PINEAPPLE DISEASE OF SUGARCANE: AN ASSESSMENT OF THREE T*richoderma Spp.* FOR USE AS POSSIBLE BIOCONTROL AGENTS

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Abstract

Sugarcane became a major foreign exchange earner for Nigeria. Pineapple disease, caused by Ceratocystis paradoxa, is however a limiting factor in its large scale cultivation. This study evaluates three *Trichoderma* spp, (*T. koningii, T. viridii and T. harzianum*) for bio-control of the disease. The laboratory study evaluated each *Trichoderma* sp against *C. paradoxa* separately using the dual culture technique in a potato dextrose agar growth medium. Three inoculation methods, simultaneous, 48 and 72 hours pre-inoculation of each *Trichoderma* sp were evaluated. The control plate was *C. paradoxa* only. The field study evaluated T. koningii, the most promising, at 10^5 and 10^7 conidia/ml as therapeutic and prophylactic agents. Experimental design was Randomized Complete Block with each plot measuring 4 m x 4 m and 20 setts/plot. The pathogen (10^7 spores/ml) and *T. koningii* were applied through spraying on cane setts. A total of 6 treatments, including the negative and positive controls were evaluated. Results showed that *T. koningii* at 72 hours pre-inoculation inhibited *C. paradoxa* mycelia growth significantly by 86.04% *in-vitro*, while its prophylactic application brought about rapid and significantly high germination percentages of 75.00 and 80.00 for TK 10^5 and TK 10^7 respectively. Percentage germination failure was significantly lowest (20.00) with prophylactic application of TK 10^7. The highest value recorded for seedling establishment (98.00%), lowest disease incidences and severities (0.00%) from the 4th to 6th months after planting were all recorded for TK 10^7 prophylactic application. *T. koningii* at 10^7 is therefore recommended as prophylactic agent against pineapple disease of sugarcane.

Keywords: Pineapple disease, Sugarcane, *Ceratocystis paradoxa, Trichoderma* spp, Bio-control.

Introduction

Amongst the many diseases afflicting sugarcane, Pineapple disease is one of the most devastating, Talukder, *et al.*, (2007). The term pineapple disease is derived from the characteristic smell of a matured pineapple associated with infected cane setts. (Chhama, *et al*., 2014). This smell is due to the production of ethyl acetate from metabolic activities of the causative organism of the disease, *Ceratocystis paradoxa*. (Chhama, *et al*., 2014). The disease is also sometimes referred to pineapple sett rot or simply sugarcane sett rot. (Yadahalli, 2006) *C. paradoxa*, is soil and sett borne (Raid, 2013) and infection usually start from the cut end of setts or wound created by insect pests on standing cane.(Wickramasinghe,

et al., 2015) The parenchyma tissue is degraded by the pathogen, causing it to turn brown initially and black as infection becomes more severe (Plate 1). The incidence of the disease has been reported in almost all sugarcane growing regions of the world (Raid, 2013) with devastating effect on setts germination, seedling establishment and cane yield. In Nigeria, very little information is available on the disease, owing largely to the fact that the cultivation of sugarcane is on small holding basis and is restricted to only a few states in the Northern parts of the country. Record shows that only 3 % of national sugar need is produced locally (Okwy, 2015). The current downturn in the price of crude oil and its adverse effect on Nigeria's economy is a wakeup call on the need for diversification and exploration of other sectors that can generate employment and provide the much needed foreign exchange required to execute pressing needs.

Agriculture has the potential to generate foreign exchange for the economy. Sugarcane is one crop that holds so much promise with the numerous uses into which the crop can be put. The Dangote groups of company recently established several hectares of sugarcane farms in some states in the northern part of the country, while the National Cereal Research Institute (NCRI) is currently carrying out field trials on several improved varieties of sugarcane across all agro-ecological zones of the country. It is hoped that in no distant future, the cultivation of sugarcane will assume a commercial scale. It therefore becomes imperative to have information on diseases associated with the crop. This well help in adopting the best agronomic practices that will prevent disease outbreaks, crop failure and yield loss that are associated with disease attack on crops. This study was design to supply such information on pineapple disease.

