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Impact on Plant Growth Promotion of Sugarcane through its Rhizospheric Mycoflora

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A B S T R A C T

The rhizosphere of sugarcane was explored for its mycoflora with the purpose of plant growth promotion. For the purpose different biochemical activites *viz.*, catalase production, ammonia production, starch hydrolysis, cellulase production, hydrogen cyanide and oxidase production. Out of the fourty one isolates characterized, twelve isolatesT4, T6, T8, T13, T14, T15, T16, T17, T18, T27, T28 and T35 were found promising and selected for their performance *in vivo* under glass house condition for in respect to plant growth promotion. Among the twelve isolates tested, isolate T16 was found best, recorded significantly higher yield (68.89 t/ha) with highest number of millable canes (76,543) followed by T8 and T17. These isolates can be used for plant growth promotion to obtain maximum yield in sugarcane.

Introduction

The rhizospheric soil holds vast diversity of inhabiting microorganisms had been explored for benefiting the sugarcane cultivation. A large number of microorganisms such as fungi, bacteria, and protozoa coexist in the rhizosphere which is also known as a hotspot of microbial activities. Soil microflora plays an important role in stimulating plant growth and evaluation of soil conditions (Singh *et al.*, 1999). The term rhizosphere for first time was described by Lorenz Hiltner in 1904. The rhizosphere is the region of soil that is near to the plant root system which is hundred percent influenced by the root exudates (Kennedy, 1999). And is the area where interactions among innumerable microorganisms affect the plant growth and the microbial population is stimulated by the root activities.

There are several continues activities going on in rhizosphere area are plant protection, pathogenesis, plant growth promotion, production of antibiotics, plant colonization and geochemical cycling of minerals (Kent *et al.*, 2002). The use of plant growth promoting

microbes offers an attractive way to replace chemical fertilizer and other chemicals with significant increase in root length, plant height and leading to increase in crop yield. Study conducted by Juma *et al.,* (2018) revealed that a number of fungi were found in the rhizosphere soil of sugarcane *viz.*, *Aspergillus, Rhizopus, Penicillium, Trichoderma* and *Alternaria*. Whereas, *Trichoderma* was found to be predominant in the rhizosphere of sugarcane. Different PGPR in Sugarcane crops in Brazil was studied (Reis *et al.,* 2000; Kennedy and Islam, 2001) they showed presence *of Aceinetobacter diazotrophicus, Azospirillum brasilence, Azospirillum lipoferum, Burkholderia, Herbaspirillum, Rhizobium leguminosarum* as dominant nitrogen fixer. Some PGPR are able to establish themselves on the crop roots especially if they are inoculated on the seed before planting.

Materials and Methods

Sample collection and isolation of rhizospheric mycoflora

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) and CoJ 64 and Co 1148 (susceptible) were explored, planted at research farm of ICAR-Indian Institute of Sugarcane Research, Lucknow, Uttar Pradesh, India. 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 9 ml 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 (Goh, 1999) and were maintained on Potato Dextrose Agar (PDA) slants. Sum total of one hundred eight different colonies were isolated belonging from the different genera *viz*., *Aspergillus, Trichoderma, Alternaria, Cladosporium, Acremonium, Mucor, Rhizopus, Penicillium, Chaetomium* and *Fusarium*. Out of total 108 isolates, forty one isolates were selected on basis of cultural growth and rest others were discarded as they were slow growing.

The selected forty one 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 by Baijal and Mehrotra, (1980) and Bisset, (1991a and b) were used to identify the microorganisms.

Enzymatic assay of sugarcane rhizospheric isolates

Cellulase production

The isolates were screened on cellulase agar medium and the culture plates of different selected mycoflora were incubated at 27±2ºC for 3-4 days. Further plates were observed for the zone formation. Formation of purple coloured zone around the colony shows that the isolate is cellulase producing (Lunge and Patil, 2012).

