Suppression of sugarcane red rot disease through its rhizosphericmycoflora

Comment [AC1]: Include in the title the location where the work was done.

Abstract

Soil samples were collected from sugarcane rhizosphere of four different varieties*viz*., CoPk 05191 and CoLk 94184 (resistant to red rot) and CoJ 64 and Co 1148 (susceptible to red rot)planted at ICAR-Indian Institute of Sugarcane Research, Lucknow experimental farm. The samples were subjected for the isolation of rhizosphericmycofloraon Potato Dextrose Agar media and antagonistic activities of the isolates were tested against red rot pathogens (Cf 07, Cf 08 and Cf 09). Isolates showing more than 50 % inhibition with all the three pathotypes (Cf 07, Cf 08 and Cf 09)were selected for further study.Among thetwelve selected isolates used for field experiment for the management of red rot, isolate T16 was found to be highlyeffective.The suppression of red rot offered by rhizospheric isolates is probably due to induced systemic resistance in sugarcane plantsor by the enzymatic action of metabolites produced by the rhizosphericmycoflora.The different levels of red rot infected seed cane*viz.*, 5%, 10%, 15%, 20%, 25%, 30%, 35% and 40% were treated with MHAT and planted along with healthy seed canes, infected seed canes, as a control to work out the losses caused by different level of red rot seed infection. The result revealed that a yield loss was recorded with the infected seed. The losses weredirectlyproportionate with the level of red rot infected seed.

Comment [AC2]: Complement the abstract with the definition of the problem and the justification of the research.Add quantitative data that supports the arguments presented.

Keywords: Sugarcane, rhizosphericmycoflora, *Colletotrichumfalcatum* and antagonistic activity.

INTRODUCTION

Sugarcane is an important agro-industrial and cash crop of India as well as the world and holds an important position in the Indian economy. It is grown in 123 countries on about 24 million hectares land[1].There are various biotic and abiotic factors responsible for its yield loss but diseases are one of the major causes of concern. About a hundred diseases of sugarcane are reported from different parts of the world[2]. Diseases in sugarcane are mainly caused by fungi, bacteria, viruses and phytoplasma. And among them, fungal diseases got international importance due to its impact on yield loss[3]. The estimated average loss in crop production due to fungal disease is about 18-31%[4].And the major fungal diseases of sugarcane are red rot, wilt and smut whereas red rot disease is considered the main constraint for sugarcane production in India.

Comment [AC3]: Start with generalities of the species, economic importance at a global, regional and national level with recent data.

Sugarcane (*Saccharumofficinarum*L.) is known to have microbial organisms associated with its rhizosphere which have potential antagonistic activity against other microbes.An antagonistic rhizospheric microbe inhibits the growth of pathogenic microorganisms and has been found to colonize the plant rhizosphere. Rhizospheric microorganisms also play an important role in many processes of crop production[5]. Keeping in view of above finding there is a need to explore the sugarcane rhizospheric microbes for the purpose of disease management. A large number of fungi had been isolated from the rhizosphere soil of sugarcaneviz., *Aspergillus*,*Rhizopus*, *Penicillium*,*Trichoderma* and *Alternaria*. *Trichoderma* was found to be predominant in the rhizosphere of sugarcane[6].*Trichoderma* is one the most commonly isolated fungi from rhizospheric soil with high bicontrol potential. The fungi *Trichoderma*may suppress the growth of the pathogen population in the rhizosphere through competition and thus reduce disease

development. Biocontrol of plant pathogens with *Trichoderma* has been established by several workers[7].*Trichoderma harzianum*strain Th 37 isolated from sugarcane rhizosphere of Kushinagar, Uttar Pradesh, was found most potent biocontrolagent for red rot disease of sugarcane[8].

Comment [AC4]: Complement the introduction with background studies considering the international, national and regional context.

MATERIALS AND METHODS

Location and soil characteristics:

Field experiments were conducted at ICAR-Indian Institute of Sugarcane Research, Lucknow located at 26.56ºN, 80.52ºE and 111 m above the sea level. The climate is semi-arid sub-tropical with dry hot summer and cold winter. The experimentation field soil was fine loamy non-calcareous mixed hyperthermicTypicHapalquept. The soil has pH 6.8, and organic carbon from 0.4 to 0.5 %. Field area per plot was 6 x 5.4 m with three replications in randomized block design. The crop was planted in the first week of February using healthy three-bud cane setts @ 38000 setts/ha at 90 cm row to row spacing[9]. The laboratory experiments were conducted in Crop ProtectionDivision, ICAR-Indian Institute of Sugarcane Research, Lucknow.

