



Management of Sugarcane Smut Disease using Triazole Fungicides and Synthetic Elicitors

A.N.W.S. Thushari^{1*} and D.M. De Costa²

¹Division of Crop Protection, Sugarcane Research Institute, Uda Walawe, Sri Lanka.

²Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Sri Lanka.

ARTICLE INFO

Article history:

Received: 30 June 2021

Revised version received: 29 September 2021

Accepted: 8 November 2021

Available online: 1 April 2022

Keywords:

Hexaconazole

Tebuconazole

Salicylic Acid

Sporisorium scitamineum

sugarcane

Citation:

Thushari, A.N.W.S., and De Costa, D.M. (2022). Management of Sugarcane Smut Disease using Triazole Fungicides and Synthetic Elicitors. *Tropical Agricultural Research*, 33(2): 213-222.

DOI: <http://doi.org/10.4038/tar.v33i2.8554>

Thushari, A.N.W.S. 

<https://orcid.org/0000-0002-3967-8375>



*Corresponding author:

asumedhathushari@yahoo.com

ABSTRACT

Sugarcane smut caused by *Sporisorium scitamineum* is a devastating disease of sugarcane. As a management strategy, seed sets of resistant varieties treat with fungicides after the hot water treatment. As a novel management strategy, we evaluated the possibility of using low concentrations of fungicides and synthetic elicitors under in vitro and field conditions. Three fungicides (i.e. Tebuconazole, Hexaconazole, and Metalaxyl 8 % + Mancozeb 64 % WP) and two synthetic elicitors (i.e. salicylic acid (SA) and jasmonic acid (JA)) were tested in vitro at four concentrations (i.e. 250, 500, 750, and 1000 ppm) for their efficacy on inhibition of the germination of smut teliospores. Tebuconazole, Hexaconazole, and salicylic acids completely inhibited the germination of the teliospores at 500 ppm, 250 ppm, and 750 ppm concentrations, respectively. Metalaxyl 8 % + Mancozeb 64 % WP and JA inhibited the teliospore germination by 52 and 58 % respectively at 1000 ppm concentration. In the field evaluation, smut pathogen was artificially inoculated to the seed sets of a resistant (Co 775) and a susceptible variety (SL 88 116) treated with fungicides and SA at selected concentrations. Disease incidence (DI) was recorded, and disease severity (DS), Area Under Disease Progress Curve (AUDPC), and Percentage Reduction of the Disease (PRD) were calculated. Tebuconazole 500 ppm, Hexaconazole 250 ppm, Hexaconazole 500 ppm, and salicylic Acid 1000 ppm successfully controlled the disease significantly ($P=0.05$) in terms of DI, DS, AUDPC, and PRD, hence can be used as a dip treatment of seed sets to control sugarcane smut disease.

INTRODUCTION

Sugarcane is a globally recognized commercial crop cultivated to produce sucrose, ethanol, and several other by-products (Ashwin *et al.*, 2017). Sugarcane smut, caused by the fungus *Sporisorium scitamineum* (formerly known as *Ustilago scitaminea*), is a major biotic constraint for the sugarcane industry in all sugarcane growing countries (Stoll *et al.*, 2003). This disease causes significant yield losses with the cultivation of susceptible varieties (Comstock, 2000) and reported a 0.6 to 0.7% yield loss for each 1% increase of infected plants (Hoy *et al.*, 1986).

The smut pathogen infects plants through the sugarcane buds or germinating shoots and then grows in association with the meristematic tissues of the apical and lateral shoots (Hoy, 1993). After infection, the fungus stimulates the production of a characteristic sorus, known as a whip which contains a mass of black-brown teliospores (Ferreira and Comstock, 1989). The whips are produced between 6-8 weeks after planting the seed setts or ratooning, while those are produced continuously throughout the season on mature stalks (Croft and Braithwaite, 2006). Plants infected with smut pathogen are stunted and produce excessive tillers. Those tillers also develop smut whips. Symptom development in latently infected plants can be induced by ratooning (Croft and Braithwaite, 2006).

Cultivation of resistant varieties is the most effective method to manage sugarcane smut (Comstock, 2000). All sugarcane varieties recommended and released by the Sugarcane Research Institute (SRI) (SL 83 06, SL 96 128, and SL 92 5588) are considered to be resistant to smut disease (Anon, 2009; Anon, 2012). However, recent surveys conducted by SRI have shown the presence of smut disease in sugarcane cultivations at varying levels. Using hot water-treated resistant varieties and applying fungicide before planting are protective methods used to control the disease. Comstock (2000) reported that hot water treatment with fungicide application to seed setts (seed cane) and rouging of infected stools or plough out of infected crops are other control measures possible to be combined with the use of resistant varieties. While rouging diseased plants is feasible where labor costs are low (Lee-Lovick, 1978), it is not an economically viable option for Sri Lanka. In Sri Lanka, the hot water treatment recommended for eliminating sugarcane smut from seed setts for commercial planting is 54 °C for 50 min (Anon, 2003). A disadvantage of hot water treatment is that it can soften the buds, making them more susceptible to re-infection

with smut spores in contaminated soil. Fungicides protect seed setts from smut infection like a cold-water dip and a combination of hot water treatment (Bhuiyan *et al.*, 2012).

