Original Research Article

Isolation and characterization of plant growth promoting rhizobacteria *Enterobacter hormaechei* and their suppression efficacy against *Colletotrichum falcatum* in combination with chitosan

ABSTRACT

Aims: This study aimed to explores the suppression efficacy of plant growth promoting rhizobacteria (PGPR) *Enterobacter hormaechei*, chitosan and its oligomers either singly or in combination on red rot disease causing pathogen *Colletotrichum falcatum* in sugarcane.

Methodology: The study was conducted to isolate twenty nine bacteria from sugarcane rhizosphere and investigate their potential for plant growth activities. Selected isolate PSC3 was characterized by biochemical and molecular identification by 16S rRNA sequencing. The study was further preceded for *in vitro* screening of plant growth promoting traits viz., production of Indole-3-acetic acid (IAA), hydrogen cyanide (HCN) ammonia (NH_3) production and antifungal activity against *C. falcatum*.

Results:

Among twenty nine isolates strain PSC3 showed highest plant growth promoting traits viz., indole-3-acetic acid, hydrogen cyanide, ammonia production and antifungal activity against *C. falcatum* among other isolated strains. Nucleotide 16S rRNA sequence analysis using clustalW program revealed that isolate PSC3 showed phylogenetic affiliation and maximum homology (99%) with *E. hormaechei*. Antifungal activity of chitosan, chitooligosaccharides (COS) and *E. hormaechei* were checked by inhibition of *C. falcatum* mycelial radial growth. Among three treatments of chitosan, COS and chitosan + *E. hormaechei*, two treatments showed significant antifungal activity ($P<0.05$). Chitosan treatment showed radial growth range from 2.5±0.07 to 1.9±0.03 cm against *C. falcatum* in comparison with control (9.1±0.09cm). The significant growth inhibition 79.0% was observed in chitosan at concentration 0.6% but the combination of chitosan with PGPR *E. hormaechei* PSC3 showed highest growth suppression of *C. falcatum* (86.8%) whereas fungal treated with only *E. hormaechei* showed growth radial inhibition 41.3%.

Conclusion: The findings reveal that chitosan and *E. hormaechei* have significant effect on *C. falcatum*. This new antifungal combination may be help to prevent red rot disease in sugarcane.

Keywards: *Enterobacter hormaechei*; *Colletotrichum falcatum;* Sugarcane; Chitosan, Chitooligosaccharides; Antifungal activity.

1. INTRODUCTION

Plant growth-promoting rhizobacteria (PGPR) are plant-associated microorganisms that are known to induce plant defenses and confer beneficial effects such as increased plant growth and low susceptibility to diseases caused by pathogens[1]. Therefore, their use as biofertilizers or control agents for agriculture improvement has been a focus of numerous researchers[2]. PGPR have been proven to counteract the activities of other harmful soil borne microorganisms, thus promoting plant growth[3]. Some PGPRs also elicit physical or chemical changes related to plant defense, a process called ‘‘induced systemic resistance’’ (ISR)[4]. ISR confers plant resistance against a large variety of attackers such as pathogens and herbivores[5].

The red rot caused by *C. falcatum* Went is the most ruinous disease of sugarcane and a big menace to both cane growers and sugar industry[6]. Conventional control of disease depends on the use of chemical inputs and resistant varieties. Development of new variants of the fungus, health hazards and environmental pollution concerned with the excessive use of agro-chemicals have resulted in adopting the biological control using native strains of PGPR as a supplemental approach to minimize pesticide usage[7]. Certain strains of PGPR have been used as ingenious weapon to protect plants from various soil borne pathogens. These bio-antagonists adopt single or multiple mechanisms of action to suppress these pathogens which include antibiosis[8], production of iron chelators, secretion of hydrolytic enzymes, synthesis of hydrogen cyanide thus disease control can be obtained by applying bacterial cells or their metabolic products[9].

Chitosan is derived from chitin, a polysaccharide found in exoskeleton of shellfish such as shrimp, lobster or crabs and cell wall of fungi[10]. Chitosan, poly (1, 4)-2-amino-2-deoxy-β-D glucose is a deacetylation product of chitin, a polysaccharide second by the prevalence in nature after cellulose[11,12]. It is a nontoxic, biodegradable biopolymer of high molecular weight. Recent studies on chitosan have attracted interest for converting chitosan to oligosaccharides[13]. In this respect, chitosan oligosaccharides, because of their shorter chain length, display a reduced viscosity and are soluble in aqueous media at pH values close to neutrality, which increases their bioavailability and opens a wide

range of new potential applications[14]. Due to its properties, various studies shown that chitosan has antifungal and antibacterial activities in different diseases [15, 16].

