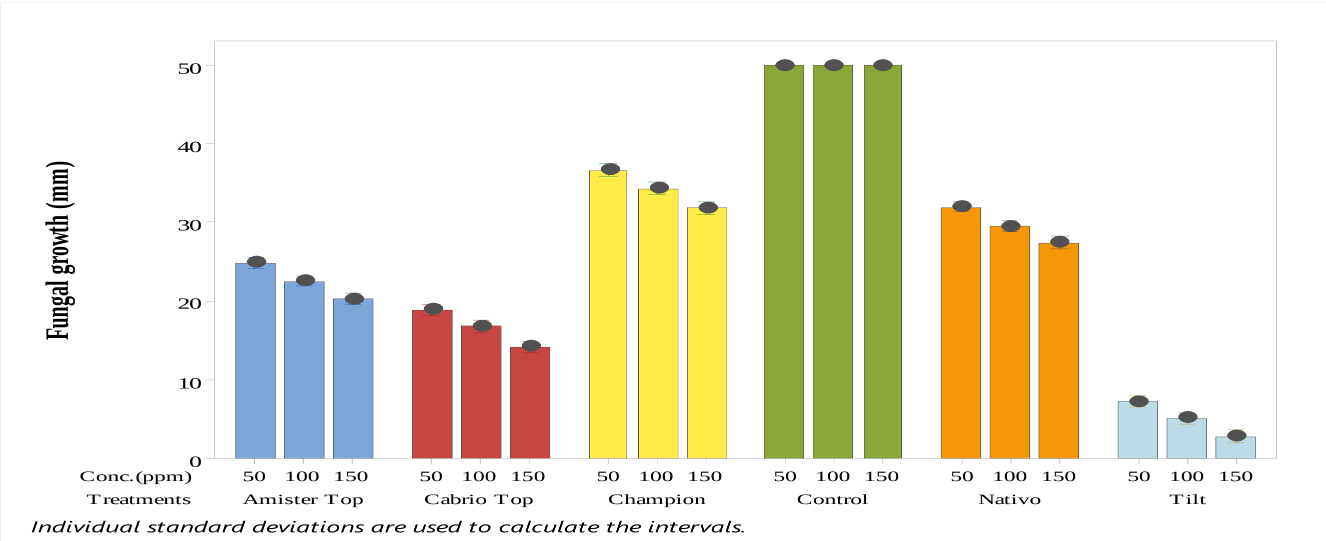

Figure 3. Evaluation of interaction between treatments and concentrations (T×C) against eyespot disease of sugarcane under *in vitro* conditions at (P ≥0.05). The bars in the figure express SD values.

Table 6. Impact of interaction between treatments and Days (T×D) against eyespot disease of sugarcane under *in vitro* conditions.

Treatment	Fungal growth (mm)		
	Days		
	1	2	3
Tilt	5.709 ij	4.474 jk	3.751 k
Champion	13.694 b	12.452 bc	11.254 cd
Nativo	11.361 cd	10.104 de	8.931 efg
Amistar Top	6.800 hi	5.733 ij	4.943 jk
Cabrio Top	9.743 def	8.289 fgh	7.311 ghi
Control	25.000 a	25.000 a	25.000 a
LDS	0.4836		

The mean value in a column sharing similar letters does not differ significantly as figured out by the LSD test (P<0.05).

