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RESEARCH ARTICLE

Comparative efficacy of different inoculation methods in screening sugarcane varieties for resistance to *Fusarium sacchari*

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Abstract

Fusarium sacchari, the causal organism of sugarcane wilt, is a major constraint to sugarcane yield in India and other countries. Unlike several other fungal diseases of sugarcane, attempts to artificially induce this disease have limited success. To address this gap, field studies were conducted at the Sugarcane Research Farm in Kalyanpur (RPCAU, Pusa, Samastipur) during the 2023-24 cropping season to simulate wilt under field conditions using thirty-five sugarcane varieties along with a highly susceptible check variety (CoV 92102). All varieties were assessed for their responses to the wilt pathogen using 0 - 4 scale of wilt severity index. Among various inoculation methods, the plug method proved to be the most effective, as it clearly differentiated resistant, moderately resistant, moderately susceptible and susceptible varieties with balanced distribution across all categories. It recorded 20 % resistant, 28.58 % moderately resistant, 37.14 % moderately susceptible and 14.29 % susceptible varieties, thereby providing a realistic assessment of varietal response. In comparison, the soil and sett dip methods showed higher proportions of resistant and moderately resistant categories but fewer susceptible varieties, while the cotton swab method largely favoured resistance (94.29 %), masking the true susceptibility of many varieties. Hence, the Plug method is considered the best and most reliable technique for evaluating sugarcane resistance against wilt disease because it exposes pathogenic variability more effectively and avoids overestimation of resistance.

Keywords: artifical inoculation; *Fusarium sacchari*; screening; sugarcane; wilt disease

Introduction

Sugarcane (*Saccharum officinarum* L.) is a large, perennial tropical grass from the Poaceae family, specifically the Andropogoneae tribe and the Saccharum genus. It is a polyploid plant primarily cultivated for its sucrose content and is propagated vegetatively using stalks with buds (1, 2). The sugar industry is India's second largest agro-processing sector, playing a key role in rural income, employment and tax revenue (3). In 2023/2024, India produced 34 million metric tons of sugar, supporting about 500000 workers and contributing ₹806 billion to the economy. It is also the third-largest sugar exporter globally (4). According to the Indian Sugar Mills Association (ISMA), Bihar ranks sixth, producing 12.74 million tonnes of sugarcane with a productivity of 60.62 tonnes per hectare (5).

Around 55 sugarcane diseases have been reported in India, causing a 10-15 % reduction in sugar production across the sugarcane-growing belt of the country (6, 7). Over 20 diseases affect sugarcane in Bihar, with red rot and wilt being major concerns recently (8). Wilt is a major disease affecting sugarcane across India, with severe cases in Gujarat Andhra Pradesh, Odisha, Bihar, Uttar Pradesh, Haryana and Punjab, reducing production and productivity (9). Wilt disease causes notable losses in germination rates (6.14 - 38.20 %), seedling mortality (4.55 - 41.24 %), cane height (6.66 -27.83 %), cane girth (2.67 - 45.16 %), cane weight (6.40 - 47.27 %), brix (4.04 - 25.58 %), sucrose (6.96 - 49.16 %) and purity (3.04 - 31.69 %) (10).

Wilt is a major sugarcane disease, managed effectively through resistant varieties. A comprehensive rating system that assesses disease severity based on pathogen presence, symptom development and root condition, using a 0-4 grade scale was proposed (11). Previous studies reported that the plug method and spore suspension were more effective in inducing sugarcane wilt than soil inoculum (12). The plug method provided direct access to the vascular system, whereas soil inoculum was less effective and only became pathogenic under favorable conditions. Susceptible varieties showed significant symptoms when inoculated at the first internode under moisture stress at 150 days after planting (DAP), while no symptoms appeared in those inoculated at the third internode or at 90 DAP (13).

Materials and Methods

The field experiments were conducted at the Sugarcane Research Farm in Kalyanpur, RPCAU, Pusa, Samastipur during 2023-24 cropping season. The region experiences a sub-humid, subtropical climate with 80-85 % of the annual rainfall (1215 mm) occurring during the monsoon. The highest rainfall, 388.5 mm, occurred in early August 2024. The maximum temperature reached 40.2 °C in early July and the lowest was 8 °C in late January 2023. Relative humidity ranged from 71.6 - 97.9 % in the morning and 34.2 - 79.6 % in the evening hours. A total 35 varieties and one susceptible variety were used during this experiment and four inoculation methods viz., soil application, setts dip, plug method and cotton swab were chosen for testing their efficacy. Inoculations were carried out on various dates and observations were made for each variety. For each inoculation method, three replications were arranged using a Randomized Block Design (RBD) and each method was applied in a distinct plot.