Sample collection and isolation of rhizosphericmycoflora:

The rhizospheric sampling has been conducted by keeping holistic approach to isolate all possible mycoflora. It includes two type of sugarcane variety, one was resistant to red rot and other was susceptible to red rot of sugarcane. In the line CoPk 05191 and CoLk 94184 (resistant),CoJ 64 and Co 1148 (susceptible) were explored, planted at research farm of ICAR-IISR, Lucknow. The samples were collected aseptically after 120 DAP and transported to lab for further process. 1 gm of air dried soil sample was suspended in 9ml of autoclaved distilled water and shaken well. After sedimentation of solid particles, the suspension was serially diluted up to 10^{-5} to 10^{-6}. 1ml of the each dilution was added to the sterile PDA plates and was spread evenly and incubated at 27±1ºC for 6-7 days. After 6-7 days incubation, fungal colonies were picked up, purified by single spore culturing[10]and were maintained on PDA slants. Sum total of sixteen isolates were selected. The selected sixteen isolates were studied for the PGPR related tests. Based on the results of biochemical and other tests twelve isolates were selected and these twelve isolates were further used for the field experiment. The selected twelve isolates belongs the genera *Trichoderma.*The isolates were identified on the basis colony morphology; colonies were selected and further purified by repeated sub culturing and maintained on PDA and stored at 4ºC. Identification keys developed byBaijal and Mehrotra, [11] and Bisset, [12, 13]were used to identify the microorganisms.

Comment [AC5]: Include the design with its repetitions and experimental units.

Growth and culturalcharacterization of rhizosphericmycoflora

Cultural characteristics such as colony appearances, mycelial textures, spores and pigmentations were observed on PDAand growth rate via colony diameter were measured. The plates were incubated at 27±ºC for 7 days.The fungal morphology was studied macroscopicallyby observing the colony features (color, shape, size and hyphae) and microscopically by a compound microscopewith a digital camera using a lactophenol cotton bluestained slide mounted with a small portion of the mycelium[14].

Antagonistic activity of rhizosphericmycofloraagainst red rot pathogens

The isolates of sugarcane rhizosphericmycoflorawere tested for the antagonistic activitiesby following the dual culture techniques against red rot pathogens Cf 07, Cf 08 and Cf 09[15].In dual cultures, 5 mm disc of 7 days old culture of red rot pathogen and the targetedmycoflora were placed in a single plate and incubated at 27ºC. The colony diameter of both the fungus was recorded after 24 h duration for five days. The rhizospheric isolates showing more than 50 % inhibition with all the three designated pathotypesCf 07, Cf 08 and Cf 09 were selected and further used for the field experimentation.

Percentage Inhibition was recorded by formula

I %= 100 (C-T)/C

Where,

I= Percent inhibition over control
C=Growth of pathogen in control
T=Growth of pathogen in treatment

Comment [AC6]: References from where they were taken or modified.

Field evaluation ofrhizosphericmycoflora for red rot management

Field experiments were conducted at ICAR-IISR farm to find out the effect of rhizospheric isolates on the productivity, millable cane and yield of sugarcane by using isolate treated seeds. Twelverhizospheric isolates showing good antagonistic activity were used for the seed treatment for managing red rot disease of sugarcane. 10% diseased seeds of sugarcane varieties CoJ 64 and Co 0238 were used for the study. Infected seeds were developed by inoculating the cane with the red rot pathogen. All the treatments were replicated thrice in a randomized block design in 6 x 5.4 m plot. Three bud setts of sugarcane variety CoJ 64 and Co0238 were dipped or treated with in spore suspension at the @ 10^6 spores/ml of bioagents for overnight and plantedat row to row distance 90 cm. Observation of percent germination (after 45 days of planting), millable canes,juice quality and yield t/ha was recorded at the time of harvest.

Comment [AC7]: Include a table showing the treatments evaluated.