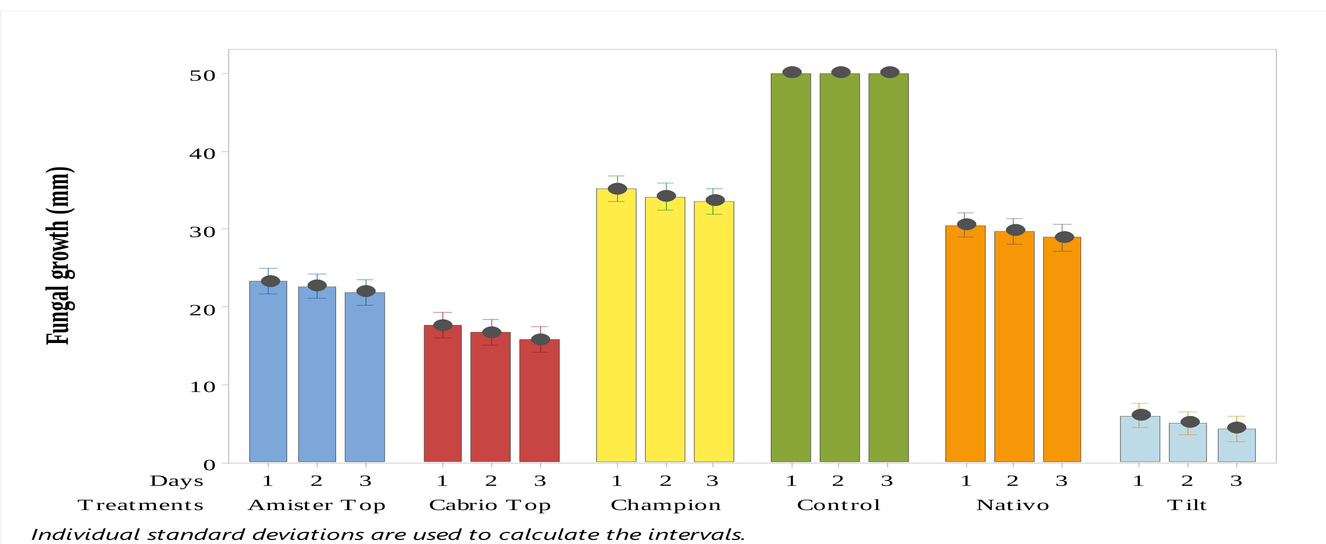

Figure 4. Evaluation of interaction between treatments and Days (T×D) against eyespot disease of sugarcane under *in vitro* conditions at (P ≥0.05).The bars in the figure express SD values.

Evaluation of plant activators against eyespot disease of sugarcane under greenhouse conditions

Salicylic acid showed the lowest disease incidence (9.064 %), followed by benzoic acid (11.867 %), K_2HPO_4 (13.922 %), KH_2PO_4 (16.171 %), and citric acid (20.864 %) compared to the control (48.173 %) (Table 7 and Figure 5). The interaction between treatments and concentrations (T×C) showed that the minimum disease incidence was observed with salicylic acid at 0.75% (7.703%), 0.5% (7.457%), and 0.25% (12.032%), followed by benzoic acid at 0.75% (11.073%), 0.5% (10.380%), and 0.25% (14.149%). For K_2HPO_4, the incidences were 9.670 %, 11.526 %, and 20.570%, respectively, while for KH_2PO_4, they were 14.012%, 14.297%, and 20.204 %. Citric acid showed incidences of 16.594%, 19.151%, and 26.848% at the respective concentrations, compared to the control group which had incidences of 48.687%, 46.907%, and 48.927% (Table 8 and Figure 6). Interaction between treatments and days (T×D) showed that minimum disease incidence (6.473%) was observed with salicylic acid after 3 weeks, and 7.483% and 13.236% after 2 and 1 weeks, respectively, followed by benzoic acid (16.637%, 10.197%, and 8.769%), K_2HPO_4 (19.481%, 12.703%, and 9.581 %), KH_2PO_4 (21.448%, 14.862%, and 12.203%), and citric acid (26.398%, 19.754%, and 16.441%) after 1, 2, and 3 weeks, respectively, as compared to control (48.837 %, 48.807%, and 46.877%) (Table 9 and Figure 7).

Table 7. Evaluation of plant activators against eyespot disease of sugarcane under greenhouse conditions.

Treatments	Chemical formula	Disease incidence (%)
Potassium hydrogen phosphate	K_2HPO_4	13.922 d
Potassium dihydrogen phosphate	KH_2PO_4	16.171 c
Citric acid	$C_6H_8O_7$	20.864 b
Benzoic acid	$C_7H_6O_2$	11.867 e
Salicylic acid	$C_7H_6O_3$	9.06 4 a
Control (Distilled water)	H_2O	48.173
LSD		0.9936

The mean value in a column sharing similar letters does not differ significantly as figured out by the LSD test (P<0.05).