Furthermore, synthetic elicitors, which can induce plant defense mechanisms, have been used to manage diseases of different crops as an alternative to synthetic fungicides (Falcioni *et al.*, 2014; Ramesh Sundar *et al.*, 2006). Salicylic acid (SA) is a synthetic elicitor (Ashwin *et al.*, 2017) and a vital defensive signal which, required for elicitor-triggered immunity and the establishment of systemic acquired resistance (SAR) in plants (Mur *et al.*, 1997; Carr *et al.*, 2010). Application of synthetic elicitors such as Benzothiadiazole (BTH), salicylic acid, and isonicotinic acid substantially reduced *Colletotrichum falcatum* colonization in sugarcane cane stalk tissues by inducing an array of defense-related compounds under controlled conditions (Ramesh Sundar *et al.*, 2006). Nevertheless, no information in sugarcane growing countries on the use of salicylic acid or other chemical elicitors to control sugarcane smut disease.

In Sri Lanka, only one systemic fungicide (i.e. Tebuconazole 500 ppm) has been recommended so far to control sugarcane smut disease (Anon, 2007). However, using the same fungicide over a long period encourages the development of resistant pathotypes hence testing for alternative fungicides is strategically vital.

Therefore, we conducted this study to identify effective fungicides and synthetic elicitors, with an inhibitory effect on spore germination of the pathogen and to determine their field efficacy to manage sugarcane smut disease.

METHODOLOGY

Spore collection and preparation of spore suspension

We collected sugarcane smut spores (teliospores) from infected plants from different sugarcane growing areas representing a wide range of agro-ecological zones (i.e. Uda Walawe, DL1a; Sevanagala, DL1a, and DL1b; Pelwatta, DL1a, and DL1b; Hingurana, DL2a, and DL2b; Kantale, DL1c) of Sri Lanka. Mature whips were cut 10-20 cm below the top visible dewlap and air-dried in separate plastic trays for two weeks. Spores were separated from the whip by scraping, and subsequent sieving through a nylon net (1 mm x 1 mm) to remove plant materials. Spores were then stored in sealed containers and maintained at 4 °C until use.

Spore suspensions were prepared by mixing 0.1 g of smut spores representing each agroecological zone in 100 ml of sterile distilled water. A drop of Tween 20 was also added and mixed thoroughly for the even distribution of the spores. The spore concentration in the suspension was determined with a hemocytometer, and the final concentration was adjusted to 1.0×10^6 spores/ml. Spore germination was assessed by incubating spores on water agar (WA) at 30 °C for 12 h. Germination percentage (> 90%) was recorded to check the viability of spores (Bhuiyan *et al.*, 2013) before inoculation to fungicide amended WA and seed sets.

***In-vitro* analysis of the effect on spore germination**

Efficacy of several fungicides and synthetic elicitors on inhibition of smut spore germination was assessed using three fungicides, namely Tebuconazole (presently used fungicide; 250g/l EW,), Hexaconazole (50g/L SC) and Metalaxyl 8 % + Mancozeb 64 % WP (pH = 7.4) at four levels of concentrations (i.e. 250, 500, 750 and 1000 ppm). As the synthetic elicitors, salicylic acid (BDH chemicals- UK) and jasmonic acid (Sigma - USA) were selected at the same concentrations. The suspension was prepared using a composite sample of viability-checked teliospores collected from different agroecological zones (i.e. DL 1a, DL 1b, DL 1c, DL 2a, and DL 2b). The prepared suspension was streaked in triplicate on water agar plates amended with different concentrations of chemicals (i.e. fungicides, SA, and JA). The water agar plates without adding fungicides or synthetic elicitors served as the negative controls. Plates incubated at 30 °C for 12h. The percentage of spore germination was quantified by the method described by Bhuiyan *et al.*, 2012. The microscopic slides were prepared from the growing culture of the smut pathogen on each water agar plate. From

each replicate, 200 spores were observed under the microscope, and the germination percentage was determined.

Evaluation of the field efficacy of the selected fungicides and synthetic elicitors

Sugarcane stalks were cut into single budded setts, and the setts were treated with hot water at 54 °C for 50 min (Anon, 2003) to remove any systemic infection. Hot water-treated seed setts were dipped separately in fungicides and elicitors at different concentrations, which were determined to be effective by the *in-vitro* spore germination test. Fungicides and synthetic elicitors treated seed sets were dried at ambient temperature (28 ± 1 °C) for an hour.