Oxidase test

The 48-72 h old fungus broth culture was

used for the study. Readymade HIMEDIA OXIDASE DISC has been taken in clean slide, with the help of loop. Then after a drop of broth culture has been rubbed in surface of Oxidase disc. Within 60-90 seconds a light purple to dark purple colour indicates positive for oxidase. If the culture does not change or it takes longer than 2 minutes it means the culture is oxidase negative.

Catalase activity

The 48-72 h old culture was taken on a clean glass slide. 2-3drops of 3% hydrogen peroxide (1 ml of 30% hydrogen peroxide mix in 9 ml of distilled water for preparing 3% H_2O_2) was added to the culture in the glass slide. The effervescence indicated positive catalase activity in culture.

Starch hydrolysis

Starch agar medium plates (Starch 20.0 g/l, peptone 5.0 g/l, yeast extract 3.0 g/l, agar 15.0 g/l; pH 7.0) were inoculated with isolated fungal cultures. The plates were incubated at 27±1ºC in inverted position for minimum 2-3 days. The plates were flooded with iodine solution for 30 seconds. The plates were observed for the appearance of clear zones around the fungal growth which defines the starch hydrolysis.

Ammonia production

Five ml peptone water (peptone 10.0 g/l, NaCl 5.0 g/l, distilled water 1.0 L, pH 7.0) was taken in test tubes and then each test tube was inoculated with the fungus culture and incubated at 30ºC for 4 days. After incubation 1 ml of Nessler's reagent was added in each tube. Presence of faint yellow colour indicates small amount of ammonia and deep yellow to brownish colour indicates maximum production of ammonia.

HCN production

The selected isolates were tested by inoculating on potato dextrose agar. Filter paper soaked in 2% sodium carbonate and 0.5% picric acid solution was placed on the top of the Petri plate. Plates were sealed with parafilm and incubated at 28±2°C for 4-5 days. Development of brown colour of the filter paper showed positive for hydrogen cyanide (HCN) production.

Efficacy of rhizospheric isolates under glass house condition

The potential isolates showing good biochemical activities i.e. enzyme production and metabolite production were selected for further studies. Pot experimentation in glass house condition was conducted to test the *in-vivo* efficacy. Earthen pots were filled with sterilized soil and three single bud setts seed of sugarcane variety Co 0238 was used for the study. The seeds were dipped for an overnight in twelve different test mycoflora spore suspension having 10^6 spores/ml. Germination percentage, cane length and girth, number of nodes and yield was recorded for the treated plants and compared with control untreated plants.

Cane juice quality analysis

The 300 days old sugarcane stalks were cut cleaned and crushed in a power crusher. The juice was strained through a muslin cloth to remove suspended impurities and was used for further analysis (Rao, 1986). Brix (total soluble solids) was recorded in a brix hydrometer. To assess sucrose percentage, 2 gm of lead acetate was added to 100 ml of juice and shakenwell. After Five minutes it was filtered through filter paper, filtrate taken in a Polaris cope observation tube (200 mm) and polariscope reading was recorded. The sucrose % was determined from the brix and

corresponding polariscope reading by referring to the Schmitz table.

The juice purity % was calculated using the following formula:

Purity = Sucrose % / Corrected brix % x 100

The commercial cane sugar (CCS) % was calculated using the following formula:

CCS % = [(S-(B-S) x 0.4] x 0.73

Where,

S= sucrose percent of juice
B= brix percent of juice

Assessment in sugarcane yield and juice quality

(i) Quality character
(ii) Juice brix percent
(iii) Juice sucrose percent
(iv) Juice purity percent

Quality parameters

Cane Yield- the cane yield was recorded at the time of harvesting

Cane yield=number of millable cane X single cane weight

Commercial cane sugar (CCS) yield-

CCS yield (kg/plot)= CCS percent X cane yield (kg/plot)

The loss in yield of sugarcane over control was calculated for each treatment by the following formula:

Percent yield loss

Percent yield loss= C-T/C X 100

Where,

C= yield in control
T= yield in treatment

Results and Discussion

Isolation of rhizospheric mycoflora

Rhizospheric mycoflora were isolated from soil samples collected from rhizosphere of red rot resistant varieties (CoPk 05191 and CoLk 94184) and susceptible varieties (CoJ 64 and Co 1148). A large numbers of different fungi were isolated from the sugarcane rhizospheric soil. During the course of purification of isolated mycoflora it has been found that sum total of forty one were found apparently good grower. Result also revealed that, *Trichoderma* was found to be predominant in the rhizosphere of sugarcane. Similar kind of study was conducted by Juma *et al.,* (2018) is in an accordance with the present study and isolated a number of fungi from the rhizospheric soil of sugarcane *viz.*, *Aspergillus, Rhizopus, Penicillium, Trichoderma* and *Alternaria* whereas, *Trichoderma* was found to be predominant in the rhizosphere of sugarcane. Number of bacterial species associated with sugarcane rhizosphere was isolated (Bhardwas and Garg, 2012). These belonging to *Azospirillum, Alcaligens, Arthrobacter, Acinetobacter, Bacillus, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Pseudomonas, Rhizobium* and *Serratia.*

Enzymatic assay of rhizospheric isolates

Sugarcane rhizospheric soil found associated with huge numbers of microbes. Out of those, several microbes have the capability of producing different enzymes for the growth and development of plant. Each and every enzyme has its own importance some may helps in plant growth promotion and some

may be useful in managing diseases. The present study was undertaken in order to isolate and identify sugarcane rhizospheric mycoflora and to perform biochemical characterization. Biochemical tests of forty one isolates *viz.*, T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, T11, T12, T13, T14, T15, T16, T17, T18, T19, T20, T21, T22, T23, T24, T25, T26, T27, T28, T29, T30, T31, T32, T33, T34, T35, T36, T37, T38, T39, T40 and T41 has been worked out. The characters studied were catalase production, ammonia production, starch hydrolysis, cellulase production, hydrogen cyanide and oxidase production. All the forty one rhizospheric mycoflora isolates results positive test for ammonia production, hydrogen cyanide and oxidase. Among the forty one isolates, thirty isolates *viz.*, T1, T2, T3, T4, T5, T6, T7, T8, T9, T13, T14, T15, T17, T18, T21, T22, T23, T24, T26, T27, T28, T30, T31, T32, T35, T37, T38, T39, T40 and T41 were catalase positive or catalase producing and rest other were non catalase producing mycoflora. Among the forty one isolates, twenty two isolates *viz.*, T5, T6, T10, T12, T18, T19, T20, T21, T22, T23, T24, T29, T31, T33, T34, T35, T36, T37, T38, T39, T40 and T41 were amylase producing and rest other were non amylase producing.

Table.1 Enzymatic assay of different sugarcane rhizospheric isolates for its plant growth promotion