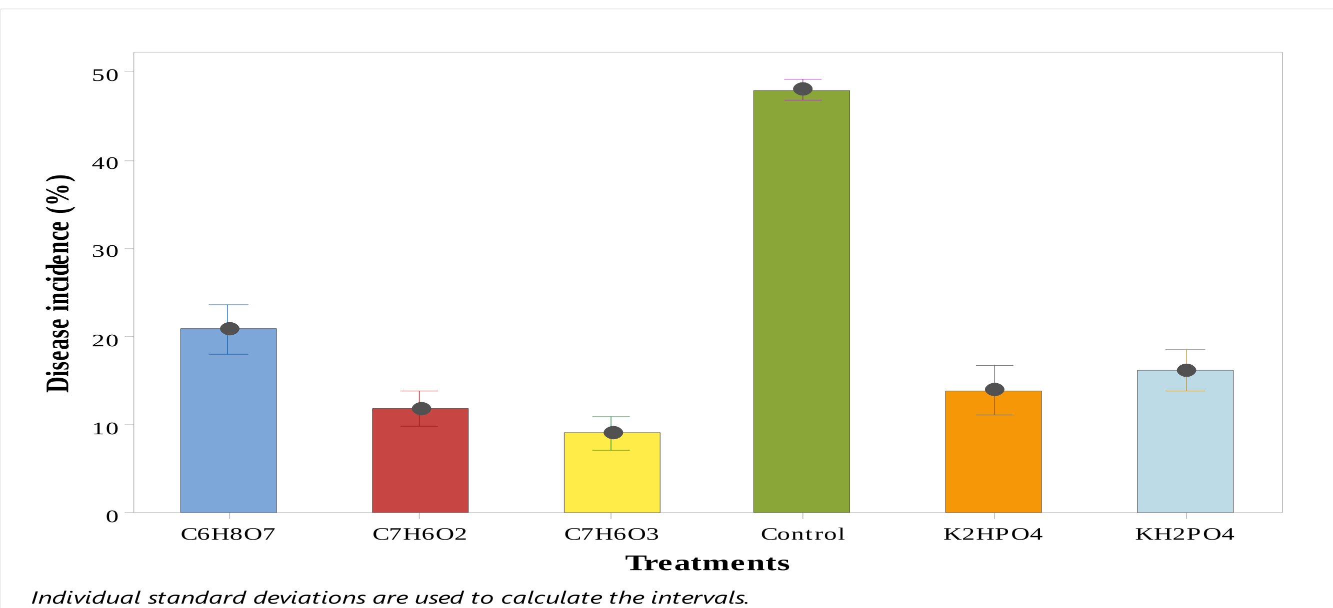

Figure 5. Evaluation of plant activators against eyespot disease of sugarcane under greenhouse conditions at (P ≥0.05). The bars in the figure express SD values.

Table 8. Impact of interaction between treatments and concentrations (T×C) against eyespot disease of sugarcane under greenhouse conditions.

Treatments	Disease incidence (%)		
	Concentrations		
	0.25 (%)	0.5 (%)	0.75 (%)
K_2HPO_4	20.570 c	11.526 fg	9.670 ghi
KH_2PO_4	20.204 c	14.297 ef	14.012 ef
Citric acid	26.8489 b	19.151 cd	16.594 de
Benzoic acid	14.149 ef	10.380 ghi	11.073 fgh
Salicylic acid	12.032 fg	7.457 i	7.703 hi
Control	48.927 a	46.907 a	48.687 a
LDS	1.7209		

The mean value in a column sharing similar letters does not differ significantly as figured out by the LSD test (P<0.05).