In view of this, the focus of the work presented in this paper is directed towards isolation and identification of PGPR from sugarcane rhizosphere. Subsequently, *in vitro* screening of the potential antagonists that control red rot disease causing pathogen. Further, this research work proceeded to check antifungal activity of chitosan and their combination with *E. hormaechei*. Therefore such type of study is necessary as it advocates that use of PGPR as inoculants or biofertilizers association with chitosan is an efficient approach to replace fungicides.

2. MATERIALS AND METHODS

2.1 Processing of soil samples for isolation of Phosphate solubilizing microrganisms

Phosphate solubilizing rhizobacteria (PSB) were isolated from sugarcane rhizospheric soil by dilution plate technique using Pikovskaya's medium[17]. Appropriate soil dilutions were plated on Pikovskaya's agar medium by spread plate technique and incubated at 30 ± 1 ºC for 2-3 days. The colonies forming halo zone of clearance (Pikovskaya's medium) around them were counted as P-solubilizers.

2.2 Morphological and Biochemical characterization

The efficient PGPR were identified on the basis of morphological, physiological and biochemical characteristics according to the standard methods described in Bergey's manual of systematic bacteriology[18] and laboratory manual of basic microbiology[19].

2.3 Molecular characterization of efficient strains

Molecular characterization of most efficient bacterial isolates was done by sequencing of their 16S rRNA gene. Bacteria PSC3 showed efficient plant growth promoting mechanism among the all other strains. Molecular characterization of bacteria PSC3 has been completed after DNA isolation of selected bacteria PSC3 followed by quantification of DNA sample; amplification of DNA by using bacterial specific primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492 R (5'-TACGGTTACCTTGTTACGACT-3'); choosing the PCR product based on concentration and processed for sequencing. Sequences have been submitted to NCBI GeneBank by Sequin.

2.4 Detection of Indole-3-acetic acid (IAA) production

Indole acetic acid production was quantitatively measured by the method given by Gordon and Weber (1951)[20]. Bacterial cultures were grown in a nutrient broth amended with tryptophan (5mM) for 3-4

days. Cultures were centrifuged at 10,000 rpm for 20 min. Two ml of supernatant was mixed with two drops of orthophosphoric acid and 4 ml of Salkowski reagent. Pink colour indicates presence of IAA.

2.5 HCN production

All isolates were subjected for the production of hydrogen cyanide (HCN) by amending 4.4 g glycine/ l media. Whatman No.1 filter paper was soaked in 2 % sodium carbonate and 0.5% picric acid solution was placed in the upper lid of the plate. The plates were sealed with parafilm and incubated at 28 ± 30°C for 5 days. The formation of orange to red colour indicates the production of hydrogen cyanide[21].

2.6 Siderophore assay

The isolates were screened for the siderophore production by adapting the universal methods explained by Schwyn and Neilands (1987)[22].

2.7 Detection of ammonia production

Qualitative detection of ammonia production was done by the method given by Bakker and Schippers, (1987)[23]. Bacterial isolates were grown in peptone water for 2-3 days at optimum growth temperature. After incubation, 1ml of Nessler's reagent was added in each tube. Tubes showing faint yellow color indicated small amount of ammonia, and deep yellow to brownish color indicated maximum amount of ammonia.

2.8 Peptone dextrose agar media preparation:

The experiment was conducted 20.0 g potato, 2.0 g dextrose, 2.0 g agar were mixed in 100 ml distilled water in a conical flask and was make a air tight with the cotton plug and wrapped with silver foil. And it is placed in the autoclave for 1 hour at 121^0C at 15 lbs. Subsequently, it was taken out from the autoclave and allowed to cool for solidify down in the laminar air flow.

2.9 Preparation of chitooligosaccharides

The enzymatic method was used for preparation of COS from chitosan [24].

2.9.1 Immobilized papain preparation

Chitin flakes (1.0 gm) were suspended in phosphate buffer (20 ml, 0.1 M, pH 6.5) and added 5mM cysteine; 2mM ethylene diamine tetraacetate (EDTA); 17.5 mg freeze-dried papain (EC3.4.22.2). The solution was kept at 5°C for 15 min in refrigerator. Then, 5% glutaraldehyde (3.1 ml) was added and the suspension was kept under mild stirring at 5°C for 14 h. The chitin–papain was filtered and washed with the same phosphate buffer trice, then stored in distilled water[24,14].