In sugarcane growing countries of the world, the control of sugarcane sett rot is mainly through the use of synthetic fungicides. The problems associated with this practice have however necessitated the search for safe, cost effective and environment friendly alternatives. Biological approach to plant disease control is an emerging trend. The effectiveness of *Trichoderma* spp as bio-control agents for the control of fungal disease of crops is widely reported in literatures. (Talukder, *et al*., 2007; Sonja, *et al*., 2013 and Febri, *et al*., 2013) The *Trichoderma* ssp evaluated in this research work were *T. hazianum*, *T. viride* and *T. koningii.*

Plate 1: Sugarcane sett infected with pineapple disease.

Study location

The study was carried out at the Department of Crop, Soil and Pest Management, Department of Microbiology and the Teaching and Research Farm, all at The Federal University of Technology, Akure (FUTA). Ondo state, Nigeria.

Materials and methods

a. Laboratory study;

Preparation of growth medium and isolation of *Ceratocystis paradoxa*: Potato Dextrose Agar (PDA) was prepared following standard procedure while *C. paradoxa* was isolated following the method described by Ajayi *et al*., 2016.

Isolation of *Trichoderma* spp: *T. viride* was isolated from dead wood. Small segments measuring 7 mm x 7 mm were cut out from a dead log obtained from the Teaching and Research Farm of FUTA. The segments were inoculated on PDA in 5 Petri-dishes at two segments/plate. Incubation at room temperature was done for 24 hours, after which sub-culturing was done to obtain a pure culture of the organism.

T. harzianum and T. koningii were obtained from the pathology unit of the International Institute of Tropical Agriculture, (IITA) Ibadan, Nigeria.

Evaluation of *Trichoderma spp* against *C. paradoxa in-vitro*

The dual culture method was employed. Exactly 5 mm agar discs from one week old cultures of *C. paradoxa* and each of the *Trichoderma* spp were inoculated at opposite ends on the same growth medium. The three *Trichoderma* spp. were evaluated separately against *C. paradoxa* and the evaluation process involved three inoculation methods; (i) Simultaneous inoculation of *C. paradoxa* and each *Trichoderma* sp (one at a time) at opposite ends of PDA in a Petri-dish separately (ii) Inoculation of *Trichoderma* sp for 48 hours before the introduction of *C. paradoxa* (iii) Inoculation of *Trichoderma* sp for 72 hours before the introduction of *C. paradoxa*. The control treatment was made up culture medium consisting of *C. paradoxa* only. Each treatment was replicated thrice. A total of 30 Petri- dishes were used and treatments were laid out in a Completely Randomized Design (CRD). Incubation was done at room temperature for 9 days.

Preparation of spore suspensions of C. paradoxa and T. koningii

Spores were dislodged from 9 days old cultures of both organisms with a bristle brush separately after adding 50 ml sterile distilled water to fully grown and well sporulated cultures in each Petri-dish. Pieces of agar discs and mycelia were filtered off with sterile muslin cloth. With the aid of a haemocytometer slide estimation and concentration of spores of C. paradoxa to 107/ml was done, while the conidia of T. koningii was concentrated to 105/ml and 107/ml. Spore and conidia concentrations were arrived at based on previous research findings.

b. Field study

This was conducted at the Teaching and Research Farm FUTA. The most promising *Trichoderma* sp from the laboratory study, *T. koningii*, was evaluated. Two concentrations of 10^5 conidia/ml and 10^7 conidia/ml were evaluated separately as therapeutic and prophylactic agents.

Therapeutic evaluation of *T. koningii*

Exactly 60 cane setts were infected with the pineapple disease pathogen by spraying 10^7 spore suspension of *C. paradoxa* on them. The setts were then kept in a polythene bag for 72 hours, to generate warmth and moisture required for spore germination and initiation of

pathogenesis, after which infected setts were sprayed with 10^5 conidia/ml suspension of *T. koningii*. Sprayed setts were kept in a new polythene bag for 24 hours before planting. The same procedure described above was repeated for 10^7 conidia/ml suspension of *T. koningii* on a second set of 60 sugarcane setts.