Effect of red rot seed borne inoculums on the performance of variety:

Eight different set of red rot infected and healthy Co 0238 sugarcane variety seeds were used as planting material *viz.*, 5% infected + 95% healthy seed, 10% infected + 90% healthy seed, 15% infected + 85% healthy seed, 20% infected+ 80% healthy seed, 25% infected + 75% healthy seed, 30%infected + 70% healthy seed, 35%infected + 65% healthy seed, 40%infected + 60% healthy seed,healthy canes and MHAT treated 10% infectedcanes.Infected seed material was developed by inoculating the red rot pathogen in mature plant of Co 0238 variety before 2 months of planting and these infected canes were used as a planting material. MHAT treatment was given for 2 h 30 min at 54ºC. Germination percentage, millable canes, average single cane weight, average length, average girth and average number of nodes, percent infection and yield t/ha was recordedat the time of harvest.

Comment [AC8]: Take into account the previous recommendation. Include an item with all the variables evaluated, describing how, where and when they were measured. At the end, the statistical analyzes and the software used must be presented.

RESULT AND DISCUSSION

Cultural characters of different sugarcane rhizosphericmycofloraon Potato Dextrose Agar media

The isolated sugarcane rhizosphericmycoflora were subjected for their morphological and cultural characterization. In the study cultural characteristics and colony growth rate of sixteen isolates has been worked out. The results summarized in table 1provides a strong evidence that sugarcane rhizosphere is full of diverse array of mycoflora exhibiting a great morphological diversity. The major and remarkable macroscopic features in species identification were the colony features, including diameter after 24h, color of conidia, mycelia colour, substrate colour, colony texture and shape[16]. Several workers have advocated using of morphology based approach in combination with variations in colony character, morphological characters and growth rates at different temperatures as an effective method for characterizing and studying inter and intra-species diversity among the *Trichoderma* strains[17, 18]. Joshi and Misra, [19] studied for the colony characters and found variability among the isolates. Green conidia was visible within 48 h for isolates STr-13 and STr-23, whereas in the other eight isolates green conidia were observed between 48 and 60 h time duration. Yellow diffusing pigment in medium was observed in isolates STr-10, 16 and 29. Similarly, yellow or orange colour pigment production in media is characteristic of *T. harzianum* strains whereas bright yellow green pigmentation in media is characteristic of *T. longibrachitaum* species[20].

Comment [AC9]: Comparative studies must have a context (international, national or regional) and in what species, country they were carried out. Use references from no more than five years ago, that is, 2019 onwards.

Table: 1. Cultural characters of different sugarcane rhizosphericmycofloraon Potato Dextrose Agar media.

S. No.	Isolates	Colony characters						Radial growth (cm)						Sporulation initiation (h)
		Colony colour	Mycelialcolour	Spore colour	Appearance	Margin	Pigment	24 h	48 h	72 h	96 h	120 h	144 h	
1.	T1	Dark green	White	Green	Flat	Smooth	Yellowish orange	0.2	1.3	2.8	4.5 (F)	F	-	72
2.	T2	Dark green	Dull white	Green	Hairy	Smooth	Yellow	0.7	1.8	3.2	F	F	-	48
3.	T3	Dark green	Cottony white	Green	Concentric	Smooth	Yellow	0.7	1.7	3.5	F	F	-	72
4.	T4	Yellowish green	White	Yellowish green	Fluffy	Smooth	Dull yellow	0.6	1.5	2.8	F	F	-	48
5.	T6	Light green	Cottony white	Light green	Hairy	Irregular	Dull yellow	0.6	1.7	3.2	F	F	-	72
6.	T8	Light green	Dull white	Yellowish green	Flat	Smooth	Yellow	0.7	1.8	3.5	F	F	-	72
7.	T9	Dark green	Off white	Green	Concentric	Smooth	Dull Yellow	0.8	1.9	3.8	F	F	-	48
8.	T13	Dark green	White	Green	Concentric	Smooth	-	0.4	1.5	2.8	4.1	F	-	48
9.	T14	Dark green	White	Dark Green	Flat	Smooth	Yellow	0.3	1.2	3.2	4.3	F	-	48
10.	T15	Light green	White	Light green	Concentric	Smooth	-	0.2	1.2	3.0	4.3	F	-	72
11.	T16	Dark green	Cottony white	Dark green	Fluffy	Irregular	Yellow	0.3	1.5	3.2	F	-	-	48
12.	T17	Green	White	Green	Concentric	Smooth	Dull yellow	0.3	1.6	3.0	F	-	-	48
13.	T18	Light green	Cottony white	Yellowish green	Hairy	Irregular	Dull yellow	0.2	1.3	2.4	3.2	F	-	48
14.	T27	Green	Dull white	Light green	Fluffy	Smooth	Dull yellow	0.8	1.6	3.2	F	-	-	72
15.	T28	Green	Dull white	Light green	Hairy	Smooth	Yellow	0.8	1.7	3.5	F	-	-	48
16.	T35	Dark green	White	Green	Concentric	Irregular	-	0.2	1.8	3.6	F	-	-	48