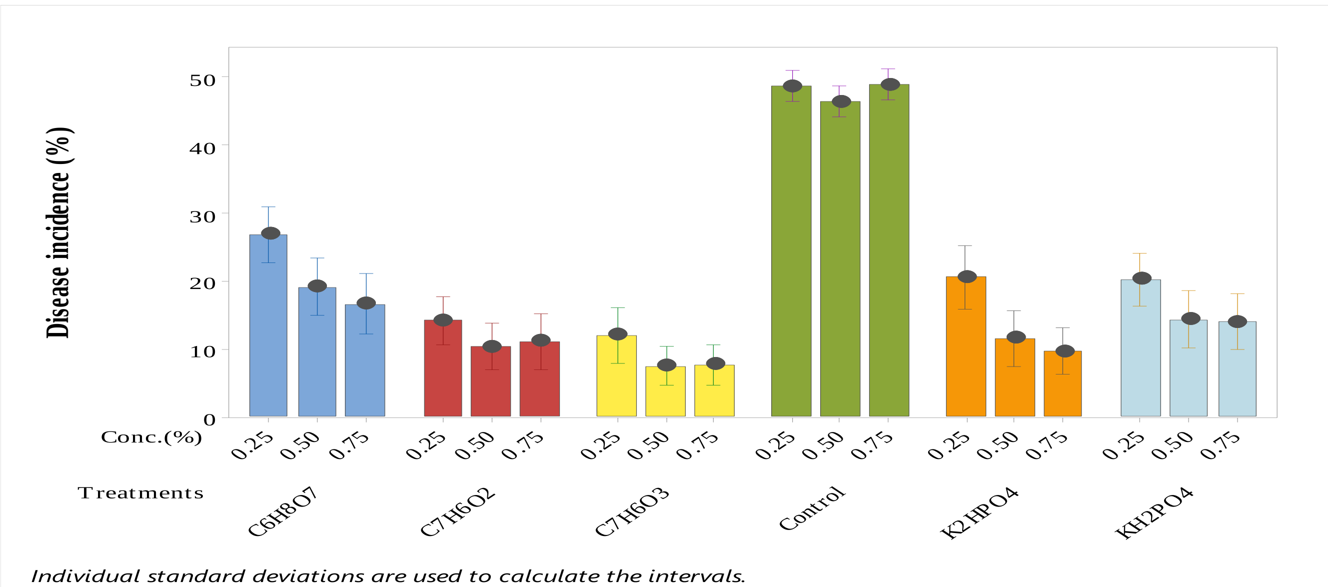

Figure 6. Evaluation of interaction between treatments and concentrations (T×C) against eyespot disease of sugarcane under greenhouse conditions at (P ≥0.05) while bars in the figure express SD values.

Table 9. Impact of interaction between treatments and days (T×D) against eyespot disease of sugarcane under greenhouse conditions.

Treatments	Disease incidence (%)		
	Days		
	15	30	45
K_2HPO_4	19.481 cd	12.703 fgh	9.581 ghi
KH_2PO_4	21.448 c	14.862 ef	12.203 fgh
Citric acid	26.398 b	19.754 cd	16.441 de
Benzoic acid	16.637d e	10.197 ghi	8.769 ij
Salicylic acid	13.236 efg	7.483 ij	6.473 j
Control (H_2O)	48.837 a	48.807 a	46.877 a
LDS	1.7209		

The mean value in a column sharing similar letters does not differ significantly as figured out by the LSD test (P<0.05).

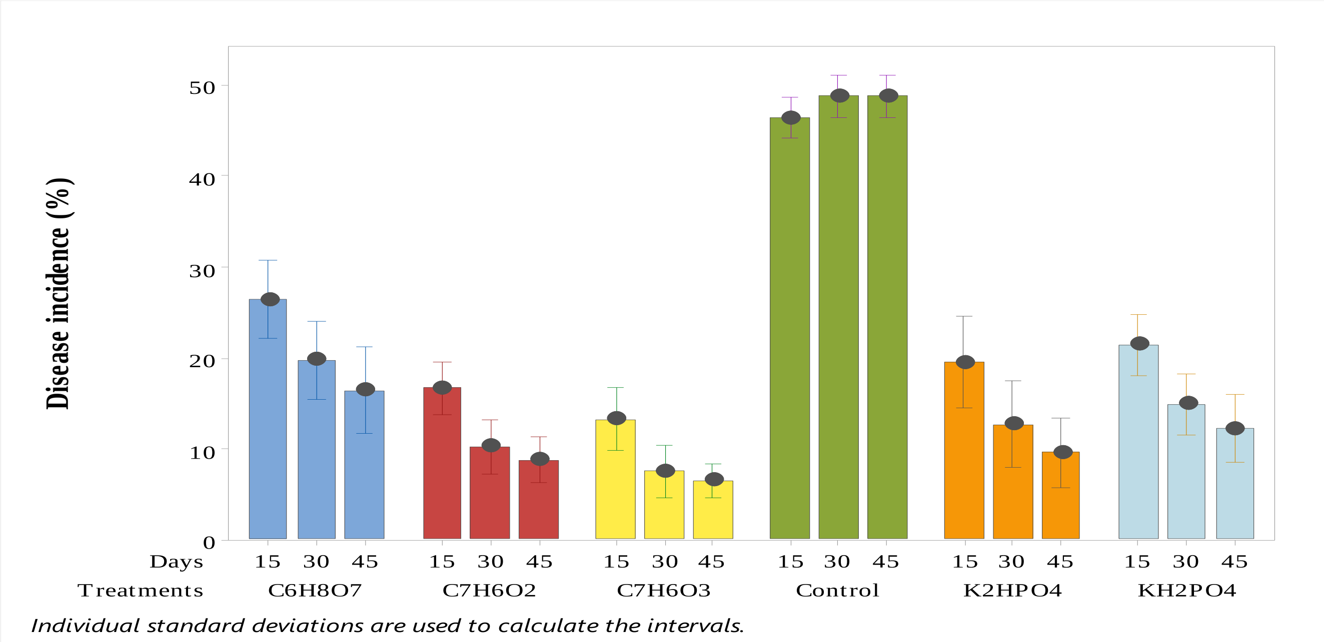

Figure 7. Evaluation of interaction between treatments and days (T×D) against eyespot disease of sugarcane under greenhouse conditions at ($P \geq 0.05$) while bars in the figure express SD values.