2.9.2 Activity determination method of papain for chitosan

An immobilized papain (3 gm is equals to 28.5 mg papain) was added to chitosan solution (1%, 10 ml) prepared by acetate buffer (0.1 M, pH 4.0). The suspension was kept under mild stirring at 45^0C for 1 h. The viscosities of the solution before and after reaction were determined by viscometer at 20°C. Under these conditions, the papain activities decreased the viscosity of substrate chitosan.

2.9.3 Determination of Chitosan and chitooligosaccharides content

The COS contents were determined by 3, 5-DNS colorimetry[25].

2.9.4 UV-Vis Spectroscopy

UV-Vis spectra of chitosan derivatives are usually recorded in aqueous acid (acetic acid) solutions in a 1.0 cm quartz cell at ambient temperature. The Diffuse Reflectance UV-Visible (DRUV) spectra of powdered or film samples are measured. Analysis in the vacuum ultraviolet through the near-infrared range has also been applied.

2.10 Effect of Chitosan on mycelia radial growth

Antifungal activity was determined by a radial hyphal growth of *C. falcatum*. Mycelium Growth Inhibition *in vitro* was performed on growth medium treated with 0.2%, 0.4%, 0.6% chitosan, COS concentration and combination of *E. hormaechei*. After 48 hr of incubation, agar piece of uniform size (diameter, 8 mm) containing fungi were simultaneously inoculated at the centre of each petri dish containing the various concentration of chitosan followed by incubation at 25 ± 2° C for 14 days. After incubation of fungi on culture medium containing chitosan, radial growth of fungal mycelium was recorded. Radial inhibition was calculated when growth of mycelia in the control plate reached the edge of the petri dish. The fungicidal effect to growth of fungi, in terms of percentage inhibition of mycelial growth was calculated by using the formula % inhibition = dc – dt/dc x 100 Where dc = Average increase in mycelial growth in control, dt = Average increase in mycelial growth in treatment[26].

Statistical Analysis & Preparation of Data

All the treatment data were statistically evaluated with SPSS/16.00 software. Hypothesis testing methods included one way Analysis of Variance (ANOVA) followed by LSD's test. $P<0.05$ was considered to indicate statistical significance. All the results were expressed as mean ± S.E. for the 3 replicate in each treatment.

3. Results and Discussion

3.1 Characterization of Chitooligosaccharides

Chitosan treated with papain releases COS. COS were preliminary confirmed by 3, 5-DNS method and with formation of brown coloured complex with sugars. The results showed that the viscosity of COS decreased upto 51.47% of the beginnings chitosan solution. This was also confirmed presence of COS.

3.2 UV-Vis Spectrum

Structure of COS was confirmed by UV-vis spectroscopy. UV-vis spectrum was recorded on Perkin Elmer Lambda 3B UV-vis spectrometer. Ultraviolet protection factor (UPF) was measured using UV Shimadzu 3101 PC spectrophotometer. UV-Vis spectra of chitosan derivatives are usually recorded in aqueous acid solutions in a 1.0 cm quartz cell at ambient temperature[8]. The Diffuse Reflectance UV-Visible (DRUV) spectra of powdered or film samples are measured[27]. Chitosan include various ratios of two far-UV chromophoric groups, N- acetylglucosamine (GlcNAc) and glucosamine (GlcN); as a result, their extinction coefficients for wavelengths shorter than approximately 225 nm are non-zero. Because GlcNAc and GlcN residues show no evidence of interacting within the chitosan chain, the monomer units contribute in a simple, additive way to the total absorbance of these polymers at a particular wavelength[28]. The UV spectra of mixtures of N-acetyl-glucosamine and glucosamine hydrochloride are quite similar to the spectra of chitosan, and the λmax is 201 nm in 0.1 M HCl solution UV-vis absorbance spectra of chitosan exhibits characteristic peak at 230 nm. After preparation of chitoligosaccharides, this peak undergoes a characteristic peak at range 360–348nm is which observed (Fig.1).