A mixture of teliospore suspension was prepared using a collection of spores from different agroecological zones. The seed sets were then inoculated with a spore suspension of 1.0×10^6 (spores/ml) by dip inoculation technique for 30 min and maintained under dark conditions at ambient temperature for 18-24 h before the planting in the field. The experiment comprised of two sugarcane varieties namely Co 775 and SL 88 116, which have been assessed as a resistant and susceptible variety respectively for smut disease under Sri Lankan conditions (Personal communication with P. Dayasena). The trial was established according to a randomized complete block design (RCBD) with three replicates for each treatment. Each block (plot) contained six treatments namely, Tebuconazole (500 ppm), Hexaconazole (250 ppm and 500 ppm), salicylic acid (750 ppm and 1000 ppm), and control for each variety. Dimensions of each plot were 60 m x 27 m with a 2 m distance between two blocks (Figure 1a and b). Irrigation, fertilizer application, and herbicide applications were done according to SRI recommendations (SRI, 2004).

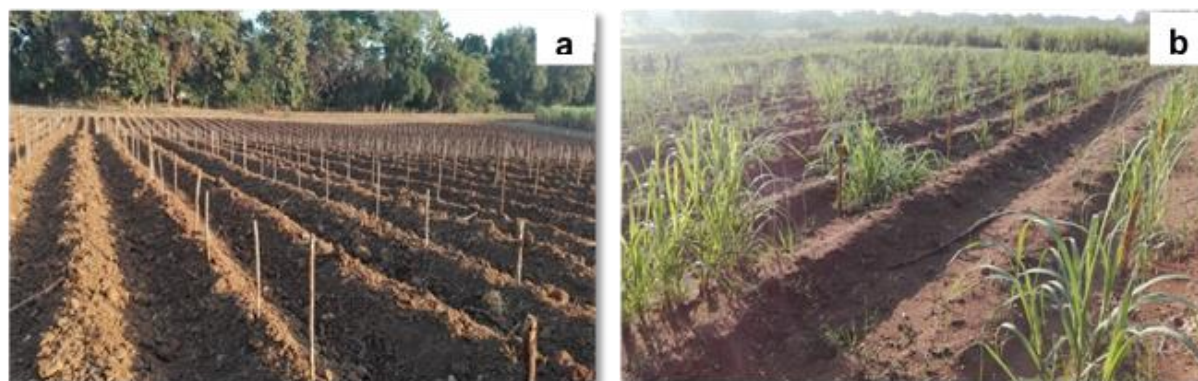


Figure 1: Field layout of the experiment. Before establishment of the crop (a), 2 months after planting of the crop (b).

Evaluation of Disease Incidence and severity

The total number of stalks and number of stalks with smut whips (Figure 2 a and b) per replicate

of each treatment was recorded from 1.5 to 10 months after planting (MAP) at regular intervals



Figure 2: Different stages of the symptoms of sugarcane smut disease. Initial stage of development of the smut whip (a), characteristic whip formed in smut infected sugarcane plant (b).

Average Disease incidence (DI) for each treatment was calculated at the end of the crop cycle based on the cumulative no. of whips in each plot during each crop cycle using the formula;

$$\text{Disease incidence (\%)} = \frac{a+b}{c+a} \times 100$$

Where; a is the cumulative number of stalks with smut whips before the final disease count, b is the number of stalks with smut whips at the final disease count and c is the total number of stalks in the plot at the final disease count (Thushari and Ariyawansa, 2019; Sumedha Thushari *et al.*, 2021).

The area under disease progress curve (AUDPC) was calculated based on the disease incidence over time using the following formula.

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left(\frac{Y_i + Y_{i+1}}{2} \right) (t_{i+1} - t_i)$$

Where Y_i is the disease incidence of the i th observation, t_i time (days) of the i th observation and n =total number of observations (Shaner and Finney, 1977).

Disease severity of the infected plants was assessed using a formula developed by Bhuiyan *et*

al., (2010). In brief, each plant was assigned to one of the following severity categories (i.e. F, L, M and S).

$$\text{Sv} = \frac{1 \times F + 2 \times L + 3 \times M + 4 \times S}{T \times 4} \times 100$$

Where, Sv = severity %, F, L, M and S = number of plants in each category and T = total number of plants per plot

F = a plant with one whip, no apparent stunting

L = a plant with slight /grassiness and 2 or 3 whips

M = a plant with moderate stunting/grassiness and >3 whips

S = a plant with severe stunting/grassiness and/or death a majority of stalks with whips

At the end of the crop cycle, the percentage reduction of the disease (PRD) was calculated concerning disease incidence (DI) using the following formula (Ayele *et al.*, 2019).

$$\text{PRD} = \frac{\text{Percentage DI in untreated plot} - \text{percentage DI in treated plot}}{\text{Percentage DI in untreated plot}} \times 100$$

Statistical Analysis

All A factorial ANOVA was conducted to compare the main effects of independent variables (i.e. different concentrations of fungicides, SA, JA, and varieties) and their interaction with the dependent variables (i.e. disease incidence, disease severity, and AUDPC). Tukey's pairwise comparisons were done for mean comparison at a 5 % significant level. The data analysis was done using Statistical Analysis Software (SAS-university version) (Kiriwaththuduwa *et al.*, (2021).