Soil inoculation

For soil inoculation, wilt-sick plots were prepared 15 days before planting by mixing chopped wilt-diseased cane pieces and debris into the soil during bed preparation. Additional inoculum was added to the setts at planting. The inoculum was prepared by mixing 250 g of ground maize grains with 750 g of sand (1:3 ratio) and 100 mL of distilled water. This mixture was divided into 250 mL flasks, with 100 g per flask and sterilized at 15 psi for 2 hrs. After 2 days, actively growing, sporulating cultures of *Fusarium sacchari* were used as inoculum. Mycelium from Fusarium growing on oat meal agar was inoculated into each flask with 4-5 mycelial discs and the flasks were incubated at 22 ± 1 °C for 15 days (All India Coordinated Research Project (AICRP)). On the 16th day, the inoculum was combined, mixed thoroughly and applied at a rate of 100 g/m of row over the setts in the furrows at planting.

Sett dip inoculation

In the sett dip inoculation method, setts were dipped in a spore suspension (4 g spores/L sterile water at 4 x 10^6 spores/mL) for 15 min at the time of planting to ensure proper exposure to the pathogen.

Plug inoculation

At 180 days after planting, the pathogen was introduced into the 3rd internode from the bottom by making a bore-hole with an iron inoculator. A 0.5 mL conidial suspension (1 × 10^6 spores/mL) was injected using a sterile needle at the 6 to 7 internode stage. The bore-hole was then sealed with the removed tissue core and waxed paper was applied to the injection site to prevent contamination.

Cotton swab inoculation

In the cotton swab inoculation method, 180 days after planting, the uppermost green leaf sheaths were removed from the canes. A cotton swab soaked in a suspension (1 × 10^6 spores/mL) of the wilt pathogen was applied around the exposed nodes, which were then sealed with wax-coated paper to retain humidity.

Observations

For the plug method and cotton swab method, the first observation was taken 240 days after planting, the second observation at 270 days and the third observation at 300 days. For soil application and sett dip methods, observations were taken 60 days after planting, with the second observation at 90 days and the third observation at 120 days. The cane stalks were examined by splitting open longitudinally and recorded wilt symptoms using 0-4 scale of wilt severity index (AICRP). The individual test cane was rated on the basis of symptoms as per scale depicted in Table 1.

The mean wilt severity index is calculated based on the number of cane samples. The formula for the mean wilt severity index is:

Table 2. Wilt severity scale (AICRP, 0–4)

S.No.	Scale range	Disease reaction
1	0-1.0	R
2	1.1- 2.0	MR
3	2.1- 3.0	MS
4	and above	S

R- Resistance, MR- Moderately resistance, MS- Moderately susceptible and S- Susceptible

Table 1. Symptoms scale rating

Scale	Symptoms
0	Healthy canes and roots with no external or internal symptoms of wilt
1	No wilting or drying of leaves, no stunting or shrinking of the stalk or rind, slight pith formation with yellow discoloration of the internal tissues in one or two lower internodes only. No cavity formation or fungal growth seen apparently normal and healthy roots.
2	Mild yellowing of top leaves and drying of lower leaves, mild stunting and shrinking of the stalk and rind. Yellowish discolouration of the internal tissues extending to three or four bottom internodes. Slight cavity formation of the pith and no fungal growth seen, slightly discoloured roots.
3	Mild yellowing of top leaves and drying of lower leaves, mild stunting and shrinking of the stalk and rind. Light brown discolouration of the internal tissues throughout the entire length of the cane except the top. Severe pith and cavity formation, sparse fungal growth observed in the pith cavities
4	Complete yellowing and death of the leaves, marked stunting, shrinking and drying of the stalk and rind, dark brown discolouration of the internal tissues extending throughout the entire length of the cane. Large pith cavities with profuse overgrowth of the associated fungi. Most of the roots necrotic with dark discolouration dislodge easily from the stalks. Roots mildly discoloured and slightly necrotic.