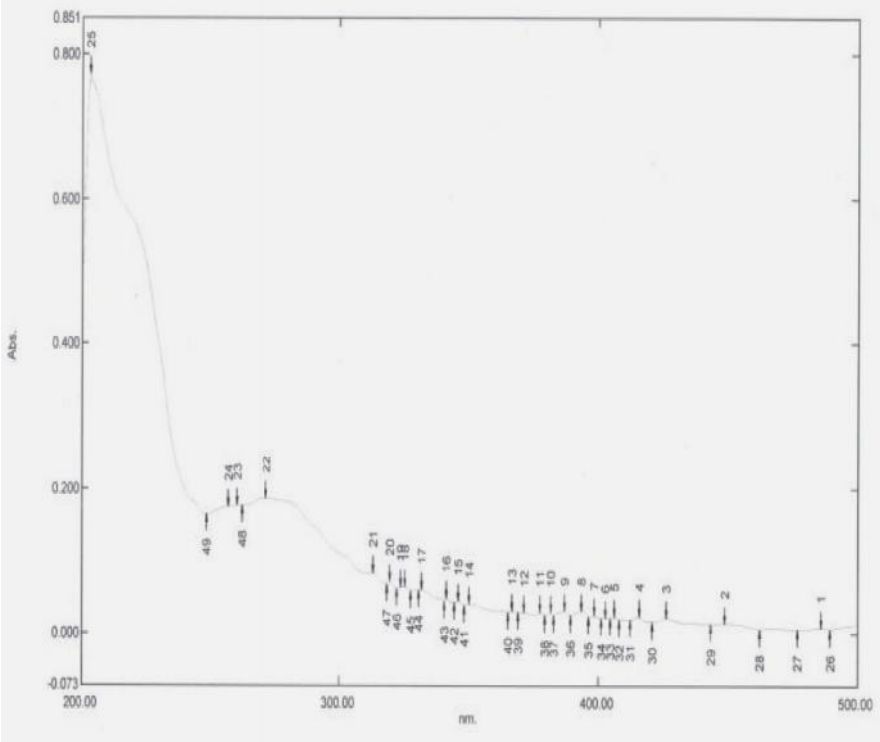


Fig 1: Characterization of chitooligosaccharides using UV- Vis spectroscopy

3.3 Isolation and Biochemical characterization of Isolates

The study was conducted to isolate PGPR from sugarcane rhizosphere and investigate their potential for plant growth activities. Twenty nine PGPR were isolated by serial dilution in selective media from two places of Uttar Pradesh. The study was further preceded for molecular identification of bacteria by 16S rRNA sequencing, and *in vitro* screening of plant growth promoting traits viz., production of Indole-3-acetic acid (IAA), Hydrogen cyanide (HCN) Ammonia (NH_3) production and antifungal activity against *C. falcatum*. Selected isolate was characterized by morphological, physiological and biochemical method. For identification and decipher their phylogenetic affiliation with bacteria, isolate was subjected to 16S rRNA (1492 bp long) gene sequencing. Nucleotide sequence analysis of test isolate using clustalW program revealed that isolate PSC3 showed maximum homology (99%) with *Enterobacter hormaechei*.

E. hormaechei strain is gram-negative rods which are motile, catalase positive, and oxidase negative and ferment D-glucose. The strain show negative Voges-Proskauer reactions. A detailed biochemical profiling of the isolate is given in Table 1. Acid is produced from the compound D-sorbitol.

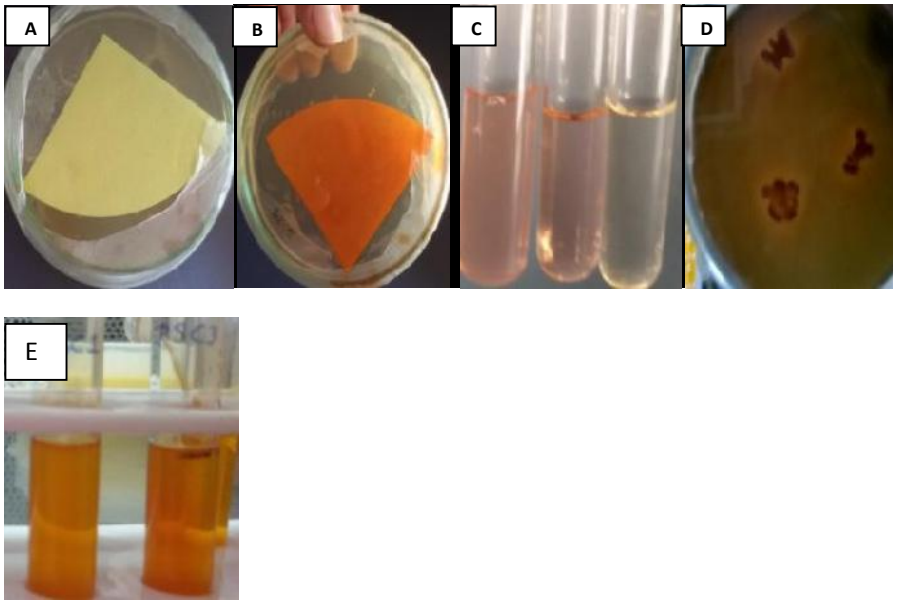


Fig 2: Screening of Plant growth promoting traits of *E. hormaechei* PSC3: (A and B) HCN production, (C) IAA production (D) siderophore production and (E) NH_3 production.