RESULTS AND DISCUSSION

In-vitro analysis of the effect on spore germination

Spore (teliospore) germination was completely inhibited by Tebuconazole, the presently recommended fungicide, at concentrations from 500 – 1000 ppm and Hexaconazole from 250 – 1000 ppm. Salicylic acid inhibited the spore germination completely from 750 ppm-1000 ppm (Table 1). Jasmonic acid and Metalaxyl 8% + Mancozeb 64 % WP at 1000 ppm concentration could inhibit spore germination 58% and 52 % respectively.

Disease incidence, severity and AUDPC

Results revealed that the incidence and severity of smut disease development significantly varied due to sugarcane variety and the treatments used ($P=0.05$). However, AUDPC did not differ significantly among the varieties and treatments ($P=0.05$). Variety SL 88 116 resulted in a significantly ($P=0.05$) higher mean disease incidence (10.9) and mean disease severity (0.99) compared to those of the variety Co 775 (3.11 and 0.31 respectively).

Table 1: Percentage germination of *Sporisorium scitamineum* spores in water agar medium consist with fungicides or synthetic elicitors.

Fungicide	0 ppm	250 ppm	500 ppm	750 ppm	1000 ppm
Tebuconazole		60.67 ^c	0.00 ^c	0.00 ^b	0.00 ^c
Hexaconazole		0.00 ^d	0.00 ^c	0.00 ^b	0.00 ^c
Jasmonic Acid		80.33 ^b	71.00 ^b	63.33 ^a	58.00 ^a
Metalaxyl 8 % + Mancozeb 64 % WP		84.33 ^{ab}	71.33 ^c	63.33 ^a	51.66 ^b
Salicylic Acid		87.66 ^a	82.00 ^a	0.00 ^b	0.00 ^c
Control	98.67 ^a				

*Each data point is the mean of three replications. Means with the same letters in a column are not significantly different at ($P= 0.05$).

In the susceptible variety, SL 88 116, disease incidence reduced considerably from the beginning of the grand growth phase (from 4th – 7th months after planting) with the treatments of Hexaconazole (250 ppm and 500 ppm) and Tebuconazole (500 ppm) with compared to the control treatment. (Figure 3a). All these fungicide treatments again showed a considerable reduction in the maturation phase (i.e. 9th month onwards). However, both treatments of Tebuconazole (500ppm) and Hexaconazole (500ppm) were able to reduce the disease incidence throughout the plant crop cycle (till 12 months from planting). Salicylic acid concentrations; 750 ppm, and 1000 ppm, effectively reduced the disease incidence, at the later part of the grand growth phase (6th, 7th, and 9th months from planting).

In variety Co 775, which is resistant for smut, all the used concentrations of Hexaconazole and Tebuconazole resulted in zero disease incidence from tillering phase to maturation phase (3rd – 10th months after planting). Both concentrations of salicylic acid reported zero disease incidence from the middle stage of the grand growth phase to the maturation phase (6th – 10th months after planting) (Figure 3b), demonstrating a late response shown by the synthetic elicitor on disease control.

In variety SL 88 116, compared to the control treatment, a significant ($P=0.05$) reduction of disease severity was reported at the later stage of the grand growth phase (7th and 9th months after planting) under all the treatments (Figure 4a). Co 775 reported zero disease severity for all the treatments from 6 months of planting onwards. In addition, all tested fungicide treatments gave zero disease severity from the tillering phase (2nd month after planting) (Figure 4b).