Mean wilt severity index = (Sum of wilt indices of individual stalks)/ (Number of stalk samples)

The scale (0-4) and disease reaction were categorized according to the AICRP on sugarcane as depicted in Table 2.

Results and Discussion

First observations

The first observation across the four inoculation methods plugs inoculation and cotton swab inoculation at 240 days and soil application and sett dip inoculation at 60 days revealed significant variations, as shown in Table 3. In the plug inoculation method, 11 varieties, including CoP 9301, CoP 22441 and CoP 19436, were resistant, while 9 varieties, such as CoP 22438 and CoP 19441, were moderately resistant. Twelve varieties, including CoLk 94184 and CoP 18436, were moderately susceptible and three varieties, including CoBln 17502 and check CoSe 16453, were susceptible. For soil application, 13 varieties, including CoP 9301 and CoP 22441, were resistant, while 11, including CoP 20438 and CoP 19459, were moderately resistant. Nine varieties, such as CoLk 20466 and CoP 19437, were moderately susceptible and two varieties, CoBln 20501 and CoSe 16453, were susceptible. In the sett dip method, 14 varieties, including CoP 9301 and CoP 22441, were resistant and 14 varieties, including CoP 19459 and CoLk 20468, were moderately resistant. Five varieties, such as CoP 19437 and CoBln 17501, were moderately susceptible, while 2 varieties CoBln 20501 and CoSe 16453, were susceptible. In the cotton swab method, 34 varieties, including CoP 9301, CoP 22441 and CoBln 19501, were resistant, with no varieties classified as moderately resistant or moderately susceptible and only one variety, CoSe 16453 were found susceptible.

Second observations

The second observation, involving 4 inoculation methods plug inoculation and cotton swab inoculation at 260 days after planting and soil application and sett dip inoculation at 90 days showed significant variation, as depicted in Table 4. In the plug inoculation method, 9 varieties, including CoP 9301 and CoP 22441, were resistant, while 10 varieties, such as CoP 22442 and CoP 20436, were moderately resistant. Twelve varieties, including CoP 06436 and CoP 18436, were moderately susceptible and 4 varieties, including Co 0238 and CoSe 16453, were susceptible. In the soil application method, 11 varieties, including CoP 9301 and CoP 22441, were resistant, while 12 varieties, such as CoP 22438 and CoP 19441, were moderately resistant. Nine varieties, including CoP 19438 and CoBln 17501, were moderately susceptible, while 3 varieties CoBln 17502, CoBln 20501 and CoSe 16453 were susceptible. In the sett dip method, 13 varieties, including CoP 9301 and CoP 22441, were resistant, while 14 varieties, such as CoP 20438 and CoP 19459, were moderately resistant. Five varieties, including CoP 20437 and CoBln 17501, were moderately susceptible and 3 varieties

Table 3. Evaluation of inoculation methods and response of sugarcane varieties on 60 and 240 DAP