Evaluation of sugarcane rhizosphericmycoflora for antagonistic potential against Cf 07, Cf 08 and Cf 09

The rhizospheric microflora have been explored for the management of red rot disease of sugarcane from the rhizospheric soil of four sugarcane varieties. The sixteenrhizospheric microflora isolated from different sugarcane variety pertains as seven isolates of CoJ 64 *viz.*, T1, T2, T3, T4, T6, T8, and T9; six isolates of Co 1148 *viz.*, T13, T14, T15, T16, T17, andT18; two isolates of CoPk 05191 *viz.*, T27, and T28; and one isolates of CoLk 94184*viz.*, T35. These isolates were subjected for the selection of potential antagonist on the basis of antagonistic activity exhibited against Cf 07, Cf 08 and Cf 09. The results summarized in table 2 representing antagonistic activity against Cf 07revealed that after 120 h time duration, maximum inhibition of red rot pathogen Cf 07 was recorded 90.00% by two isolates namely T13 and T28; against Cf 08 maximum inhibition of 96.77% was recorded with isolate T2 and against Cf 09 isolate T3 recorded maximum inhibition of 93.33% followed by minimum of 53.33%,51.61%,50.00% inhibition by isolate T35 withpathogen Cf 07, Cf 08 and Cf 09 respectively. Overall, it is evident from the experiment result that the two isolates T13 and T28 exhibited consistently high potential to antagonize the red rot pathogen Cf 07; isolate T2 against red rot pathogen Cf 08; whereasisolate T3 exhibited high potential to antagonize the red rot pathogen Cf 09 at 48h, 72h, 96h and 120h.

Thus, the results obtained from the study clearly indicates that sugarcane rhizospheric soils harbor a diversity of beneficial mycoflora which may be used as a biocontrol agents due to their interesting metabolic activity and their antifungal potential displayed toward target pathogenic fungi *C. falcatum*pathotypesCf 07, Cf 08 and Cf 09. Different mode of action are involved in acting as a biocontrol agents and these action may be competition, antibiosis, production of lytic enzymes, mycoparasitism and induced systemic resistance (ISR). All the above observations reviewed supported our findings that soil rhizosphericmycoflora are potential enough to antagonize pathogenic fungi *Colletotrichum falcatum* and these finding will help in future endeavor on biocontrol of *Colletotrichum falcatum*pathotypes. Sumana and Singh, [15], screened 231 microbial isolates for their antifungal activity against the red rot pathogen *Colletotrichum falcatum* using dual culture technique. 12 bacterial isolates, 4 fungal isolates and 5 *Actinomycete* isolates were found to be antagonistic to *C. falcatum* while none of the mould isolates could inhibit the growth of *C. falcatum*. Haque*et al.*[21] finding revealed that among many of the *Trichoderma species* very few of them are reported to be useful as a biocontrol and those few *Trichoderma species* are *T. viridae, T. haziarum, T. atroviridae*and *T. asperellum.* Out of the ten isolates, eight isolates which were isolated from the sugarcane agro-ecosystem exhibited significant reduction in *C. falcatum* growth over control with a maximum of 51.5% inhibition in colony area and inhibition of 30.3% in colony diameter[19].The fungi *Trichoderma* interacts with the other microorganisms, mainly with pathogenic fungi and these interactions include hyperparasitism, antibiosis or competition[22]. The competition may be for food, nutrients or space by modifying environmental conditions which suppresses the activity of other pathogenic fungi [23].These *Trichoderma* fungi produce a rich mixture of antifungal enzymes such as chitinases and β-1, 3 glucanases. Our findings are in accordance with study conducted by Joshi and Misra, [19] different *Trichoderma* isolates may vary considerably in their inhibitory activity against the specific pathogen.