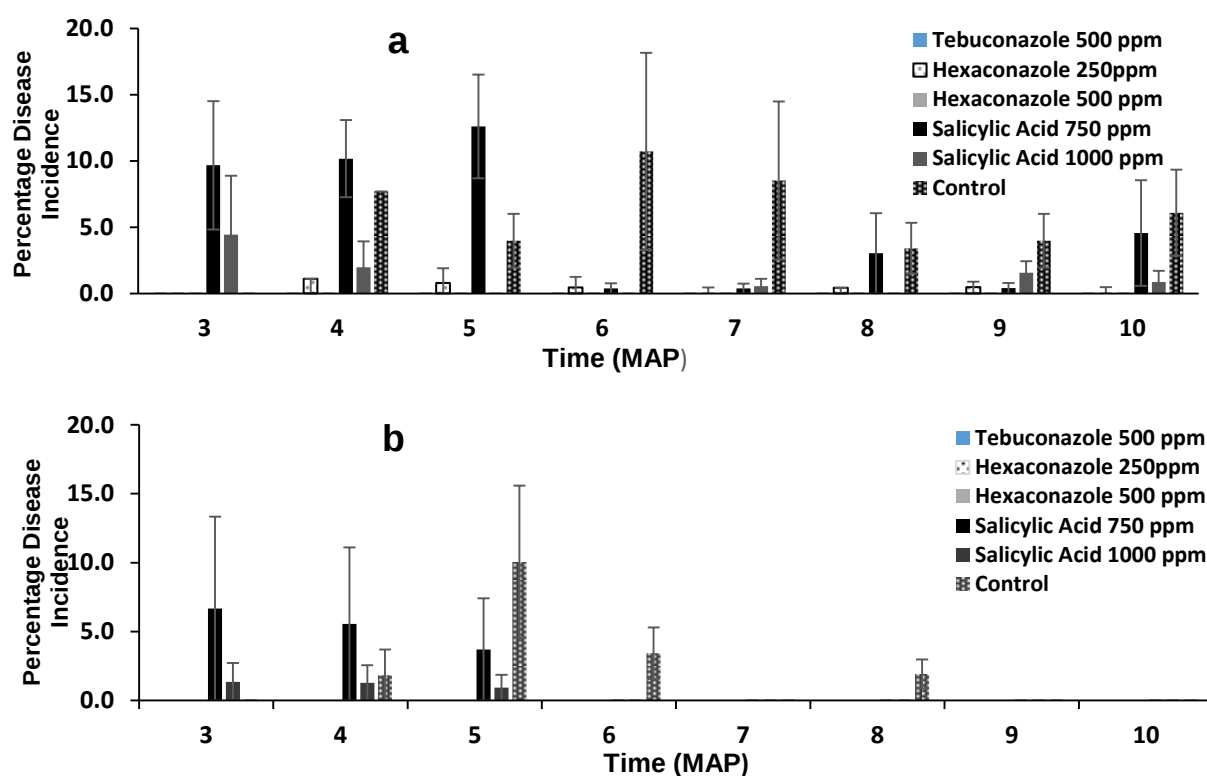


Figure 3: Variation of percentage disease incidence of variety SL 88 116 with different treatments (a) and variation of percentage disease incidence of variety Co 775 with different treatments (b). Each data point for percentage disease incidence is the mean of three replicates. Error bars are the standard error of mean for each data point.

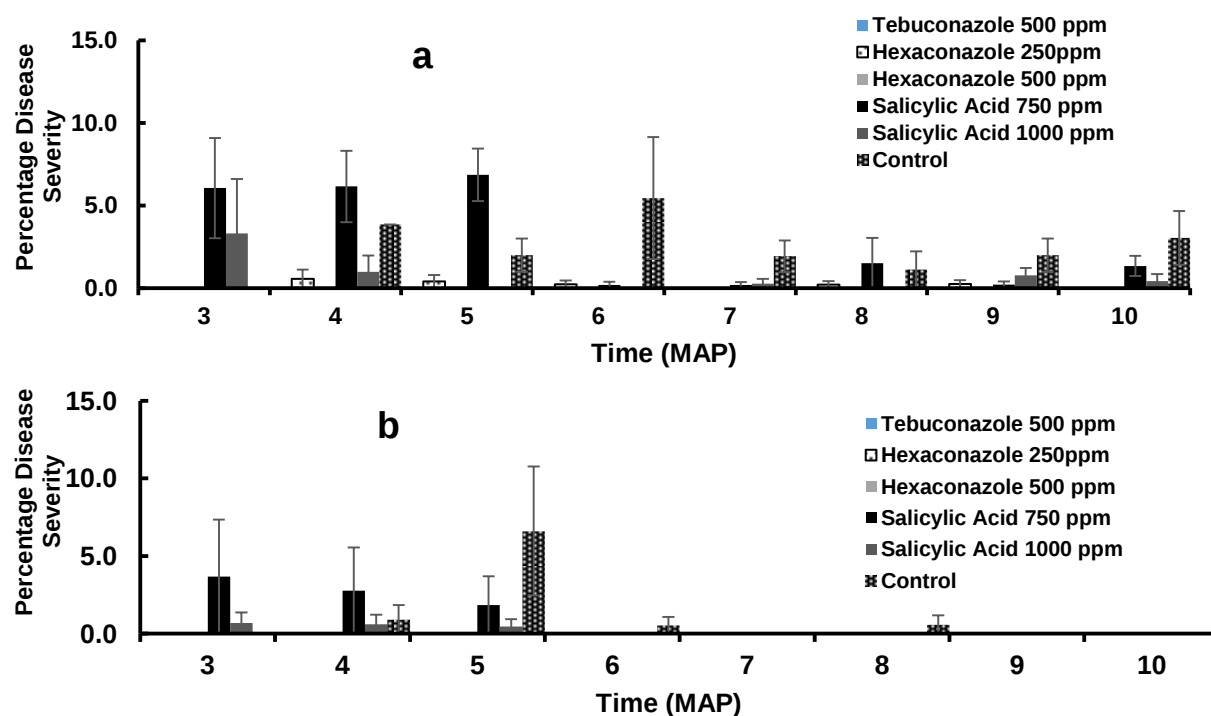


Figure 4: Variation of percentage disease severity of variety SL 88 116 with different treatments (a) and variation of percentage disease severity of variety Co 775 with different treatments (b). Each data point for percentage disease severity is the mean of three replicates. Error bars are the standard error of mean for each data point.

Based on the findings, Tebuconazole 500 ppm and Hexaconazole at 500 ppm effectively reduce disease incidence and severity to zero in both susceptible and resistant varieties. As a non-fungicidal alternative, salicylic acid at 1000 ppm concentration can be effectively used to get the same level of disease management in both resistant and susceptible varieties.

