The Journal of Rural and Agricultural Research Vol. 14 No. 2, 70-74 (2014)
Received July 2014; Acceptance October 2014

Synergistic effect of chitosan and *Trichoderma viride* against *C. paradoxa*, the causal agent of pineapple disease in sugarcane

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Abstract

Pineapple disease is one the major disease in sugarcane. Normally chemical pesticides and fertilizers are used for controlling this disease but they have negative consequences on soil and plant in long run. In this disease we have tried to show that biological agents can be used successfully to reduce the effect of C.paradoxa *on sugarcane. We have used chitosan and* Trichoderma viride *against C.paradoxa, the causal agent of the pineapple disease in sugarcane. It was observed that chitosan and* Trichoderma viride *could control the growth of* C.paradoxa *up to 84%.*

Keywords- chitosan; *Trichoderma viride; C.paradoxa*; pineapple disease; potato dextrose agar

Introduction

Sugarcane is one of the most important commercial crops of the tropical and sub-tropical countries in the world. It contributes nearly 70% of the world's sugar production. Sugarcane is prone to more than 150 diseases caused by many fungal bacterial, viral, phytoplasma, nematode pathogens as well as abiotic factors right from planting to harvest. Since, sugarcane is vegetatively propagated (*i.e.,* through setts) and more than a yearlong crop, there are more chances of it being exposed to these pathogens. They are estimated to cause a loss of 10-25 per cent annually throughout the world, amounting to millions of loss of dollars (Mohan Raj *et al*, 2002). In India about 100 diseases of sugarcane have been reported so far. One of the disease affecting sugarcane production is the Pineapple Disease caused by *Ceratocystis paradoxa*. The disease was first reported from Java (Indonesia) by Went, who named it the Pineapple disease because the affected setts emit a smell resembling that of the mature pineapple fruit, which is due to ethyl acetate formed by metabolic activity of the pathogen. The disease has been reported to cause 15-20 per cent losses in sett germination and 10-15 tons losses per hectare in yield in India. The disease are both sett borne and soil borne affecting germination at early stages of planting and enters stalk pieces cut for seed through the cut ends of the seed pieces and can reduce cane yield by 31-35 %. Pineapple disease primarily affects sugarcane setts in the 2-3 weeks of sugarcane planting, fungus affects sugarcane planting mainly through the cut ends and from there spreads rapidly through the parenchyma, the parenchyma then breaks down and the interior of the setts become hollow and blackened. Setts affected by pineapple disease may decay before buds germinates or young shoots may die back shortly after emergence.

Pineapple disease can result in crops having a patchy, uneven appearance. When severe, the disease may seriously reduce the germination over large areas. *Ceratocystis paradoxa* occurs mainly in heavy textured soil and in poor drainage fields and it reduces the germination by 47% if left untreated. *C. paradoxa* is white in color in its early stages and later it turns to dark brown, it has round or oval spores... Bio-control of plant pathogen involves the use of biological processes to reduce the inoculums density of pathogen and to maintain their soil population below the disease threshold level. This reduces crop losses while interfering minimally with the ecosystem and damaging the environment. Bio-control measures are the best measure that can be taken to control pineapple disease of sugarcane. *Trichoderma* is a biological microorganism for controlling the soil borne plant pathogens and has been considered natural and environmentally acceptable alternative to the existing chemical treatment methods (Baker and Paulitz, 1996). The antagonistic activity of *Trichoderma* species against plant pathogen has been studied extensively (Sabalpara *et al*, 2009; Charati *et al*, 1998; Rangeshgwan and Prasad, 2000; Burns and Benson, 2000; Ushamalini *et al* 1997; Pandey and Upadhyay, 1999; Calistru, *et al*., 1997; Etabarian, 2006; Hjeliord, *et al*., 2001). *Trichoderma* inhibits the growth of other fungi by producing some antibiotics that penetrates into pathogen and release some enzymes that directly attack other fungi, thereby controlling disease. It is usually green or greenish brown in color. Chitosan have been

indicated for the preservation of foods juices and other material from microbial deterioration due their action against different groups of microorganisms, such as bacteria, fungi and yeast. Chitosan significantly affected both growth and toxin production at higher concentrations. In this study we have focused on observing the synergistic effect of Trichoderma and chitosan against *C.paradoxa*.