S.No	Varieties	Plug method (240 DAP)		Soil application (60 DAP)		Sett dip (60 DAP)		Cotton swab (240 DAP)	
		Disease score	Disease reaction	Disease score	Disease reaction	Disease score	Disease reaction	Disease score	Disease reaction
1	CoP 19437	2.66	MS	2.46	MS	2.16	MS	0.80	R
2	CoP 19438	2.26	MS	1.95	MR	1.54	MR	0.70	R
3	CoP 19440	1.75	MR	1.53	MR	1.28	MR	0.61	R
4	CoP 19436	0.23	R	0.13	R	0.11	R	0.15	R
5	CoP 06436	1.92	MR	1.65	MR	1.38	MR	0.63	R
6	CoP 19459	1.18	MR	1.13	MR	1.10	MR	0.54	R
7	CoP 19441	1.13	MR	0.98	R	0.65	R	0.50	R
8	CoBln 20501	3.24	S	3.14	S	3.12	S	0.92	R
9	CoBln 17501	2.78	MS	2.67	MS	2.46	MS	0.85	R
10	CoBln 17502	3.12	S	2.98	MS	2.89	MS	0.87	R
11	CoBln 19501	2.69	MS	2.54	MS	2.23	MS	0.82	R
12	Co 0238	2.95	S	2.75	MS	2.68	MS	0.87	R
13	CoP 20440	1.56	MR	1.46	MR	1.23	MR	0.60	R
14	BO 153	0.75	R	0.62	R	0.47	R	0.37	R
15	CoSe 18451	2.37	MS	2.26	MS	1.65	MR	0.74	R
16	CoLk 20466	2.29	MS	2.13	MS	1.59	MR	0.72	R
17	CoP 20436	0.97	R	0.79	R	0.53	R	0.45	R
18	CoP 20438	1.15	MR	1.11	MR	0.94	R	0.52	R
19	CoP 20437	2.51	MS	2.42	MS	1.95	MR	0.79	R
20	CoSe 18452	2.17	MS	1.73	MR	1.48	MR	0.67	R
21	CoP 9301	0.14	R	0.13	R	0.12	R	0.11	R
22	CoP 20439	0.36	R	0.30	R	0.29	R	0.19	R
23	CoLk 20469	0.68	R	0.54	R	0.44	R	0.36	R
24	CoLk 20468	1.34	MR	1.30	MR	1.18	MR	0.58	R
25	CoP 22439	0.28	R	0.23	R	0.21	R	0.17	R
26	CoP 22436	0.24	R	0.15	R	0.13	R	0.14	R
27	CoP 22438	1.10	MR	0.91	R	0.58	R	0.48	R
28	CoP 22437	1.21	MR	1.19	MR	1.16	MR	0.57	R
29	CoP 22440	0.45	R	0.43	R	0.39	R	0.32	R
30	CoP 22442	0.86	R	0.76	R	0.50	R	0.40	R
31	CoP 22441	0.23	R	0.14	R	0.12	R	0.14	R
32	CoP 18436	2.20	MS	1.84	MR	1.51	MR	0.69	R
33	CoLk 94184	2.15	MS	1.69	MR	1.46	MR	0.64	R
34	CoSe 16453	3.35	S	3.25	S	3.22	S	0.96	R
35	CoLk 16467	2.45	MS	2.32	MS	1.74	MR	0.77	R
36	CoV 92102 (Check)	3.56	S	3.49	S	3.44	S	3.35	S

R- Resistant, MR- Moderately Resistant, MS- Moderately Susceptible, S- Susceptible

Table 4. Evaluation of inoculation methods and response of sugarcane varieties on 90 and 270 DAP

S.No	Varieties	Plug method (270 DAP)		Soil application (90 DAP)		Sett dip (90 DAP)		Cotton swab (270 DAP)	
		Disease score	Disease reaction	Disease score	Disease reaction	Disease score	Disease reaction	Disease score	Disease reaction
1	CoP 19437	2.73	MS	2.59	MS	2.24	MS	0.85	R
2	CoP 19438	2.31	MS	2.10	MS	1.69	MR	0.74	R
3	CoP 19440	1.95	MR	1.64	MR	1.43	MR	0.66	R
4	CoP 19436	0.32	R	0.29	R	0.27	R	0.26	R
5	CoP 06436	2.13	MS	1.73	MR	1.54	MR	0.68	R
6	CoP 19459	1.26	MR	1.23	MR	1.18	MR	0.59	R
7	CoP 19441	1.19	MR	1.13	MR	0.98	R	0.55	R
8	CoBln 20501	3.43	S	3.32	S	3.26	S	3.12	S
9	CoBln 17501	2.90	MS	2.81	MS	2.57	MS	0.89	R
10	CoBln 17502	3.34	S	3.24	S	3.18	S	0.92	R
11	CoBln 19501	2.78	MS	2.72	MS	2.45	MS	0.88	R
12	Co 0238	3.16	S	2.95	MS	2.85	MS	0.91	R
13	CoP 20440	1.89	MR	1.58	MR	1.31	MR	0.64	R
14	BO 153	0.94	R	0.83	R	0.64	R	0.48	R
15	CoSe 18451	2.52	MS	2.34	MS	1.77	MR	0.80	R
16	CoLk 20466	2.43	MS	2.23	MS	1.74	MR	0.78	R
17	CoP 20436	1.11	MR	0.92	R	0.65	R	0.50	R
18	CoP 20438	1.20	MR	1.16	MR	1.12	MR	0.57	R
19	CoP 20437	2.65	MS	2.57	MS	2.18	MS	0.83	R
20	CoSe 18452	2.26	MS	1.89	MR	1.58	MR	0.71	R
21	CoP 9301	0.27	R	0.25	R	0.24	R	0.22	R
22	CoP 20439	0.50	R	0.48	R	0.46	R	0.37	R
23	CoLk 20469	0.85	R	0.72	R	0.63	R	0.46	R
24	CoLk 20468	1.65	MR	1.46	MR	1.35	MR	0.63	R
25	CoP 22439	0.48	R	0.35	R	0.32	R	0.31	R
26	CoP 22436	0.34	R	0.30	R	0.29	R	0.25	R
27	CoP 22438	1.17	MR	1.12	MR	0.74	R	0.52	R
28	CoP 22437	1.43	MR	1.32	MR	1.26	MR	0.61	R
29	CoP 22440	0.68	R	0.56	R	0.54	R	0.41	R
30	CoP 22442	1.13	MR	0.95	R	0.61	R	0.49	R
31	CoP 22441	0.32	R	0.29	R	0.26	R	0.24	R
32	CoP 18436	2.35	MS	1.95	MR	1.67	MR	0.72	R
33	CoLk 94184	2.23	MS	1.78	MR	1.50	MR	0.69	R
34	CoSe 16453	3.52	S	3.51	S	3.48	S	3.25	S
35	CoLk 16467	2.63	MS	2.46	MS	1.95	MR	0.81	R
36	CoV 92102 (Check)	3.61	S	3.60	S	3.54	S	3.46	S