Table: 2. Antagonistic potential of rhizosphericmycofloraagainst Cf 07, Cf 08 and Cf 09

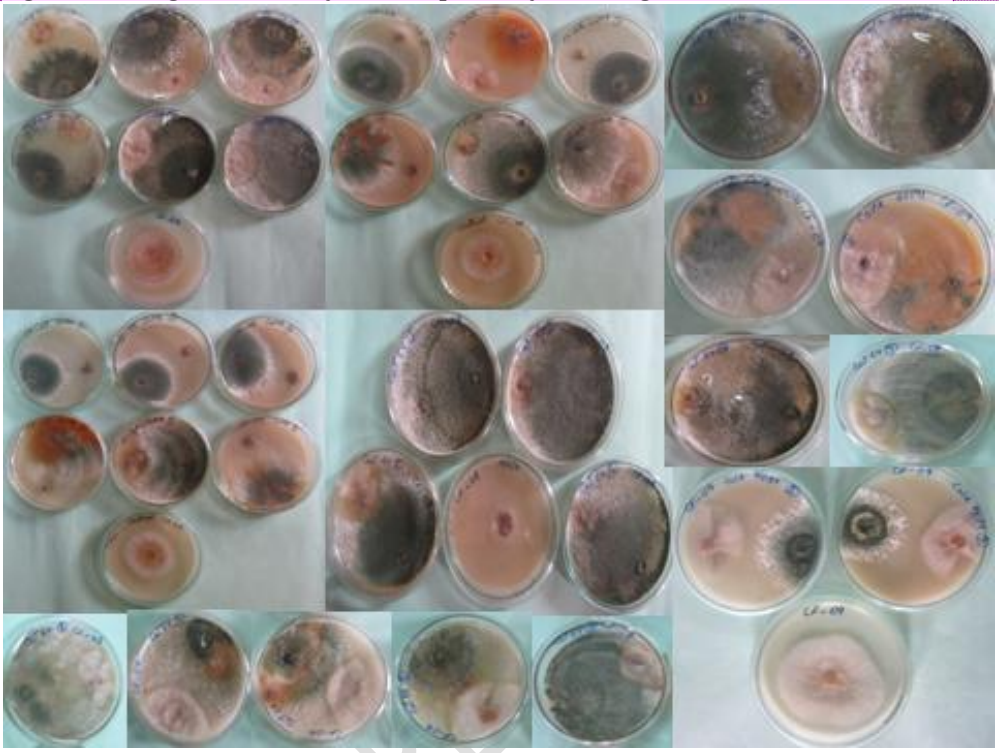
S.	Isolate	120 h	120 h	120 h

Comment [AC10]: Take into account the previous observations.

Comment [AC11]: The tables must be related in the text. The information in Table 2 is best presented in a figure.

No.	code	Mycelial growth CF 07 (cm)	% Inhibition	Mycelial growth CF 08 (cm)	% Inhibition	Mycelial growth CF 09 (cm)	% Inhibition
1.	T1	1.1	63.33	0.9	70.96	0.6	80.00
2.	T2	1.2	60.00	0.1	96.77	0.4	86.67
3.	T3	0.8	73.33	0.3	90.32	0.2	93.33
4.	T4	1.0	66.67	0.4	87.09	1.1	63.33
5.	T6	1.5	50.00	0.8	74.19	1.0	66.67
6.	T8	1.3	56.67	0.2	93.54	0.3	90.00
7.	T9	0.9	70.00	0.9	70.96	1.0	66.67
8.	T13	0.3	90.00	0.3	90.32	1.4	53.33
9.	T14	0.5	83.33	0.6	80.64	0.9	70.00
10.	T15	0.7	76.67	0.2	93.54	1.1	63.33
11.	T16	0.7	76.67	0.2	93.54	0.6	80.00
12.	T17	1.1	63.33	0.4	87.09	0.8	73.33
13.	T18	0.6	80.00	0.7	77.41	1.2	60.00
14.	T27	0.4	86.67	1.2	61.29	1.2	60.00
15.	T28	0.3	90.00	1.3	58.06	0.7	76.67
16.	T35	1.4	53.33	1.5	51.61	1.5	50.00
Control (Cf 07)		**3.0**	-	-	-	-	-
Control (Cf 08)		-	-	**3.1**	-	-	-
Control (Cf 09)		-	-	-	-	**3.0**	-

Figure: 1. Antagonistic activity of rhizosphericmycoflora againstCf 07, Cf 08 and Cf 09.



Comment [AC12]: The figures must be referenced in the document.