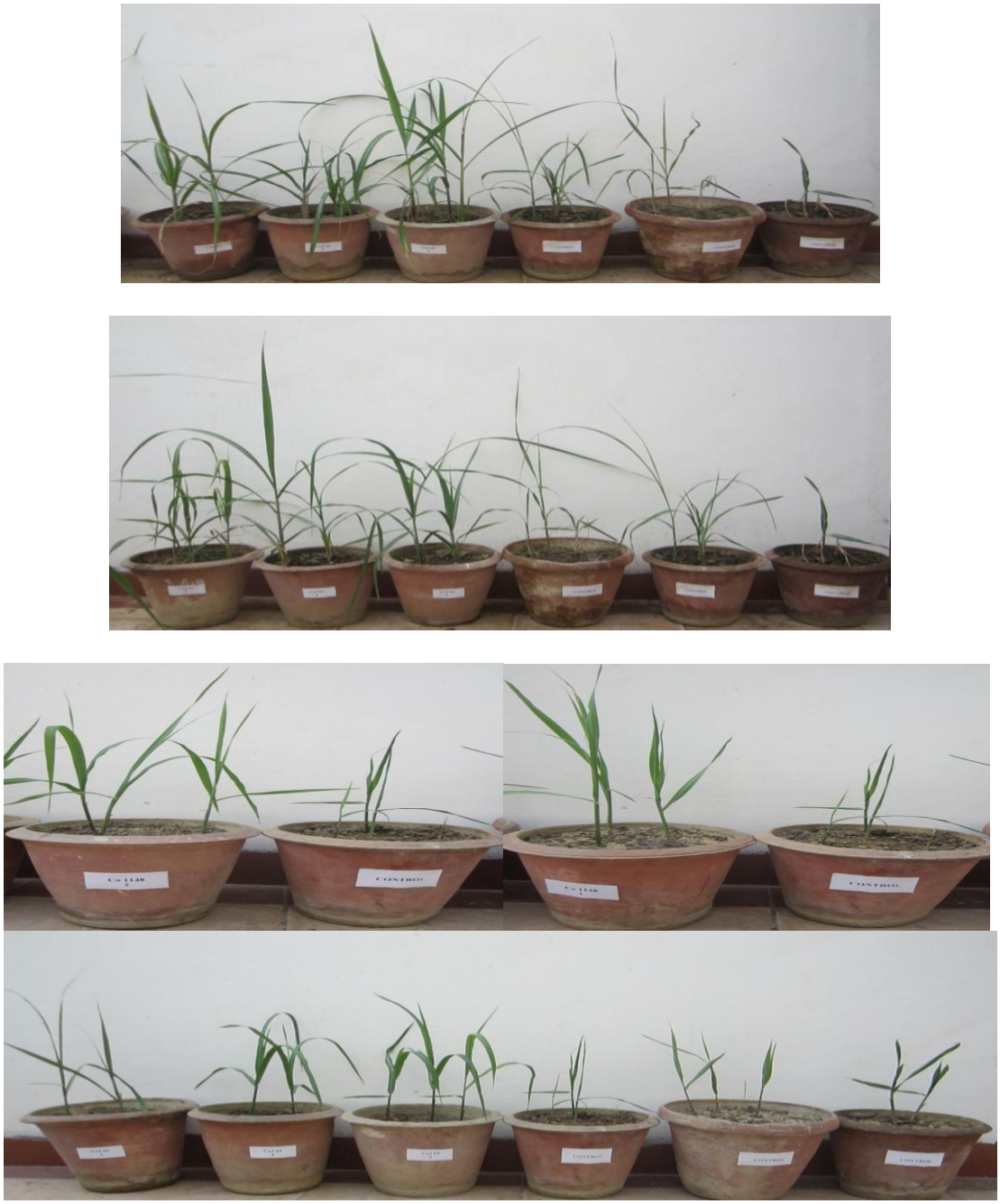
S. No.	Isolate	Catalase	Ammonia	Starch hydrolysis	Cellulase	HCN	Oxidase
1.	**T1**	+	++	-	+++	+	+
2.	**T2**	+	+	-	++	+	+
3.	**T3**	+	+	-	+	+	+
4.	**T4**	+	+	-	+	+	+
5.	**T5**	+	+	+	+++	+	+
6.	**T6**	+	+++	+	+	+	+
7.	**T7**	+	+	-	++	+	+
8.	**T8**	+	++	-	+++	+	+
9.	**T9**	+	++	-	++	+	+
10.	**T10**	-	++	+	-	+	+
11.	**T11**	-	++	-	-	+	+
12.	**T12**	-	+++	+	-	+	+
13.	**T13**	+	++	-	++	+	+
14.	**T14**	+	++	-	+	+	+
15.	**T15**	+	+	-	+	+	+
16.	**T16**		++	-	++	++	+
17.	**T17**	+	++	-	++	+	+
18.	**T18**	+	++	+	+	+	+
19.	**T19**	-	+++	+	-	+	+
20.	**T20**	-	+++	+	-	+	+
21.	**T21**	+	+++	+	-	+	+
22.	**T22**	+	++	+	-	+	+
23.	**T23**	+	++	+	+	+	+
24.	**T24**	+	++	+	-	+	+