Evaluation of the most effective plant activator and chemical, alone and in combination, against eyespot disease of sugarcane under field conditions

Among all the treatments, the minimum disease incidence was observed with the combination of salicylic acid and Tilt at 12.395 %, followed by salicylic acid at 15.654 % and Tilt at 22.848 %, compared to the control at 47.636 % (Table 10 and Figure 8). The interaction between treatments and concentrations (T×C) showed that the minimum disease incidence (7.016 %) was observed with the combination of salicylic acid and Tilt at 2%, followed by incidences of 10.649% and 19.520% at 1.5% and 1%, respectively. Salicylic acid alone showed incidences of 20.354%, 14.447 %, and 12.162 %, while Tilt showed incidences of 29.998%, 22.301%, and 16.244% at concentrations of 1%, 1.5%, and 2%, respectively, compared to the control with incidences of 40.982%, 48.520%, and 53.407% (Table 11 and Figure 9). Interaction between treatments and days (T×D) showed that the minimum disease incidence (5.316%) was observed with the combination of salicylic acid and Tilt after 3 weeks, followed by 8.371% and 23.498% after 2 and 1 weeks, respectively. This was followed by salicylic acid alone (26.998%, 11.312%, and 5.316%) and Tilt alone (34.448%, 18.704%, and 15.391%) after 1, 2, and 3 weeks, respectively, compared to the control (45.877%, 47.807%, and 49.226%) (Table 12 and Figure 10).

Table 10. Evaluation of most effective plant activator and chemical alone and in combination against eyespot disease of sugarcane under field conditions.

Treatments	Disease incidence (%)
Salicylic acid	15.654 c
Tilt	22.848 b
SA + Tilt	12.395 d
Control (H_2O)	47.636 a
LSD	1.2448

The mean value in a column sharing similar letters does not differ significantly as figured out by the LSD test ($P<0.05$).

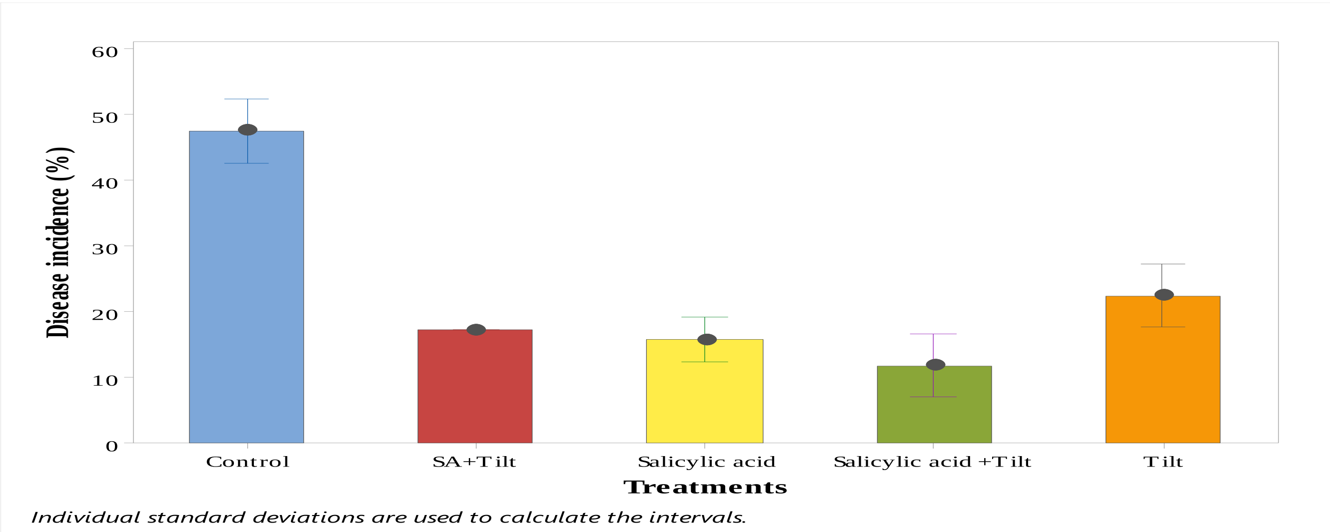

Figure 8. Evaluation of most effective plant activator and chemical alone and in combination against eyespot disease of sugarcane under field conditions at (P ≥0.05) while bars in the figure express SD values.