Efficacy evaluation of rhizosphericmycoflora for red rot management under field condition

Red rot is one of the major sugarcane diseases and for the control of this disease, microbes are being evaluatedas a biocontrol agent to manage it. In the line the sugarcane seed were treated with twelve different isolates and planted with 10% untreated infectedseed and healthy seeds. Results obtained from the field experiments is being summarized in table 3which reveals that out of twelve isolates T16 was recorded for the maximum yield with both the varieties CoJ 64 and Co 0238 for both healthy and 10% infectedcane seeds. Treating healthy canes with T16 resulted 85,185 millable canes owing yield of 102.22 t/ha with an increase yield of 4.45 % as compared to the healthy control without any treatments. The highest yielding isolate was found significant as compared to lowest yielding isolate T18. Control of red rot disease may be due to direct parasitic action of *Trichoderma* isolates or may be due to systemic resistance induced in sugarcane plant. Penetration of the fungal (biocontrol agent) hyphae increasing the concentrations of bioactive compounds and subsequent development of the fungus is suppressed. Reduction in the symptom severity of the disease can also be considered as an important aspect for improving the tolerance of the plants. Natural spread of the red rot disease of sugarcane during the month of July- August in field should be kept under check during the application of seed treatment.

Comment [AC13]: This information must be referenced.

A similar study was conducted bySingh *et al*.[8]and reported for *Trichoderma harzianum* strain T37 to be efficient in controlling red rot disease of sugarcane. Howell *et al*.[24]examined the role of terpenoid compounds in disease control. And also found the terpenoid synthesis and peroxidase activity was increased in the roots of plants treated with *Trichoderma virens*. Biological control of the plants by induction of defense response i.e., terpenoid synthesis by *Trichoderma virens* can be an important mechanism. Secondary metabolites produced by the *Trichoderma* isolates *viz.,* STr-52, STr-83 and STr-108 had shown high inhibitory activity against the red rot pathogen *Colletotrichum falcatum*[25].All the six *Trichoderma* treatments resulted in considerable reduction (29.5-56.3 %) in red rot disease over untreated control[26]. Germination failure was found to be much less (13.7-27.7 %) in case of the *Trichoderma* treatments. *Trichoderma* strains resulted in improved germination as compared to the untreated control with varying germination percent from 26.4- 31.5 % across the six different treatments[26].The results obtained from the field studies conducted by Joshi *et al*.[26]revealed that the application of talc-based formulation of the *Trichoderma* isolates by the three different methods *viz.,* sett treatment, soil application and combination of sett and soil treatment was found effective in suppressing sugarcane red rot disease. Also, most of the studies have focused on use of spores and/or use of secondary metabolites of *Trichoderma* for the management of red rot disease of sugarcane [27, 28, 25].However, in the previous studySingh *et al*.[28] in case of sugarcane, use of*Trichoderma harzianum* have been explored for suppression of red rot disease of sugarcane. Strains of *Trichoderma longibrachiatum* have been previously reported as an effective biocontrol agent to a number of plant pathogens[29, 30].

Comment [AC14]: Take into account the previous observations.

Table: 3. Biocontrol of red rot disease of sugarcane by rhizosphericmycofloraisolates

Comment [AC15]: Improve the presentation of all the tables in the document as well as their legends, which should be more informative.