25.	**T25**	-	+++	-	-	+	+
26.	**T26**	+	++	-	+	+	+
27.	**T27**	+	++	-	+	+	+
28.	**T28**	+	+++	-	++	+	+
29.	**T29**	-	++	+	+	+	+
30.	**T30**	+	++	-	+	+	+
31.	**T31**	+	++	+	+	+	+
32.	**T32**	+	+	-	+	+	+
33.	**T33**	-	++	+	+	+	+
34.	**T34**	-	++	+	+	+	+
35.	**T35**	+	+++	+	++	+	+
36.	**T36**	-	+++	+	-	+	+
37.	**T37**	+	++	+	+	+	+
38.	**T38**	+	+++	+	+	+	+
39.	**T39**	+	+++	+	+	+	+
40.	**T40**	+	++	+	-	+	+
41.	**T41**	+	++	+	+	+	+

Table.2 Evaluation of different parameters related to plant growth promotion and increase in yield of sugarcane

S. No.	Isolates	No. of single bud sett planted	Germination percentage (%)	Millable cane	Average Single cane wt. (kg)	Average length (m)	Average girth (cm)	Average node	Yield (t/ha)
1.	**T4**	3	100	74,691	0.80	1.5	2.0	18	59.96
2.	**T6**	3	100	72,222	0.90	1.6	2.1	18	64.99
3.	**T8**	3	100	75,925	0.90	1.7	2.0	17	68.33
4.	**T13**	3	100	73,839	0.80	1.7	1.9	19	59.07
5.	**T14**	3	100	71,604	0.70	1.6	1.8	17	50.12
6.	**T15**	3	100	74,074	0.80	1.8	2.0	19	59.25
7.	**T16**	3	100	76,543	0.90	1.8	1.9	20	68.89
8.	**T17**	4	100	75,308	0.90	1.7	2.0	19	67.78
9.	**T18**	4	25	18,518	0.45	1.0	1.5	18	08.33
10.	**T27**	4	100	77,777	0.80	1.6	2.0	19	62.22
11.	**T28**	3	100	70,987	0.75	1.7	1.8	19	53.24
12.	**T35**	4	100	70,370	0.75	1.6	1.9	18	52.77
13.	**Control diseased**	4	75	61,111	0.65	1.5	1.7	16	39.72
14.	**Control healthy**	4	100	74,074	0.90	1.7	2.0	20	66.67
Mean		**3.428**	**92.85**	**69,075**	**0.785**	**1.607**	**1.9**	**18.35**	**55.81**
$CD_{0.05}$		**NS**	**-**	**13.786**	**0.111**	**0.132**	**0.186**	**1.269**	**4.854**
CV		**18.971**	**-**	**0.012**	**8.438**	**4.881**	**5.839**	**4.118**	**5.181**
SEm±		**0.423**	**-**	**67.436**	**0.004**	**0.006**	**0.012**	**0.571**	**8.359**

Fig.1 Efficacy test of selected rhizospheric mycoflora under glass house



Among the forty one isolates, thirty isolates *viz.*, T1, T2, T3, T4, T5, T6, T7, T8, T9, T13, T14, T15, T16, T17, T18, T23, T26, T27, T28, T29, T30, T31, T32, T33, T34, T35, T37, T38, T39 and T41 were cellulase producing isolates and rest eleven isolates were non cellulase producing. Screening of their ability to synthesize chemical compounds revealed that the majority of the mycoflora isolated, were able to produce catalase, ammonia, amylase, cellulase, hydrogen cyanide and oxidase. Zyani, (2009) in his study found that *Penicillium chrysogenum* showed the highest cellulase