R- Resistant, MR- Moderately Resistant, MS- Moderately Susceptible, S- Susceptible

CoBln 17502, CoBln 20501 and CoSe 16453, were susceptible. For the cotton swab method, 33 varieties, including CoP 9301 and CoP 22441, were resistant, with none being moderately resistant or moderately susceptible. Only two varieties CoBln 20501 and CoSe 16453, were susceptible to wilt disease.

Third observations

The third observation, which involved plug inoculation and cotton swab inoculation methods at 270 days after planting and soil application and sett dip inoculation methods at 120 days, revealed significant variation, as depicted in Table 5. Using the plug method, seven varieties, including CoP 9301 and CoP 22441, were resistant, while 10 varieties, such as CoLk 20469 and BO 153, were moderately resistant. Thirteen varieties, including CoP 20440 and CoLk 94184, were moderately susceptible and six varieties CoBln 17501, Co 0238 were susceptible. For soil application, nine varieties, including CoP 9301 and CoP 22441, were resistant, while 12, including CoP 22442 and CoP 20436, were moderately resistant. Ten varieties, such as CoSe 18452 and CoP 18436, were moderately susceptible and four CoBln 17502 and CoSe 16453, were susceptible. In the sett dip method, 11 varieties, including CoP 9301 and CoP 22441, were resistant, while 15 varieties, including CoP 22438 and CoP 19441, were moderately resistant. Five varieties, including CoLk 16467 and CoP 19437, were moderately susceptible and 4 varieties CoBln 17502 and CoSe 16453, were susceptible. Using the cotton swab method, 33 varieties, including CoP 9301 and CoP 22441, were resistant, with none being moderately susceptible. Only 2 varieties CoBln 20501 and CoSe 16453, were susceptible to wilt disease.

Based on the third observations response of various inoculation methods in terms of percentage were calculated as depicted in Table 6 and Fig. 1. Among the four inoculation techniques, the plug method showed that 20.00 % of the varieties exhibited a resistant reaction, 28.58 % a moderately resistant reaction, 37.14 % a moderately susceptible reaction and 14.29 % exhibited a susceptible reaction to wilt disease. In the soil application inoculation method, 25.71 % of the varieties exhibited a resistant reaction, 34.29 % a moderately resistant reaction, 28.58 % a moderately susceptible reaction and 11.42 % exhibited a susceptible reaction to wilt disease. In the sett dip inoculation method, 31.43 % of the varieties exhibited a resistant reaction, 42.86 % a moderately resistant reaction, 14.29 % a moderately susceptible reaction and 11.42 % exhibited a susceptible reaction to wilt disease. In the cotton swab inoculation method, 94.29 % of the varieties exhibited a resistant reaction, none exhibited a moderately resistant and moderately susceptible reaction and 5.71 % exhibited a susceptible reaction to wilt disease.