Table 1: Biochemical characteristics of *Enterobacter hormaechei* strain isolated from sugarcane rhizosheric soil

Biochemical test	***E. hormaechei* PSC3**	**Biochemical test**	***E. hormaechei* PSC3**
Colony shape	Irregular spreading	Methyl Red test	+
Colony colour	Yellow	Voges-Proskauer test	-
SIM (Motality)	+	Sucrose	-
Oxidase	-	D-Lactose	-
Catalase	+	Mannitol	-
TSI	R/R	D-Sorbitol	+
Citrate	+	Innositol	-
Nitrate	+	Maltose	-

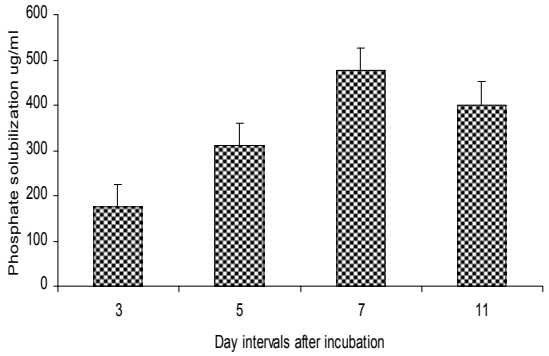
Gelatin	+	Dextrose	-
Starch	-	Galactose	-

191

192 **3.4 Evaluation of isolates for their Plant growth promoting activities and physiological traits:**

193 The phosphorus solubilizing activity was evaluated (Fig 3). Phosphate solubilizing activity of
194 bacterial isolates PSC3 showed the highest phosphate solubilizition efficiency 475.51 µg/ml at 7th day
195 of intervals and lowest at 3rd day of intervals. *E. hormaechei* KU196780 was showing plant growth
196 promoting activities like Indole-3-acetic acid production hydrogen cyanide production and ammonia
197 production (Fig 2).

198 PGPR isolate PSC3 grew up to 250 mM and none grew at 300 mM of NaCl conditions, but
199 the concentrations of 50 to 100 mM NaCl were critical as the isolate showed discriminatory
200 performances in these NaCl concentrations. At 50 mM NaCl, isolate exhibited very luxuriant good
201 growth comparison with other concentration. At 250 mM and 300 mM NaCl, the isolates show very
202 less or no any growth respectively. PEG of 20, 40 and 60 % were found high for the growth of isolate
203 PSC3 (Table 2). The isolate PSC3 showed greatest growth at pH 7.0, 9.0. There was no any growth
204 on pH 5.0, 11.0. The finding showed that this strain might be help in drought and saline stress in
205 plants.



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207 **Fig.3:** Quantification of Phosphate solubilization in g/ml by *E. hormaechei* PSC3 strains in different
208 day intervals (Data are expressed as mean ± SE, n=3)

209 **Table 2:** Plant growth promoting characteristics of *Enterobacter hormaechei* strain isolated from
210 sugarcane rhizospheric soil (+ Good, ++ Strong, +++ Very strong)

PGP traits	***E. hormaechei***	**Stress tolerance**	***E. hormaechei* PSC3**

	PSC3	traits	
Phosphate solubilization	+	pH	+
NH_3 Production	++	NaCl	+
HCN Production	+++	PEG	+
IAA Production	+++	Cu	-
Siderophore Production	+	Hg	-

211

212 **3.5 Molecular identification of isolate PSC3**

213 Molecular tools for the identification of soil bacteria were used and 16S rDNA gene analysis was
214 intensively used to understand the phylogenetic relationships. The accession numbers of the 16S
215 rDNA sequences is KU196780. Bacterial phylogenetic classification is based on sequence analysis of
216 the 16S rRNA molecule or its genes. For further identification at genus level, bacterial isolates were
217 identified through homology search with BLAST and FASTA using partial sequence of 16S rDNA[29].
218 Sequencing data showed that the isolates belonged to genus, *Enterobacter spp*. being a dominant
219 species. Nucleotide sequence analysis of test isolates using clustalW program revealed that isolate
220 PSC3 showed maximum homology (99%) with *Enterobacter hormaechei* (Fig. 5).