S. No.	Treatment	% Germination		Millable cane		Average Single cane wt. (kg)		Average length (m)		Average girth (cm)		Average node		Yield (t/ha)		Brix		Sucrose %		Purity		% Increase/ Decrea in yield	
		Co 0238	CoJ 64	Co 0238	CoJ 64	Co 0238	CoJ 64	Co 0238	CoJ 64	Co 0238	CoJ 64	Co 0238	CoJ 64	Co 0238	CoJ 64	Co 0238	CoJ 64	Co 0238	CoJ 64	Co 0238	CoJ 64	Co 0238	CoJ 64
1.	T4 (HC)	41.98	31.24	77,395	85,185	1.20	0.90	2.0	1.5	2.5	2.0	20	18	92.87	76.66	21.48	19.74	18.83	17.48	87.69	88.53	4.90 %(D)	3.21 % (I)
2.	T4 (DC)	42.40	28.59	69,135	79,629	1.05	0.75	1.7	1.3	2.1	1.8	20	19	72.59	59.72	21.21	19.67	18.75	17.45	87.54	88.45	5.31 % (I)	4.42 % (I)
3.	T6 (HC)	37.46	34.96	75,925	83,950	1.20	0.85	2.1	1.5	2.4	1.9	19	16	91.11	71.35	20.81	19.87	18.09	17.63	86.92	88.79	6.66 % (D)	2.10 % (D)
4.	T6 (DC)	39.87	28.73	69,753	80,246	1.00	0.65	1.6	1.3	2.1	1.5	20	21	69.75	52.15	20.23	19.32	17.94	17.52	86.89	88.80	2.47 % (I)	3.15 %(D)
5.	T8 (HC)	38.33	38.48	83,950	88,271	1.20	0.85	2.1	1.5	2.4	1.7	20	19	100.74	75.03	21.25	19.78	18.58	17.59	87.45	88.95	2.97 % (I)	1.58 % (I)
6.	T8 (DC)	36.62	31.28	77,777	80,864	1.00	0.75	1.8	1.4	2.0	1.2	18	20	77.77	60.64	20.15	19.34	18.46	17.58	87.41	88.98	10.49 % (I)	5.34 % (I)
7.	T13 (HC)	26.30	29.00	82,098	86,419	1.15	0.70	1.8	1.4	2.3	1.5	19	17	94.41	60.49	20.41	18.77	18.43	16.64	90.30	88.58	3.36 % (D)	12.96 % (D)
8.	T13 (DC)	27.68	31.00	70,370	80,246	1.00	0.70	1.6	1.5	2.2	1.6	18	16	70.37	56.17	20.45	18.15	18.49	16.69	90.15	88.57	3.09 % (I)	0.87 % (I)
9.	T14 (HC)	29.67	22.22	82,716	89,375	1.10	0.80	1.8	1.5	2.4	1.8	18	15	90.98	71.50	20.24	18.98	17.53	16.49	86.61	86.84	6.79 % (D)	1.95 % (D)
10.	T14 (DC)	28.79	29.74	74,074	72,222	0.90	0.60	1.5	1.4	1.9	1.0	17	18	66.66	43.33	20.13	18.67	17.50	16.48	86.49	86.89	0.62 % (D)	11.97 % (D)
11.	T15 (HC)	27.93	28.56	81,481	82,716	1.10	0.80	1.9	1.6	2.3	1.8	20	18	89.62	66.17	20.42	19.10	18.42	16.76	90.21	87.86	8.15 % (D)	7.28 % (D)
12.	T15 (DC)	29.45	29.32	70,987	81,481	0.90	0.70	1.4	1.3	2.0	1.6	18	17	63.88	57.03	20.23	18.85	18.40	16.69	90.24	87.79	3.40 % (D)	1.73 % (I)
13.	T16 (HC)	35.87	39.38	85,185	87,037	1.20	0.90	2.0	1.6	2.5	1.9	22	23	102.22	78.33	21.48	19.47	19.24	17.39	89.61	89.31	4.45 % (I)	4.88 % (I)
14.	T16 (DC)	32.93	35.43	72,839	79,629	1.00	0.80	1.8	1.4	2.0	1.9	19	22	72.83	63.70	21.16	19.21	19.19	17.41	89.59	89.29	5.55 % (I)	8.40 % (I)
15.	T17 (HC)	34.28	37.26	80,864	84,567	1.15	0.85	2.0	1.2	2.2	2.0	21	18	92.99	71.88	20.89	19.42	19.08	17.32	91.37	89.25	4.78 % (D)	1.57 % (D)
16.	T17 (DC)	31.76	35.69	66,667	80,864	1.00	0.70	1.8	1.3	2.0	1.6	19	20	66.66	56.60	20.32	19.03	19.05	17.35	91.39	89.23	0.62 % (D)	1.3 % (I)
17.	T18 (HC)	23.67	19.34	18,518	21,604	0.90	0.60	1.7	1.3	1.9	1.2	20	14	16.66	12.96	20.31	18.84	17.57	16.61	86.52	88.27	81.11 % (D)	60.49 % (D)
18.	T18 (DC)	20.45	17.54	14,197	17,283	0.80	0.50	1.4	1.2	1.9	1.0	17	12	11.35	08.64	20.13	18.67	17.46	16.59	86.49	88.30	55.93 % (D)	46.66 % (D)
19.	T27 (HC)	35.87	41.66	83,333	87,654	1.20	0.90	2.2	1.6	2.3	1.8	22	21	99.99	78.89	21.44	19.78	19.01	18.06	88.72	91.31	1.89 % (I)	5.4 % (I)
20.	T27 (DC)	32.65	37.96	68,518	79,012	1.10	0.75	1.8	1.5	2.4	1.5	20	20	68.51	59.25	20.89	19.62	18.89	18.10	88.75	91.28	1.23 % (I)	3.95 % (I)
21.	T28 (HC)	31.29	34.92	82,716	86,419	1.20	0.85	2.1	1.4	2.5	1.8	21	21	99.25	73.45	20.43	19.73	18.55	17.76	90.79	90.03	1.48 % (I)	0.0 %
22.	T28 (DC)	28.58	33.16	62,962	78,395	1.00	0.70	1.8	1.3	2.4	1.6	21	20	62.96	54.87	20.02	19.71	18.42	17.78	90.78	90.00	4.32 % (D)	0.43 % (I)
23.	T35	28.30	35.78	81,613	85,802	1.10	0.85	1.9	1.3	2.3	2.0	20	17	89.77	72.93	20.16	19.66	17.71	17.20	87.84	87.48	8.00 %	0.52 %