Among the tested inoculation methods, the plug method emerged as the most efficient, outperforming soil application and sett dip methods, while the cotton swab method was the least effective. This aligns with findings of previous studies (14, 15), who also highlighted the plug method's superior performance. This method is recommended for the inoculation of *Fusarium sacchari*

Table 5. Evaluation of inoculation methods and response of sugarcane varieties on 120 and 300 DAP

S.No	Varieties	Plug method (300 DAP)		Soil application (120 DAP)		Sett dip (120 DAP)		Cotton swab (300 DAP)	
		Disease score	Disease reaction	Disease score	Disease reaction	Disease score	Disease reaction	Disease score	Disease reaction
1	CoP 19437	2.84	MS	2.63	MS	2.46	MS	0.91	R
2	CoP 19438	2.41	MS	2.38	MS	1.76	MR	0.82	R
3	CoP 19440	2.28	MS	1.78	MR	1.53	MR	0.74	R
4	CoP 19436	0.44	R	0.40	R	0.38	R	0.38	R
5	CoP 06436	2.25	MS	1.81	MR	1.61	MR	0.77	R
6	CoP 19459	1.54	MR	1.46	MR	1.31	MR	0.63	R
7	CoP 19441	1.36	MR	1.23	MR	1.19	MR	0.61	R
8	CoBln 20501	3.68	S	3.65	S	3.62	S	3.13	S
9	CoBln 17501	3.42	S	2.92	MS	2.84	MS	0.95	R
10	CoBln 17502	3.61	S	3.57	S	3.52	S	0.98	R
11	CoBln 19501	2.93	MS	2.84	MS	2.65	MS	0.93	R
12	Co 0238	3.54	S	3.48	S	3.41	S	0.97	R
13	CoP 20440	2.20	MS	1.72	MR	1.44	MR	0.72	R
14	BO 153	1.28	MR	0.96	R	0.87	R	0.54	R
15	CoSe 18451	2.64	MS	2.45	MS	1.89	MR	0.87	R
16	CoLk 20466	2.56	MS	2.32	MS	1.84	MR	0.85	R
17	CoP 20436	1.20	MR	1.09	MR	0.87	R	0.56	R
18	CoP 20438	1.38	MR	1.35	MR	1.24	MR	0.66	R
19	CoP 20437	2.74	MS	2.72	MS	2.37	MS	0.89	R
20	CoSe 18452	2.38	MS	2.18	MS	1.65	MR	0.79	R
21	CoP 9301	0.38	R	0.36	R	0.33	R	0.30	R
22	CoP 20439	0.67	R	0.61	R	0.59	R	0.45	R
23	CoLk 20469	1.23	MR	0.92	R	0.89	R	0.50	R
24	CoLk 20468	1.74	MR	1.64	MR	1.47	MR	0.70	R
25	CoP 22439	0.62	R	0.56	R	0.55	R	0.41	R
26	CoP 22436	0.48	R	0.46	R	0.45	R	0.36	R
27	CoP 22438	1.30	MR	1.17	MR	1.15	MR	0.58	R
28	CoP 22437	1.67	MR	1.57	MR	1.38	MR	0.68	R
29	CoP 22440	0.91	R	0.88	R	0.76	R	0.49	R
30	CoP 22442	1.25	MR	1.13	MR	0.83	R	0.53	R
31	CoP 22441	0.40	R	0.39	R	0.36	R	0.33	R
32	CoP 18436	2.46	MS	2.26	MS	1.73	MR	0.80	R
33	CoLk 94184	2.32	MS	1.86	MR	1.59	MR	0.76	R
34	CoSe 16453	3.76	S	3.73	S	3.55	S	3.25	S
35	CoLk 16467	2.79	MS	2.52	MS	2.23	MS	0.86	R
36	CoV 92102 (Check)	4.00	S	3.84	S	3.75	S	3.46	S

R- Resistant, MR- Moderately Resistant, MS- Moderately Susceptible, S- Susceptible

Table 6. Response of various inoculation methods in terms of percentage based on final observation