Significant reduction of the AUDPC in both varieties by all treatments except salicylic acid 750 ppm concentration is possible (Figure 5a and 5b). Interestingly, in the smut susceptible variety, AUDPC with the salicylic acid treatment of 1000 ppm concentration was lower than that was developed by Hexaconazole 250 ppm concentration. The percentage Reduction of Disease concerning DI under field conditions is presented in Table 2.

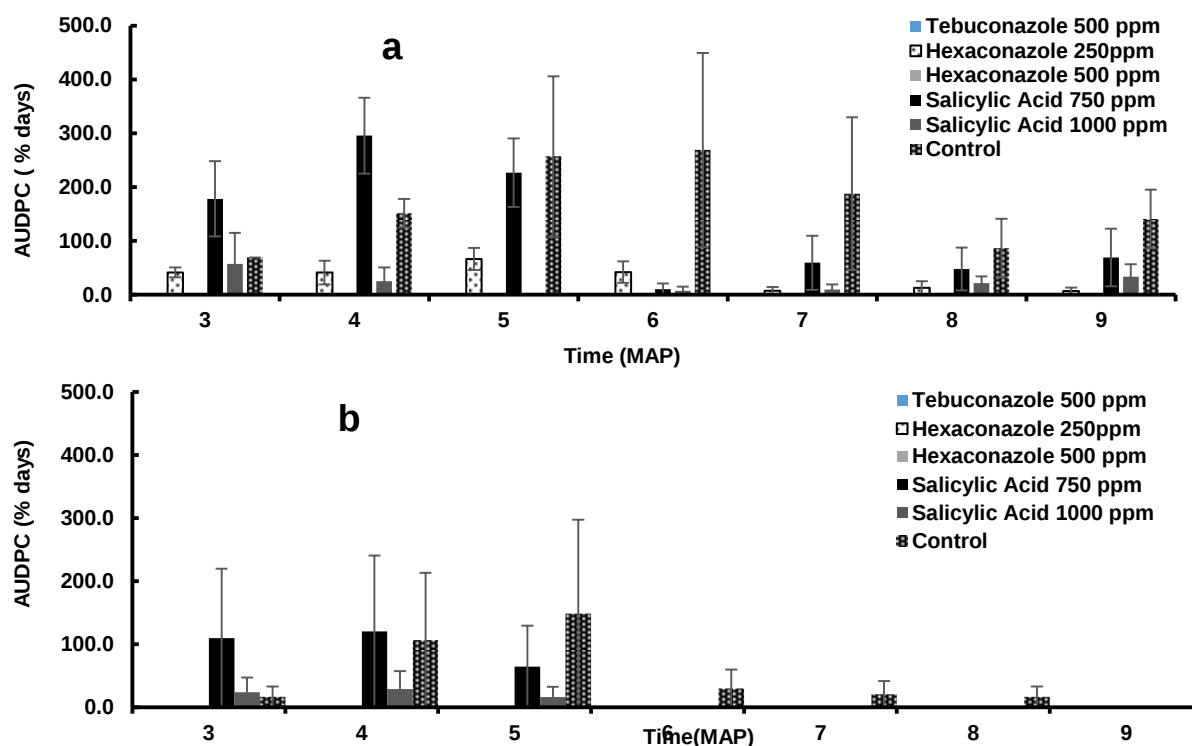


Figure 5: Variation of AUDPC in the variety SL 88 116 under different treatments (a) and variation of AUDPC in the variety Co 775 under different treatments (b). Each data point is the mean of three replicate in experimental period. Error bars are the standard error of mean for each data point.

Interestingly, Hexaconazole 250 ppm treatment has shown different responses in terms of PRD in resistant and susceptible varieties (Table 2). In contrast, SA 1000 ppm treatment has given equal PRD in both susceptible and resistant varieties. It is a better attribute of SA. However, SA 750 ppm and Hexaconazole 250 ppm concentrations did not result in equal efficacy in both sugarcane varieties.

Non-systemic (i.e. mancozeb) and systemic (i.e. triazole, metalaxyl) fungicides with both curative and systemic action (i.e. some triazoles such as Flutriafol) have been used for successful control of sugarcane smut disease in other sugarcane growing countries (Wada, 2003; Bhuiyan *et al.*,

2012). Our results revealed the complete inhibition of the germination of smut spores by the two triazole fungicides used in this study. Interestingly, the ability of complete inhibition of spore germination by the newly-tested fungicide, Hexaconazole, was at a lower concentration (i.e. 250 ppm) than the currently used concentration of Tebuconazole (i.e. 500 ppm). In contrast to the results of Wada (2003), we observed a lower efficiency of Metalaxyl 8 % + Mancozeb 64 % WP on spore germination.

Plant growth hormones, (i.e. salicylic acid and jasmonic acid (JA)), could act as inducers of plant defense responses, have been used for plant disease control through various application

methods (e.g. seed priming, exogenous foliar application). Considering the environmentally friendly nature of the hormones compared to the field application of fungicides, we investigated the possible use of SA and JA to control sugarcane smut disease. The findings of our in vitro and field

experiments are in agreement with previous work that salicylic acid can be used to inhibit germination of smut spores (da Rocha *et al.*, 2015; Amborabe *et al.*, 2002). Further, it reduces disease incidence, disease severity, and AUDPC even in a susceptible sugarcane variety in field applications.