	(HC)																					(D)	(D)
24.	T35 (DC)	29.45	34.67	73,456	77,778	0.95	0.70	1.8	1.2	2.0	1.6	20	19	69.78	54.44	20.12	19.49	17.65	17.03	87.82	87.50	2.50 % (I)	0.86 % (D)
25.	10%Diseased Control	35.50	30.00	67,283	79,012	1.00	0.70	1.7	1.3	2.0	1.6	20	18	67.28	55.30	20.19	18.98	18.82	17.25	87.88	87.79		
26.	Healthy Control	37.77	36.10	81,481	86,419	1.20	0.70	2.1	1.5	2.4	1.7	22	20	97.77	73.45	21.46	19.63	18.07	17.89	88.42	89.32		

HC- healthy canes; DC - diseased canes (10 %); I- increase in yield; D- decrease in yield

Table: 4. Effect of red rot seed borne inoculums on the performance of sugarcane variety Co 0238

S. No.	Treatment	Bud number		Germination %	Millable cane per hectare	Average Wt. of single (kg)	Average length (m)	Average girth (cm)	Average node	Yield (t/ha	% Yield loss	Brix	Sucrose%	Purity
		Planted	Germinated											
1.	5% infected cane	135	49	36.29	70,370	1.10	1.8	1.9	21	77.40	20.83	21.50	19.20	89.32
2.	10 % infected cane	135	48	35.50	67,283	1.00	1.7	1.9	20	67.28	31.21	21.41	18.82	87.88
3.	15 % infected cane	135	45	33.33	55,500	0.95	1.5	1.6	18	52.72	46.07	20.81	18.04	86.66
4.	20 % infected cane	135	42	31.11	53,703	0.90	1.5	1.5	19	48.33	50.56	20.31	17.96	88.41
5.	25 % infected cane	135	36	26.67	44,444	0.88	1.4	1.6	18	39.11	59.99	20.68	17.72	85.78
6.	30 % infected cane	135	33	24.44	38,888	0.80	1.4	1.5	17	31.11	68.18	20.23	17.49	86.46
7.	35 % infected cane	135	30	22.22	35,185	0.77	1.2	1.4	15	27.09	72.29	20.05	17.36	86.57
8.	40 % infected cane	135	24	17.77	31,481	0.70	1.0	1.4	13	22.03	77.46	20.02	17.32	86.54
9.	MHAT treated 10 % infected cane	135	49	36.29	76,456	1.15	1.7	1.8	20	87.92	10.07	21.30	18.51	88.72
10.	Healthy cane	135	51	37.77	81,481	1.20	1.8	1.9	22	97.77	00.00	21.46	18.07	88.42

Effect of red rot seed borne inoculums on the performance of variety:

Red rot of sugarcane is primarily seed transmitted disease followed by other sources of secondary transmission. Keeping in view to harvest optimum potential of variety, healthy seed is one of the inputs. To determine the qualitative and quantitative loss due toinfected seeds,an experiment is being conducted. Different level of seed infection *viz.*, 5%, 10%, 15%, 20%, 25%, 30%, 35% and 40% along with healthy canes, and MHAT treated 10% infected canes of variety Co 0238 have been planted for the evaluation. Results obtained from table 4 revealed thatMHAT treated diseased cane seeds showed higher germination percentage as compared to the untreated seed.

Comment [AC16]: Expand the discussion with comparative studies.

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Comment [AC17]: The number, quality and timeliness of the references are not the most appropriate. Include more up-to-date references.