S. No.	Rating score	Disease reaction	Plug method		Soil method		Sett dip method		Cotton swab method	
			Number of varieties	Percentage	Number of varieties	Percentage	Number of varieties	Percentage	Number of varieties	Percentage
01	0.0-1.0	R (Resistant)	7	20.00	9	25.71	11	31.43	33	94.29
02	1.1-2.0	MR (Moderately resistant)	10	28.58	12	34.29	15	42.86	0	0
03	2.1-3.0	MS (Moderately susceptible)	13	37.14	10	28.58	5	14.29	0	0
04	3.1 and above	S (Susceptible)	5	14.29	4	11.42	4	11.42	2	5.71

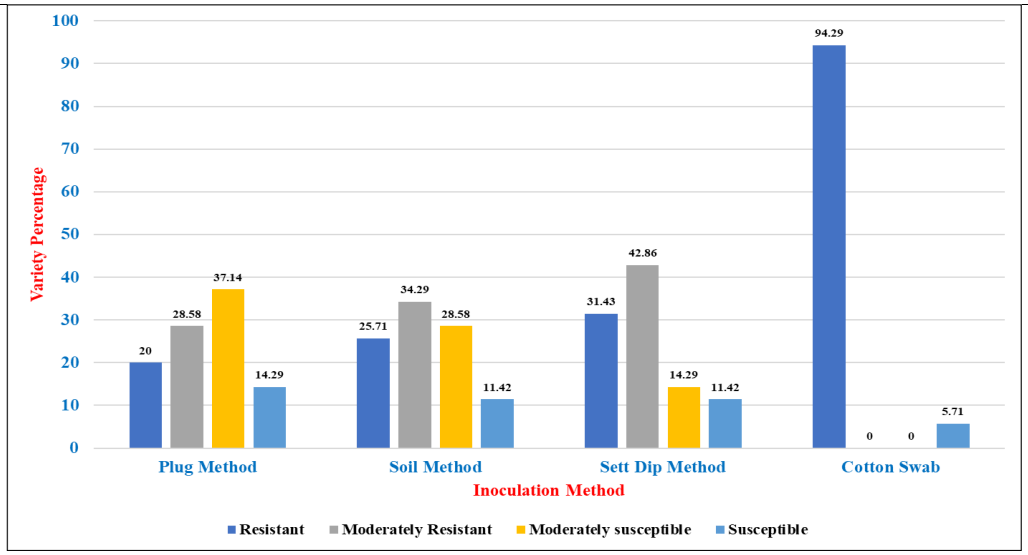

Fig. 1. Response of various inoculation methods in terms of percentage.

into the first internode from the base at 150 days after planting under water stress conditions to accurately simulate natural wilt disease (13). This approach offers a reliable framework for studying wilt in controlled environments. Additionally, it was found that the plug method, utilizing spore suspension, effectively induces disease in stalks (16). In contrast, soil inoculation was observed to impede sett germination and increase mortality, while inoculating wilt-infected soil into the root zone resulted in typical wilt symptoms in susceptible varieties. The plug method's reliability and effectiveness in simulating disease conditions are supported by previous studies (9, 11), validating its use in sugarcane disease research and management.

Conclusion

Based on the research conducted during the cropping season 2023-2024, the plug inoculation method stands out as the most effective for assessing resistance to wilt disease in sugarcane varieties. This method identified the fewest number of resistant varieties (7 varieties), indicating a more stringent and selective assessment of resistance. The lower number of resistant varieties suggests that the plug inoculation method provides a more accurate evaluation of a variety's true susceptibility to wilt, distinguishing between resistant and susceptible varieties more clearly. In comparison, the soil application and sett dip methods identified a slightly higher number of resistant varieties, which may indicate less stringent criteria or varying levels of pathogen exposure. The cotton swab method was the least effective method of inoculation of wilt pathogen, as most varieties appeared resistant and only a few were susceptible. Therefore, the plug inoculation method's ability to identify fewer resistant varieties may reflect a more precise and reliable assessment of wilt resistance, making it the preferred method for evaluating sugarcane varieties in this context. Currently, resistance is tested by planting in wilt-infested plots or using artificial inoculation methods, which are suited mainly to endemic areas. However, a universal screening technique for all locations is still needed.

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Authors' contributions

PK performed experiments. CSC, MM designed the research. SPS and AK wrote the manuscript. SP revised and corrected the manuscript. SAB, SK, CC and H have helped me during my research experiments. All authors have contributed to different sections of writing, reviewing, correction and statistical analysis. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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