Table 11. Impact of interaction between treatments and their concentrations (T×C) against eyespot disease of sugarcane under field conditions.

Treatments	Disease incidence (%)		
	Concentrations		
	1 %	1.5 %	2 %
Salicylic acid	20.354 ef	14.447 gh	12.162 gh
Tilt	29.998d	22.301e	16.244 fg
SA + Tilt	19.520 ef	10.649 hi	7.016 i
Control	40.982 c	48.520 b	53.407 a
LDS	2.1561		

The mean value in a column sharing similar letters does not differ significantly as figured out by the LSD test (P<0.05).

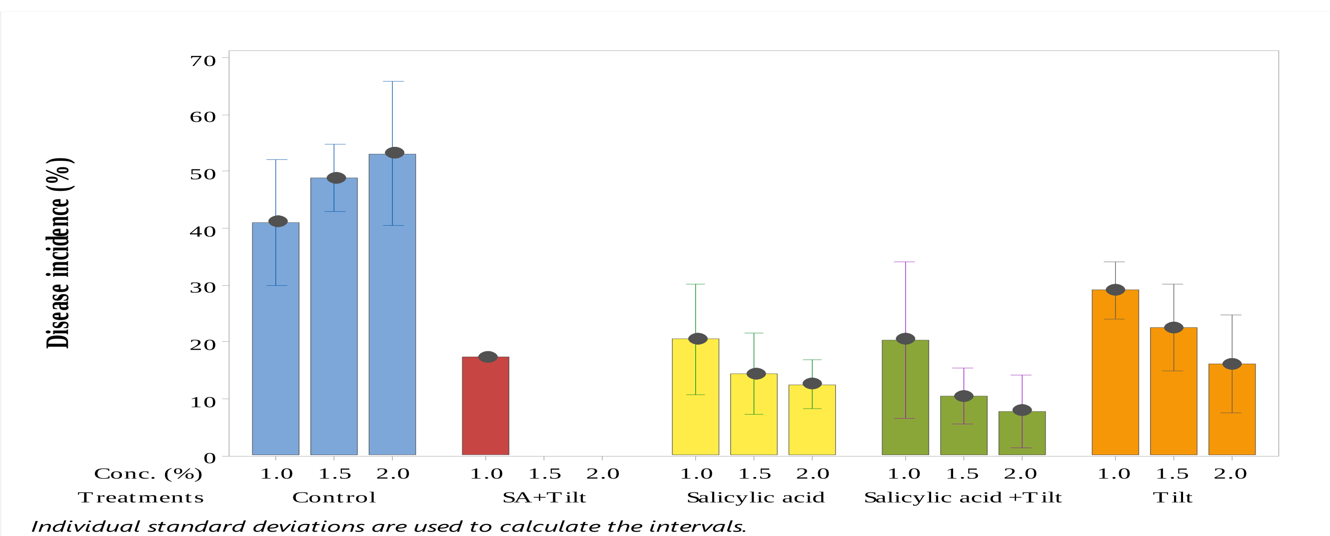

Figure 9. Evaluation of interaction between treatments and concentrations (T×C) against eyespot disease of sugarcane under field conditions at (P ≥0.05) while bars in the figure express SD values.

Table 12. Impact of interaction between treatments and days (T×D) against eyespot disease of sugarcane under field conditions.

Treatments	Disease incidence (%)		
	Weeks		
	1	2	3
Salicylic acid	26.998 c	11.312 ef	5.316 g
Tilt	34.448 b	18.704 d	15.391d e
SA + Tilt	23.498 c	8.371 fg	5.316 g
Control	45.877 a	47.807 a	49.226 a
LDS		2.1561	

The mean value in a column sharing similar letters does not differ significantly as figured out by the LSD test (P<0.05).