Methodology

Isolation of C. pardoxa

1. Diseased setts (pineapple) of sugarcane collected from various fields were brought to the laboratory and washed thoroughly in running tap water.
2. These diseased specimens were blot dried and cut with sharp sterilized blade into small bits (5mm) keeping half healthy and half diseased portion intact.
3. These pieces were surfaces sterilized with 0.1% aqueous solution of mercuric chloride ($HgCl_2$) for one minute and then washed by giving three changes with sterile distilled water to remove traces of mercuric chloride, it was then blot dried.
4. The surface sterilized diseased pieces were then inoculated on the solidified and cooled PDA (Potato dextrose Agar) medium in petri plates under aseptic conditions of Laminar-air-flow cabinet.
5. Inoculated plates were then incubated in BOD incubator at 28 ± 2^0C temperature. Seven to ten days after incubation, the well-developed mycelial growth free from any contaminant was obtained.
6. Following single hyphal-tip technique, the fungus was transformed/sub-cultured aseptically onto the PDA slant in test tubes.
7. Through frequent sub-culturing, the fungus was purified and pure culture was maintained on agar slants in test tubes stored in refrigerator for further studies.

Isolation of Trichoderma viride

For isolation of Trichoderma species soil samples were taken, sample I was diluted 10^5 times then 0.5ml of dilution was taken from each tube and was inoculated. All the plates were incubated for 5 days, after 5 days we checked for the pure culture, pure culture was again inoculated on PDA medium for better growth and purification.

Comparison of the growth of *C. paradoxa* on addition of chitosan, acetic acid and derivatives of chitosan in media

- PDA was prepared by adding 3.9gms of PDA and 1gm agar to 100ml of d/w
- Similarly PDA media with six different concentration of chitosan/acetic acid i.e. 5%,7.5%,10%,15%,20% and 25% were prepared, each having 3.9g of PDA,1g agar and 95ml,92.5ml,90ml,85ml,80ml,75ml of d/w respectively
- Each media was autoclaved for 15minutes at 121^0C
- Media was poured into petriplates, plates were kept for some time so that media may get solidify
- After solidification *C. paradoxaa* was inoculated on each plate
- Plates were kept for 7 days to record the observation.

Comparison of *Trichoderma* spp. and *C. paradoxa* on media with different concentration of chitosan or acetic acid

- 100ml PDA was prepared by adding 3.9gms of PDA and 1gm agar to 100ml of d/w
- Similarly PDA media with six different concentration of chitosan/acetic acid i.e. 5%,7.5%,10%,15%,20% and 25% were prepared, each having 3.9g of PDA,1g agar and 95ml,92.5ml,90ml,85ml,80ml,75ml of d/w respectively
- Each media was autoclaved for 15minutes at 127^0C
- Media was poured into petriplates, plates were kept for some time so that media may get solidify
- After solidification *C. paradoxa* and *Trichoderma* spp. were inoculated on each plate by cork borer method. They are inoculated on the opposite sides of the plate with 2mm gap from the edge
- Plates were kept for 7 days to record the observation

Results and Discussion

Present studies on the pineapple (*Ceratocystis paradoxa*) of sugarcane (*Saccharum officinarum*) were undertaken on the aspects *viz*., isolation, effect of chitosan, derivatives of chitosan and acetic acid and bio agents like *Trichoderma* spp. The results obtained on all these aspects are being presented in the following paragraphs.