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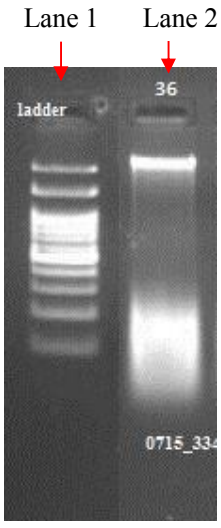

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224 **Fig. 4** : Agarose gel electrophoresis of the 16S rDNA PCR products of bacterial isolate .Lane 1: 1kb
225 DNA ladder; Lane 2: bacterial isolate PSC3

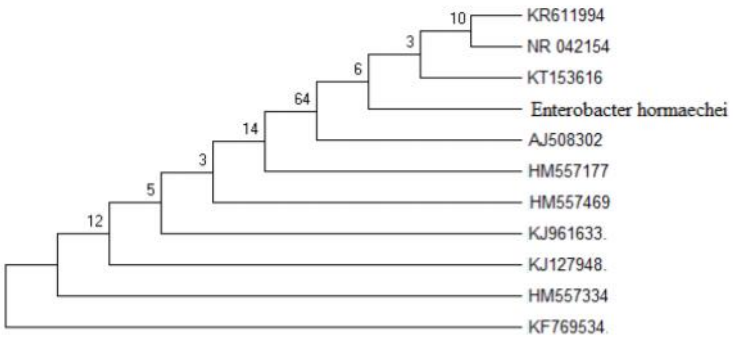

Fig 5: Neighbor-joining phylogenetic dendrogram based on a comparison of the 16S rRNA gene sequences of some of their closest phylogenetic taxa.

3.6 Growth Inhibition

Antifungal activity of chitosan, COS and in combination with *E. hormaechei* were evaluated based on the diameters of growth inhibition percentage against *C. falcatum*. If there is no inhibition, it is assumed that there is no antifungal activity. The validation of potential antifungal activity has been validated against known organisms, such as *C. falcatum*. *In vitro* prescreening showed noticeable antagonistic activity of isolate PSC3 against *C. falcatum* with a variable range of percentage inhibition. Fig.7 shows representative radial growth plates with red rot causing fungus *C. falcatum* after 7 day incubation. Initially we found the significant result of individual effect of chitosan, COS with 0.5 %, *E. hormaechei and antifungal drug clotrimazole* after 10 days of incubation (Fig.6). In which *E. hormaechei* showed 41.3 % growth inhibition of fungal pathogen *C. falcatum*. The radial growth inhibition of *C. falcatum* is larger in 0.6 % chitosan than other concentration 0.2%, 0.4% chitosan. These findings indicated that *C. falcatum* is more susceptible at the dose of 0.6%. But this 0.6 % chitosan is showing highest antifungal activity when combined with *E. hormaechei*. Fig 8 also showed highest growth inhibition in chitosan combination with *E. hormaechei*. Chitosan with various concentrations demonstrated effective inhibition on the fungi growth ($P < 0.05$). The average mean of radial growth is 1.87 to 9.13 cm against *C. falcatum*. Microscopic analysis of lactophenol blue stained fungal mycelia showed structural aberration in chitosan treated *C. falcatum* (Fig.9). These findings support the fact that, virtually, all the agricultural soils possess some suppressive effect on various soil borne pathogens causing diseases in plants which may be because of the antagonistic activities of microbes existing in soil. This phenomenon is also known as “general suppression” or “general antagonism”. This may be possible due to production of HCN. Michelsen and Stougaard, 2012

reported that HCN is a secondary metabolite produced by many antagonistic *Pseudomonas* species[30]. He also found that production of HCN inhibited growth of hyphae of *Rhizoctonia solani* and *Pythium aphanidermatum*.