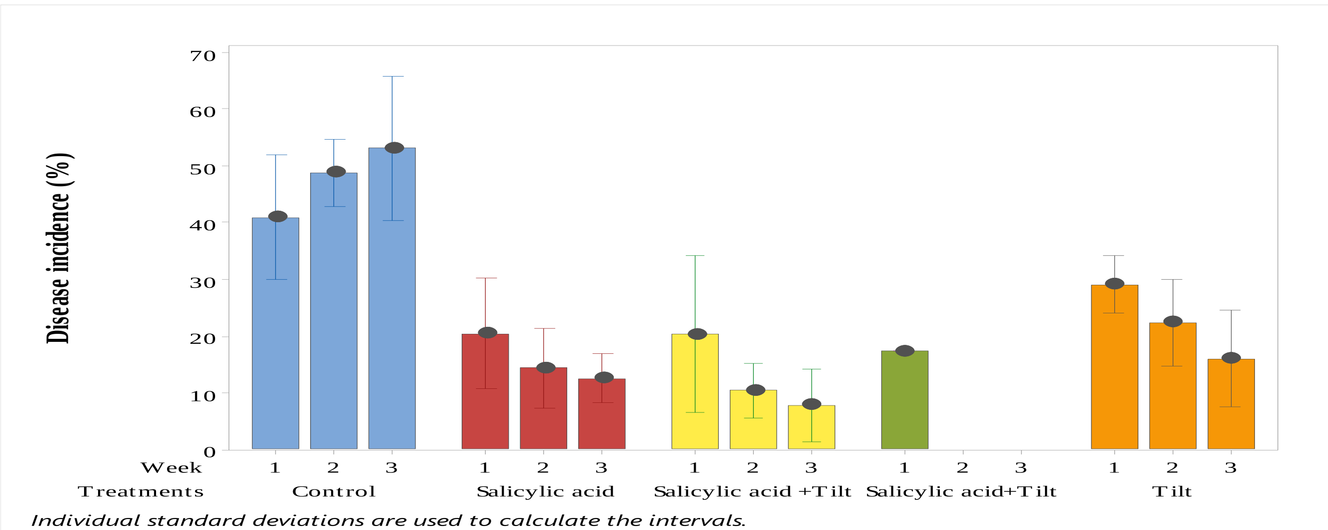

Figure 10. Evaluation of interaction between treatments and days (T×D) against eyespot disease of sugarcane under field conditions at (P ≥0.05) while bars in the figure express SD values.

DISCUSSION

For the management of eyespot disease, several approaches have been used, such as resistant varieties, chemicals, and plant activators. However, the use of resistant varieties is the most effective method to control the disease. Therefore, ten sugarcane varieties were screened against eyespot disease. Among these varieties, CPF-246 showed an HR reaction; CPF-247 and CPF-77400 expressed an R reaction; one variety, CPF-252, showed an MR response; CPF-249 and CPF-251 showed an MS response; HSF-240, CPF-248, and CPF-250 showed an S response; and CPF-253 showed an HS response. Analysis of quantitative transcript indicated that mycoprotective gene (SUGARWIN1 and SUGARWIN2) expression was highest in genotype CPF-246. Among the observed genotypes, the most susceptible one showed the lowest SUGARWIN expression, while the most resistant genotypes showed comparatively higher gene expression. The expression of SUGARWIN genes indicates the tolerance level to fungal pathogens (Parvaiz et al., 2019). The total phenolic content of healthy foliage in the resistant variety was considerably higher than that of the susceptible variety. The resistant cultivar has higher peroxidase activity in healthy leaves compared to the susceptible cultivar. The susceptible progeny has downregulated PR10, HCT1, and ScChi, while these genes are upregulated in the resistant progeny. This could be an early attempt to halt pathogen development by increasing lignin deposition at the infection site, and they were extremely upregulated in the susceptible progeny. This indicates that PR10 is involved in the resistance mechanism of the resistant progeny (Hidayah et al., 2021). Results of the present study are supported by Pervaiz et al. (2019), who identified some resistant varieties of sugarcane. Nine varieties were evaluated, with CPF-246 showing resistance, CPF-248 and CP-77400 showing moderate resistance, and CPF-247 showing a moderately susceptible response. Viswanathan et al. (2021) screened 27 sugarcane varieties and observed that 14 of them were susceptible, while 13 varieties were resistant to eyespot disease.