Effect of different concentration of chitosan against Ceratocystis paradoxa

A total of six concentration of chitosan were used in the experiment. Results (Table 1) revealed that all the concentration of chitosan tested exhibited a varied range of radial mycelial growth of the test pathogen (PLATE I), depending upon their concentrations used and it was decreased with increase in concentrations of the chitosan tested.

The result (Table 1 and Fig. 1) indicated that the chitosan concentration of 25 ml/ ltr. of water significantly reduced the mycelium growth (MG) of 3rd and 7th day. The observed growth was 9.00 mm on 3rd day and 41.67 mm on 7th day, mean mycelium growth was 25.33 mm and control's growth was 88.33 mm and 90.00 mm on day three and seventh respectively. However, the highest per cent inhibition of mycelium growth was recorded for 25 ml/ ltr. of water (71.25 %).

Table 1: Effect of different chitosan concentration against *Ceratocystis paradoxa*

Treatment	Treatment Details	MG of 3rd days (mm)	MG of 7th days (mm)	Mean (mm)	% Inhibition of 3rd days	% Inhibition of 7th days	Mean (mm)
T-1	5 ml chitosan/ ltr. of water	86.00	90.00	88.00	2.75	0.00	1.37
T-2	7.5 ml chitosan/ ltr. of water	59.00	89.00	74.00	33.07	1.11	17.09
T-3	10 ml chitosan/ ltr. of water	52.00	89.00	70.50	41.07	1.11	21.09
T-4	15 ml chitosan/ ltr. of water	34.00	87.67	55.83	61.44	2.59	31.97
T-5	20 ml chitosan/ ltr. of water	24.67	77.00	50.83	72.09	12.22	42.15
T-6	25 ml chitosan/ ltr. of water	9.00	41.67	25.33	89.80	53.70	71.75
T-7	Control	88.33	90.00	89.16	0.00	0.00	0.00
	S.E. ±	2.52	2.15	-	2.20	1.83	-
	C.D. @ 5%	7.65	6.54	-	6.67	5.52	-

Table 2: Effect of different concentration of acetic acid against

Treat-ment	Treatment Details	MG of 3rd days (mm)	MG of 7th days (mm)	Mean (mm)	% Inhibition of 3rd days	% Inhibition of 7th days	Mean (mm)
T-1	5 ml AA / ltr. of water	46.33	65.00	55.66	17.08	27.78	22.43
T-2	7.5 ml AA/ ltr. of water	41.83	53.33	47.58	25.06	40.74	32.90
T-3	10 ml AA/ ltr. of water	35.00	49.67	42.33	37.67	44.81	41.24
T-4	15 ml AA/ ltr. of water	17.00	53.33	35.16	69.03	40.74	54.88
T-5	20 ml AA/ ltr. of water	8.67	33.33	21.00	84.30	62.96	73.63
T-6	25 ml AA/ ltr. of water	8.00	14.67	11.23	86.43	83.70	85.06
T-7	Control	61.33	90.00	75.66	0.00	0.00	0.00
	S.E. ±	5.04	3.14	-	8.62	2.18	-
	C.D. @ 5%	15.31	9.53	-	26.16	7.55	-

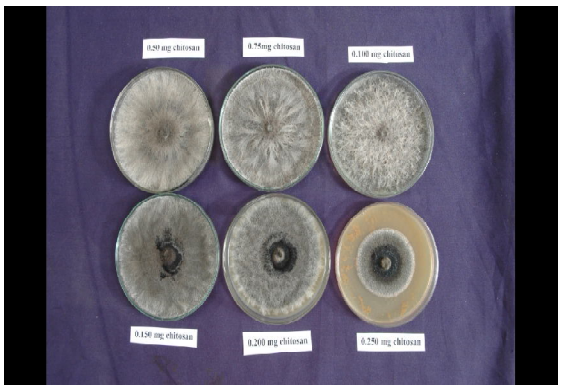

Fig. 1: Effect of chitosan against *C. paradoxa* after 7th days of inoculation

Effect of different concentration of acetic acid against C.paradoxa

A total of six concentration of acetic acid were used in the experiment. Results (Table 2) revealed that all the concentration of acetic acid tested exhibited a varied range of radial mycelial growth of the test pathogen (PLATE III), depending upon their concentrations used and the growth was decreased with increase in concentration of the acetic acid.