Table 2. Mean Percentage Reduction of Disease (PRD) by different treatments in two sugarcane varieties under field condition.

Variety	Treatment	PRD
SL88 116	Tebuconazole 500 ppm	100.0
	Hexaconazole 250 ppm	88.5
	Hexaconazole 500 ppm	100.0
	Salicylic Acid 750 ppm	6.8
	Salicylic Acid 1000 ppm	80.5
	Control	0.0
Co 775	Tebuconazole 500 ppm	100.0
	Hexaconazole 250 ppm	100.0
	Hexaconazole 500 ppm	100.0
	Salicylic Acid 750 ppm	39.5
	Salicylic Acid 1000 ppm	80.6
	Control	0.0

Glazebrook, 2005 reported that salicylic acid is involved in the activation of plant defense responses against biotrophic and hemibiotrophic pathogens and the establishment of systemic acquired resistance against leaf-chewing insects and necrotrophic microbes mediated by Jasmonic acid (JA)-dependent signaling. Smut is a biotrophic fungus, and our results confirm the possibility of using SA as a control measure.

Viljanen and colleagues reported that varietal resistance is vital to determine the frequency and effectiveness of the chemical application (Viljanen *et al.*, 2002). Results of the present study revealed the importance of using resistant varieties like Co 775 and its ability to reduce sugarcane smut incidence, severity, and AUDPC by salicylic acid instead of fungicide application. Therefore, for more efficient disease control, integrating the resistance varieties with SA can be proposed. Further, our findings demonstrated that fungicides can be used to protect the early stage of the crop from smut pathogen infection.

Results revealed that the treatment with fungicides or SA protects the crop for about ten months in disease severity and DI, especially in

resistant varieties. Sugarcane is a perennial or multiyear crop, where plant crops are harvested and regrown as ratoon crops for about 3 to 7 years. It is unclear whether the protection from smut could carry over to the ratoon crop. Further investigations are warranted to understand the efficacy of the fungicides and synthetic elicitors on ratoon crops towards the management of smut disease.

CONCLUSION

This research confirmed that triazole fungicides Tebuconazole (500 ppm) and Hexaconazole (250 and 500 ppm), and synthetic elicitor, salicylic acid (1000 ppm), when used as a pre-planting dip treatment, are effective in protecting sugarcane from smut disease under field conditions. Therefore, the above treatments are recommended to treat seed cane after the usual practice of hot water treatment

ACKNOWLEDGEMENT

This research was funded by Sugarcane Research Institute (SRI). Assistance given by the Board of Governors of SRI, former Directors, and the staff of

the Crop Protection Division is gratefully acknowledged. The contribution of Dr. Wasana Wijesuriya, Principal Research Officer (Biometry), Rubber Research Institute of Sri Lanka, Agalawatte, Sri Lanka in the statistical analysis of data is gratefully acknowledged.