Although resistant varieties are the most effective in managing eyespot disease, if the disease prevails in epidemic form, farmers have no choice but to use chemicals. Chemical use is effective in managing eyespot disease as it is quick, easy, and low-cost. Among chemicals, Tilt was the most effective in inhibiting the mycelial growth of fungus. Propiconazole works by demethylating C-14 during ergosterol biosynthesis, causing an accumulation of C-14 methyl sterols. The biosynthesis of these ergo sterols are essential for the formation of fungal cell walls. The growth of the fungus is slowed or stopped due to the lack of normal sterol production, effectively preventing further infection and invasion of host tissues (Atiq et al., 2023). It also enhances photochemical activity and superoxide dismutase (SOD) activity. Plants can metabolize propiconazole by hydroxylating the n-propyl group on the dioxolane ring, yielding four beta-hydroxy isomers that form sugar conjugates. The dioxolane ring is then cleaved and deketalized to form the alkanol metabolite. Cleavage of the alkyl bridge between the phenyl and triazole rings results in the formation of a triazole-alanine conjugate, which may undergo oxidation, potentially leading to gradual degradation of the toxicant in plant tissues (Saroha et al., 2022). It stops the development of fungi by interfering with the biosynthesis of sterols in cell membranes (Uppala and Zhou, 2018). The results of the current study align with the findings of Ramesh et al. (2021), who described the fungicidal effect of Tilt against *B. sacchari*. The outcomes of the present study are also supported by Gupta et al. (2013), who evaluated five fungicides and concluded that Tilt exhibited maximum growth inhibition against *B. sacchari*.

Continuous use of chemicals has resulted in environmental pollution and toxic effects on non-target organisms, including humans. The high cost, possible adverse health effects, and environmental pollution caused by these fungicides have triggered immense interest in searching for alternatives. Plant activators have been shown to be one of the effective alternatives to chemical agents. They contain antimicrobial compounds that are less toxic, eco-friendly, safe, and easily biodegradable.

Five plant activators (salicylic acid, benzoic acid, potassium hydrogen phosphate, potassium dihydrogen phosphate, and citric acid) were evaluated in greenhouse conditions for their effectiveness against eyespot disease of sugarcane. Among these plant activators, salicylic acid was the most effective. The most effective chemical and plant activator were also evaluated alone and in combination against eyespot disease under field conditions. Among all the treatments, the minimum disease incidence was observed with the combination of salicylic acid and Tilt, followed by salicylic acid and Tilt individually. Salicylic acid increases the accumulation of Cd in the cell wall and prevents its translocation into other cell organelles (Janda et al., 2020).

Salicylic acid plays a crucial role in plant growth and development, including essential physiological functions such as enhancing the plant's response to stress conditions (both biotic and abiotic) and increasing resistance through systemic acquired resistance (SAR). These physiological functions are accomplished by stimulating or altering internal endogenous signaling to withstand various stresses (Simaei et al., 2012). The salicylic acid (SA)-dependent pathway is associated with SAR that acts on plant pathogen infection. The SA-dependent pathway promotes the expression of defense genes and a set of pathogenesis-related (PR) proteins, including basic-1,3-glucanase (PR-2) and basic chitinase (PR-3). This pathway results in the expression of genes for a different set of PR proteins, most notably PR-1. The relationship between disease symptoms and the level of salicylic acid occurs when the elicitor treatment changes and increases the level of salicylic acid, as signaling is an important process that induces resistance in plants (Inchaya et al., 2013).

The results of the current study are supported by the findings of Shabana et al. (2008), who investigated the antifungal activity of plant activators against eyespot disease of sugarcane caused by *B. sacchari*. Salicylic acid and benzoic acid were found to be the most effective plant activators against eyespot disease of sugarcane in both field and greenhouse conditions. Similar results were reported by Singh et al. (2019) who studied the exogenous application of salicylic acid alone and in combination with tricyclazole against *Bipolaris* spp.

under lab and field conditions. The study concluded that it significantly increased the activation and accumulation of phenylalanine ammonia-lyase (PAL), peroxidase, 1,3-glucanase, and chitinase activities in *Bipolaris* spp. Integrating chemotherapy, plant defense activators, and genetic resistance offers a promising strategy for combating eye spot disease in sugarcane. It is pivotal to highlight study limitations, such as specific conditions and potential variability across sugarcane cultivars or environments. Future research could explore the long-term field-scale efficacy of these disease intervention tools and the underlying molecular mechanisms driving resistance in the host.

CONCLUSION

In the current investigation, the minimum disease incidence was exhibited by the integration of salicylic acid and Tilt. Among all tested varieties of sugarcane, CPF-246 expressed a highly resistant response. As a result, it is concluded that salicylic acid and Tilt are strongly recommended for farmers as an effective approach for managing eye spot disease in sugarcane.

AUTHORS' CONTRIBUTIONS

FY wrote the manuscript; MA designed, formulated and laid out the study; NAR and AN collected, arranged and analyzed the data; MJM provided technical assistance; AJ supervised the work; WD and FA conducted the experiments; AU proofread the paper.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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