The result (Table 2 and Fig. 2) indicated that the acetic acid concentration of 25 ml/ ltr. of water significantly reduced the mycelium growth (MG) of 3rd days and 7th days i.e. 8.00 mm and 14.67 mm, also mean 11.23 mm and control 61.33 mm and 90.00 mm respectively. However, the highest per cent inhibition mycelium growth was recorded in 25 ml/ ltr. of water (85.06%). The synergistic effects (Table 4 and Fig. 4) of acetic acid and *Trichoderma* spp. against *C. Paradoxa* the lowest mycelium growth were recorded in treatment of 25 ml/ ltr. of water in 3rd and 7th days observation (9.00 mm and 15.00 mm) and control (50.33 mm and 89.33 mm respectively). However, the highest percent inhibition mycelium growth was recorded in 25 ml/ ltr. of water (83.63 %).

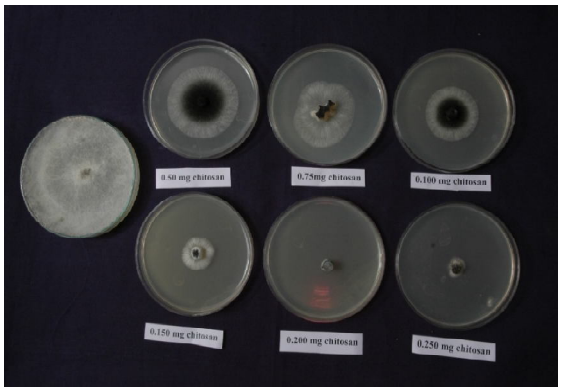

Fig. 2: Effect of acetic acid against *C. Paradoxa*

Table 3: Synergistic effects of chitosan and *Trichoderma* against against*Ceratocystisparadoxa*.

Treat-ment	Treatment Details	Mycelium Growth(mm)	%Inhibition of MG(mm)
T-1	5 ml chitosan/ ltr. of water	86.00	3.72
T-2	7.5 ml chitosan/ ltr. of water	85.33	4.49
T-3	10 ml chitosan/ ltr. of water	67.33	24.60
T-4	15 ml chitosan/ ltr. of water	54.00	39.56
T-5	20 ml chitosan/ ltr. of water	36.00	59.68
T-6	25 ml chitosan/ ltr. of water	14.00	84.34
T-7	Control	89.33	0.00
	S.E. ±	2.22	2.39
	C.D. @ 5%	6.75	7.26

Synergistic effect of chitosan and Trichoderma

Same procedure as given above was followed except acetic acid was replaced with chitosan and Trichoderma. Chitosan and Trichoderma were placed opposite to each other on a plate and it was observed that the synergistic effect was greater than the individual effects. 84.34 percent inhibition of mycelial growth was observed Table 3 and Fig. 3.

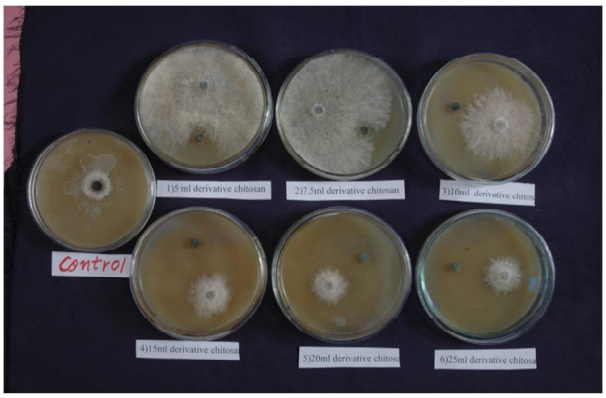

Fig. 3: Synergistic effect of chitosan and *Trichoderma* against *C. Paradoxa*

Synergistic effect of acetic acid and Trichoderma *against* C. paradoxa *7th day*