One way ANOVA analysis indicated significant difference among the treatments ($F_{3, 11}=2.298$ $P<0.05$). The treatment T2 (Chitosan 0.2%), T3 (Chitosan 0.4%), T4 (Chitosan 0.6%), showed significant result compare with T1 (Control) somewhat treatment T4 showed greatest significant in this treatment. Which indicate 0.2% chitosan solution showing very efficient antifungal result against *C. falcatum.* Chitosan at the rate of 0.6 % showed 79.0% growth inhibition of *C. falcatum* (Fig.10). Also our study coincided with those of Meng *et al.,* 2012 who demonstrated that Chitosan and COS had stronger inhibitory effect on mycelia growth of two fungal pathogens *A. kikuchiana* and *P. piricola*[31]. Numerous studies on antifungal activity of chitosan against plant pathogens have been carried out and reviewed[32]. Chitosan's inhibition was observed on different development stages such as mycelial growth, sporulation, spore viability and germination, and the production of fungal virulence factors. It has been commonly recognized that antifungal activity of chitosan depends on its molecular weight, deacetylation degree, pH of chitosan solution and, of course, the target organism. Mechanisms proposed for the antifungal activity of chitosan focused mainly on its effect on fungal cell wall[33] and cell membrane[34].

Analysis of variance was used to determine whether levels of significant with chitosan treated in *C. falcatum* fungal strain different among control. The analysis showed no significant difference among the treatment ($F_{3, 11}= 3.89$ $P<0.05$). The treatment T2 (COS 0.2%), T3 (COS 0.4%), T4 (COS 0.6%), showed significant result with other treatment somewhat treatment T2 showed lowest radial growth in this treatment which indicate 0.2% showing no efficient antifungal result against *C. falcatum fungal* (Fig.10).

Chitosan and its derivatives offer a great potential as natural biodegradable nontoxic substances which have anti-microbial and eliciting activities. In the present study, the Chitosan was showing antifungal activity and highly effective in managing the red rot disease in sugarcane. The 0.6% of Chitosan was showing significant dose compared control. The Chitosan was showing more effect than COS. That indicate chitosan was efficient antifungal agent and highly effective in managing the complication associated with red rot disease. The 0.2% chitosan and COS was showing significant dose compared 0.2%, 0.4%., 0.6%. The study revealed that chitosan was

effective in inhibiting mycelial growth of *C. falcatum*. However, when compared to chitosan and *E. hormaechei* is relatively more effective than chitosan (Table 3). Furthermore, our results indicated that both chitosan and plant growth promoting rhizobacteria were effective in controlling diseases caused by *C. falcatum*.

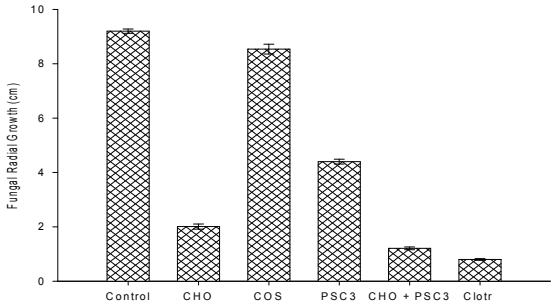

Fig.6 Growth inhibition of *Colletotrichum falcatum* in presence of initial chitosan, COS, *E. hormaechei* and their combination (Data are expressed as mean ± SE, n=3)

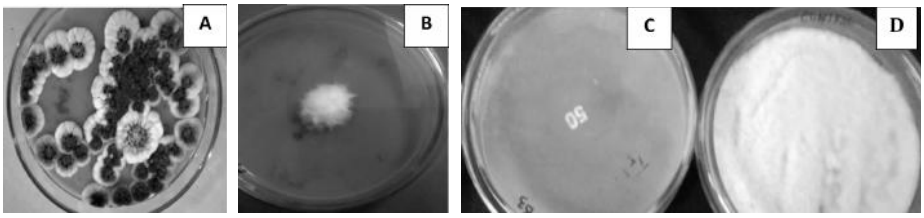

Fig.7 Growth of *Colletotrichum falcatum* in presence of initial screening of different concentration of chitosan after 7 days. (A) 0.1% Chitosan (B) 0.5% Chitosan(C) 1.0% Chitosan (D) without chitosan control