productivity as confirmed by CMCase values. According to the research conducted by Mirzaakhmedov *et al.*, (2007), *Aspergillus terrus* was found to be the most active producer of cellulolytic enzymes than *Penicillum, Fusarium, Trichoderma* and other *Aspergillus* species. Yalpani, (1987) stated that *Trichoderma ressei, Trichoderma koningii, Fusarium, Aspergillus* and *Penicillium* sp. are the most potent and common cellulase producers. Seventeen fungal species belonging to three genera i.e. *Trichoderma, Aspergillus* and *Penicillium* were isolated from different sources, screened and compared for their ability to degrade cellulose (Khokhar, 2012). The results of the enzymatic assays identified three isolates of *Trichoderma viz.*, STr-52, STr-83 and STr-108, which had exhibited high production of cellulase enzymes (Joshi *et al.*, 2018). Joshi and Misra, (2013) compared inhibitory activity of soluble and volatile metabolites produced by *Trichoderma* isolates.

Glasshouse evaluation of rhizospheric mycoflora for plant growth promotion

Field performance of mycoflora is being influenced by the several weather and soil parameter as compared to the laboratory (control condition). For the said evaluation, twelve best performing (enzyme assay) mycoflora were selected for i*n-vivo* study (pot experiments) under glass house condition. The data on germination percentage, millable cane per hectare, average weight of single cane, average length, average girth, average node and per hectare yield (Table 2) reveals that 100 % germination was recorded for healthy control and all the eleven treatments (T4, T6, T8, T13, T14, T15, T16, T17, T27, T28 and T35) except T18 (25 %) and disease control (75 %). Out of the twelve treatments (T4, T6, T8, T13, T14, T15, T16, T17, T18, T27, T28 and T35), treatment T16 was significantly recorded for the highest yield (68.89 t/ha) having highest number of millable canes (76,543) with average single cane weight of 0.90 kg, 1.8 m average length, 1.9 cm average girth and 20 average nodes against the lowest yield (08.33 t/ha) recorded for treatment T18 having least number of millable canes (18,518) with average single cane weight of 0.45 kg, 1.0 m average length, 1.5 cm average girth and 18 average node. Treatment T8 was recorded for the second highest in terms of yield (68.33 t/ha) and millable cane (75,925) having average single cane weight of 0.90 kg, average length of 1.7 m, average girth of 2.0 cm and average node of 17 when compared with the healthy control. The results of other treatments were T17 (yield- 67.78 t/ha and 75,308 millable canes), T6 (yield- 64.99 t/ha and 72,222 millable canes), T27 (yield- 62.22 t/ha and 77,777 millable canes), T4 (yield- 59.96 t/ha and 74,691 millable canes), T15 (yield- 59.25 t/ha and 74,074 millable canes), T13 (yield- 59.07 t/ha and 73,839 millable canes), T28 (yield- 53.24 t/ha and 70,987 millable canes), T35 (yield- 52.77 t/ha and 70,370 millable canes) andT14 (yield- 50.12 t/ha and 71,604 millable canes). The fig. 1 revealed that seed treated with different isolates shows good results except isolate T18 interms of plant growth and height as compared to the untreated seeds and can be used for plant growth promotion. Muthukumar *et al.*, (2011) study reveals that treating seeds of chilli with *Trichoderma* culture filtratewas effective in inhibiting *P. aphanidermatum* growth which resulted in an increase in germination percent, root length, shoot length and vigour index. Similar kind of study was conducted by Srivastava, (2006) to test the two isolates *Trichoderma harzianum* (T 24) and *Trichoderma viride* (T 6) for growth promotion and enhancing the yield of the variety CoS 94257 and the isolates were found significantly effective in improving germination (6-14%), tiller population (21-78 %), millable canes (5-30 %) and yield (6-

38%) over the control incane of variety CoS 94257. Metabolites @ 2.5 % were found to be significantly more efficient then spore suspension (10^6 conidia/ml) for both the species (Srivastava, 2006). Singh *et al.*, (2004) selected two potent isolates of *Trichoderma viride* and *Trichoderma harzianum*, on the basis of higher efficacy against red rot pathogen *in vitro* which were used for improving the growth and yield of sugarcane plant and ratoon.

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