Pineapple disease is mainly controlled by chemical pesticides and fertilizers all around the world but these chemical pesticides and fertilizers have negative impact on soil, hence, nowadays focus has been shifted to biological control of the disease. For biological control, *Trichoderma* spp. is used by various scientists for the control of this disease. *Trichoderma* spp. is used in combination with *some* individual species of the soil fungi *viz Aspergillus awamori, A. niger, Gliocladium virens, Penicillium citrinum, Trichothecium sp, Trichoderma glaucum, T. harzianum.* It happens because *Trichoderma* does not allow other fungi to grow, it acts as a parasite on other fungus thereby checking their growth. The colony interactions between the pathogen and the soil fungi were assessed and it was found that biological agents control the growth of *C. paradoxa* up to significant levels, porter (1924) and Diekinson and Broadman (1971). The mycelium of *T. koeningii, Gliocladium virens and T. viride were* found growing over the pathogen. The antagonistic properties of different species of *Aspergillus, penicillium, Trichothecium* and *Trichoderma* against different pathogens have also been reported. In this study we have used 1% chitosan in combination with *Trichoderma viride, Trichoderma viride* grew over *C. paradoxaa*. Chitin and chitosan are naturally occurring compounds that have potential in agriculture with regard to controlling plant diseases. These molecules display toxicity and inhibit fungal growth and development. They have eliciting activities leading to variety of defence responses in plants. So in this study we have used chitosan in combination with *Trichoderma viride* against *C. paradoxa* and we found better inhibition of the growth of *C. paradoxa.* It was noted that *C. paradoxa* grew at a faster rate in absence of *Trichoderma* and chitosan. *C. paradoxa* in the presence of chitosan and in the absence of

Table 4: Synergistic effects of acetic acid against against*Ceratocystis paradoxa*

Treat-ment	Treatment Details	MG of 3rd days (mm)	MG of 7th days (mm)	Mean (mm)	% Inhibition of 3rd days	% Inhibition of 7th days	Mean (mm)
T-1	5 ml AA/ ltr. of water	36.67	43.33	40.00	51.43	26.29	38.86
T-2	7.5 ml AA/ ltr. of water	30.67	37.67	34.17	57.77	38.67	48.22
T-3	10 ml chitosan/ ltr. of water	21.67	33.33	27.50	62.66	56.49	59.57
T-4	15 ml AA/ ltr. of water	18.67	31.33	25.00	64.87	62.81	63.84
T-5	20 ml AA/ ltr. of water	12.33	22.00	17.16	75.35	75.52	75.43
T-6	25 ml AA/ ltr. of water	9.00	15.00	12.00	83.22	82.02	82.63
T-7	Control	50.33	89.33	69.83	0.00	0.00	0.00
	S.E. ±	1.63	2.43	-	1.75	2.48	-
	C.D. @ 5%	4.95	7.39	-	5.31	7.55	-

Trichoderma also grew at a moderate rate and covered most of the portion of a 90 mm plate. Plate with least concentration of chitosan i.e. 5ml showed maximum growth and minimum growth was observed in the plate with highest concentration of chitosan i.e. 25ml of chitosan. Growth of *C. paradoxaa* was least when both chitosan and *Trichoderma* acted against it. Least growth was seen when grown in higher concentration of chitosan in PDA along with *Trichoderma*. Even *Trichoderma* could not grow to its extent in higher concentration of chitosan i.e. 20 ml and 25 ml chitosan showed very less growth of *Trichoderma* and neglible growth of *C. paradoxa*. Thus for efficiently controlling the growth of *C. paradoxa* 20 to 25ml of chitosan and *Trichoderma* can be used (Table 4).

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