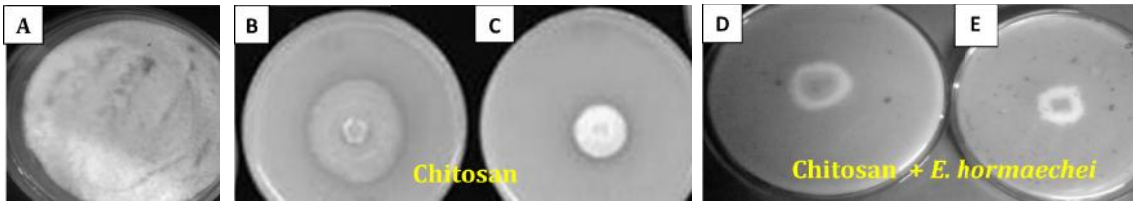

Fig.8 Growth of *Colletotrichum falcatum* in presence of chitosan and chitosan with *E. hormaechei* and their combination after 7 days of inoculation (A) Control, (B) 0.2% Chitosan, (C) 0.6% Chitosan,(D) 0.2% Chitosan with *E. hormaechei,* (E) 0.6% Chitosan with *E. hormaechei*

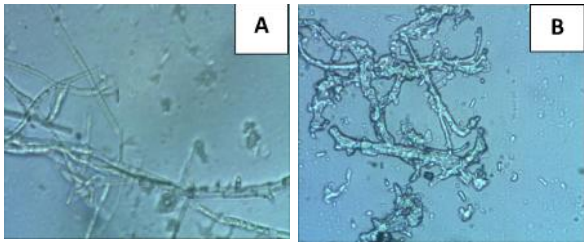

Fig.9 Microscopic analysis of lactophenol blue stain mycelia (a) Control (b) Chitosan treated mycelia showing deformed structure.

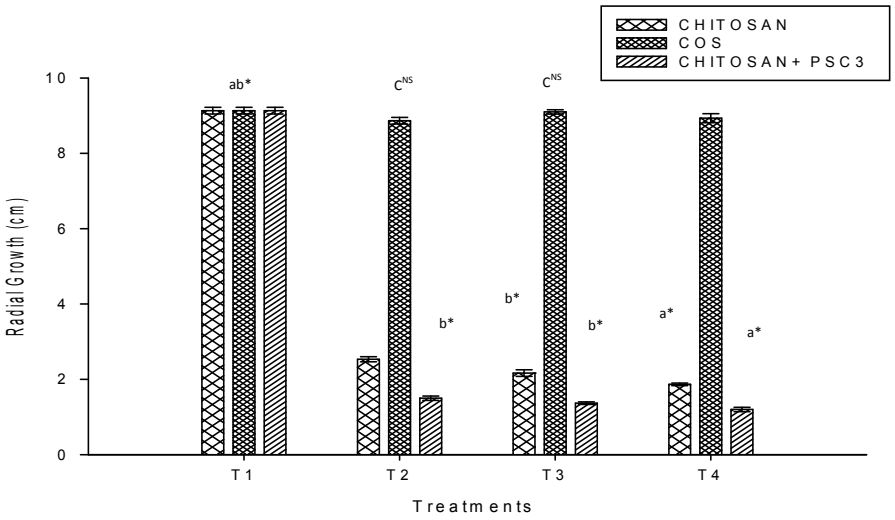

Fig.10 Antifungal Activity of different concentration of chitosan against *Colletotrichum falcatum* (Data are expressed as mean ± SE, n=3).

Values are statistically significant at *p<.05. Significance determined by ANOVA was compared within the treatments as follows: a Control vs 0.6% chitosan and chitosan+ *E. hormaechei*; b 0.2%, 0.4%, 0.6% Chitosan vs. Control and ^{CNS} Not significant

Table 3. Percentage of radial growth inhibition of *Colletotrichum falcatum*

Concentration of / COS Chitosan	**Growth Inhibition % by Chitosan**	**Growth Inhibition % by COS**	**Growth Inhibition % by Chitosan + *E. hormaechei***
0.2 % Chitosan/COS	71.3%	2.7%	83.5%
0.4% Chitosan/COS	76.1%	1.0%	84.9%
0.6% Chitosan/COS	79.0%	3.0%	86.8%

4. Conclusion and Future Prospects

This present research findings proved that this study is helpful for developing new biocontrol combination from chitosan and plant growth promoting rhizobacteria for managing fungal diseases and associated complications. The study reveals that chitosan solution and their concentration have significant effect of antifungal activity but their combination with plant growth promoting rhizobacteria *E. hormaechei* showed greatest growth inhibition of *C. falcatum* (86.8%). The chitosan and *E. hormaechei* seems promising for the development of a new formulation for fungal infection in plants.

A further investigation of the best antifungal result of chitosan and *E. hormaechei* like time of application, concentration, combination with other components, physiological changes in plants and molecular mechanism are needed and provide future line of work for controlling red rot disease of sugarcane for sustainable agriculture.

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