REFERENCES

- Amborabé, B. E., Fleurat-Lessard, P., Chollet, J. F., & Roblin, G. (2002). Antifungal effects of salicylic acid and other benzoic acid derivatives towards *Eutypa lata*: structure-activity relationship. *Plant Physiology and Biochemistry*, 40(12), 1051-1060.
- Anon (2003). Annual Research Progress 2003. Sugarcane Research Institute, Uda Walawe, Sri Lanka.
- Anon (2007). Annual Research Report 2007. Sugarcane Research Institute, Uda Walawe, Sri Lanka.
- Anon (2009). Annual Research Progress 2009. Sugarcane Research Institute, Uda Walawe, Sri Lanka.
- Anon (2012). Annual Research Progress 2012. Sugarcane Research Institute, Uda Walawe, Sri Lanka.
- Ayele, A., Chala, A., & Shikur, E. (2019). Effect of Varieties, Fungicides and Application Frequencies to Wheat Yellow Rust Disease (*Puccinia striiformis* f. sp. tritici) Management in Arsi Highlands of Ethiopia. *American Journal of BioScience*, 7(6), 113.
- Ashwin, N. M. R., Barnabas, E. L., Sundar, A. R., Muthumeena, M., Malathi, P., & Viswanathan, R. (2017). Disease suppressive effects of resistance-inducing agents against red rot of sugarcane. *European Journal of Plant Pathology*, 149(2), 285-297.
- Bhuiyan, S. A., Croft, B. J., Cox, M. C., & Bade, G. E. O. R. G. E. (2010, January). Varietal resistance of sugarcane to natural infection of smut-preliminary results. In Proceedings of the Australian Society of Sugar Cane Technologists (Vol. 32, pp. 355-365).
- Bhuiyan, S. A., Croft, B. J., James, R. S., & Cox, M. C. (2012). Laboratory and field evaluation of fungicides for the management of sugarcane smut caused by *Sporisorium scitamineum* in seedcane. *Australasian Plant Pathology*, 41(6), 591-599.
- Bhuiyan, S. A., Croft, B. J., Deomano, E. C., James, R. S., & Stringer, J. K. (2013). Mechanism of resistance in Australian sugarcane parent clones to smut and the effect of hot water treatment. *Crop and Pasture Science*, 64(9), 892-900.
- Carr, J. P., Lewsey, M. G., & Palukaitis, P. (2010). Signaling in induced resistance. *Advances in virus research*, 76, 57-121.
- Comstock, J.C. (2000). Smut. In P.Rott, Bailey, R.A., Comstock, J.C., Croft, B.J. & Saumtally, A.S. (Eds.), A guide to sugarcane diseases (pp 181-185). Montpellier, France. CIRAD and ISSCT.
- Croft, B. J., & Braithwaite, K. S. (2006). Management of an incursion of sugarcane smut in Australia. *Australasian Plant Pathology*, 35(2), 113-122.
- da Rocha Neto, A. C., Maraschin, M., & Di Piero, R. M. (2015). Antifungal activity of salicylic acid against *Penicillium expansum* and its possible mechanisms of action. *International journal of food microbiology*, 215, 64-70.
- Falcioni, T., Ferrio, J. P., Del Cueto, A. I., Giné, J., Achón, M. Á., & Medina, V. (2014). Effect of salicylic acid treatment on tomato plant physiology and tolerance to potato virus X infection. *European journal of plant pathology*, 138(2), 331-345.
- Ferreira, S. A. & Comstock, J. C. (1989). Smut. In C Ricaud, Egan, B.T., Gillaspie Jr, A.G. and Hughes, C.G. (Eds.), Diseases of sugarcane (pp 211-229). Amsterdam. Elsevier.
- Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annual Review Phytopathology.*, 43, 205-227.
- Hoy, J. W., Hollier, C. A., Fontenot, D. B., & Grelén, L. B. (1986). Incidence of sugarcane smut in Louisiana and its effect on yield. *Plant Disease (EUA)* v. 70 (1) p. 59-60.
- Hoy, J. W. (1993). Sugarcane smut in Louisiana: biology and control (No. 839, 1-31). Louisiana. Agricultural Experimental Station.
- Kiriwaththuduwa, B., Wijesuriya, A., Silva, T. D., Ranwala, S. W., & Wijesuriya, W. (2021). Characterization of Conserved Sugarcane (*Saccharum* spp.) Germplasm for Parental Selection in Directional Breeding of

- Economically Important Traits. *Tropical Agricultural Research*, 32(2), 179-190.
- Lee-Lovick, G. (1978). Smut of sugarcane–*Ustilago scitaminea*. *Review of Plant Pathology*, 57, 181–188.
- Mur, L. A., Bi, Y. M., Darby, R. M., Firek, S., & Draper, J. (1997). Compromising early salicylic acid accumulation delays the hypersensitive response and increases viral dispersal during lesion establishment in TMV-infected tobacco. *The Plant Journal*, 12(5), 1113-1126.
- Shaner, G., & Finney, R. E. (1977). The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. *Phytopathology*, 67(8), 1051-1056.
- Sugarcane Research Institute. (2004). Methods of Sugarcane cultivation (No. 01). Sri Lanka. Sugarcane Research Institute.
- Sumedha Thushari, A. N. W., Wijesuriya, A., Wijesuriya, B. W., Perera, A. M. M. S., & De Costa, D. M. (2021). Identification of Sugarcane Germplasm in Sri Lanka for Breeding of Varieties Resistant to Smut Disease (ca *Sporisorium scitamineum*). *Sugar Tech*, 23(5), 1025-1036.
- Sundar, A. R., Viswanathan, R., Malathi, P., & Padmanaban, P. (2006). Mechanism of resistance induced by plant activators against *Colletotrichum falcatum* in sugarcane. *Archives of Phytopathology and Plant Protection*, 39(4), 259-272.
- Stoll, M., Piepenbring, M., Begerow, D., & Oberwinkler, F. (2003). Molecular phylogeny of *Ustilago* and *Sporisorium* species (Basidiomycota, Ustilaginales) based on internal transcribed spacer (ITS) sequences. *Canadian Journal of Botany*, 81(9), 976-984.
- Thushari, A. N. W. S., and Ariyawansha, B. D. S. K. (2019). Evaluation of Selected Sugarcane (*Saccharum* spp. Hybrid) Varieties for Smut Disease Reaction and its Effect on Cane and Sugar- Yield Components. In Dr. VHL Rodrigo, Dr. BW Wijesuriya, Dr. DG Edirisinghe, Dr. NMC Nayanakantha (Eds.), *Towards Achieving Sustainable Development Goals in the Plantation Sector :Proceedings of the Seventh Symposium on Plantation Crop Research Sri Lanka 2019* (pp. 163-169).
- Viljanen-Rollinson, S. L. H., Parkes, R. A., Armour, T., & Cromey, M. G. (2002). Fungicide control of stripe rust in wheat protection or eradication. *New Zealand Plant Protection*, 55, 336-340.
- Wada, A. C. (2003). Control of sugarcane smut disease in Nigeria with fungicides. *Crop protection*, 22(1), 45-49.