Prophylactic evaluation of *T. koningii*

The same procedure described for therapeutic evaluation was adopted. *T. koningii* conidia suspension was however sprayed first on cane setts, with the spraying of *C. paradoxa* occurring 72 hours later. Planting was also done after 24 hours of infection with spore suspension of *C. paradoxa.*

The positive and negative controls consisted of 60 cane setts sprayed with sterile distilled water and another 60 setts sprayed with 10^7 spore suspension of *C. paradoxa* respectively. Both were kept in separate polythene bags for 96 hours before planting.

Treatments evaluated

T 1 = Positive control. (Cane setts sprayed with sterile distilled water only)

T 2 = Negative control. (Cane setts sprayed with 10^7 spore suspension of *C. paradoxa* only)

T 3 = Therapeutic application of 10^5 conidia/ml suspension of *T. koningii*

T 4 = Therapeutic application of 10^7 conidia/ml suspension of *T. koningii*

T 5 = Prophylactic application of 10^5 conidia/ml suspension of *T. koningii*

T 6 = Prophylactic application of 10^7 conidia/ml suspension of *T. koningii*

Land preparation and field layout

The total land area was 31m x 16m = $496m^2$. After ploughing and harrowing, it was divided into three blocks/replicates consisting of 6 plots per block. Each plot was 4m x 4m. Exactly 1 m. spacing was observed between plots and blocks. The borders were also 1m.

Planting of cane setts/cultural practices

Twenty cane setts in 4 rows of 5 setts/row were planted in each plot. The setts were spread out evenly on each row and the rows were 1 m apart. Planting was done in shallow grooves of about 2cm depth. Earthning up, to prevent lodging, was done at 4 months after planting, while NPK 15:15:15 fertilizer was applied at the rate of 120kg/ha. in split application. At planting and 6 months after planting. Weeding was done manually as required.

Data collection

Data were collected on the following parameters;

Germination: Counting of sprouted setts was done once in a week from the 3rd to the 5th week after planting. The values obtained for each treatment weekly were converted to percentage sett germination using the formula;

$$Percentage\ sett\ germination = \frac{Number\ of\ germinated\ setts}{Total\ number\ of\ setts\ planted}\ x\ \ 100$$

The values obtained at 5 weeks after planting was taken as overall percentage germination for each treatment.

Germination failure: The number of un-germinated setts per plot was counted at 5 weeks after planting. The values obtained were converted to percentage germination failure using the formula;

$$Percentage\ germination\ failure = \frac{Number\ of\ ungerminated\ setts}{Total\ number\ of\ setts\ planted}\ x\ 100$$

Seedling establishment: The number of seedlings stand in each plot at 2 months after planting were counted and converted to percentage seedling establishment using the formula;

$$Percentage\ sedling\ establishment = \frac{Nunber\ of\ established\ seedlings}{Number\ of\ germinated\ cane\ setts}\ x\ 100$$

Disease incidence: The numbers of cane stands showing symptoms of pineapple disease in each plot were counted each month from the 2nd to 6th month after planting. The values obtained for each plot was converted to percentage disease incidence using the formula;

$$Percentage\ disease\ incidence = \frac{Number\ of\ infected\ stands\ in\ each\ plot}{Total\ number\ of\ stands\ in\ the\ plot}\ x\ 100$$

Disease severity: The severity of infection in each plot was estimated once in a month through visual observation from the 2nd to the 6th month after planting. A disease rating scale of 0-5 was developed as follows;

0 = No symptoms of infection

1 = 1-25% of leaf area showing symptom of infection

2 = 26-50% of leaf area showing symptom of infection

3 = 51-75% of leaf area showing symptom of infection

4 = 76% and above of leaf area showing symptom of infection

5 = Death of cane

Percentage disease severity in each plot was calculated using the formula;

$$DSisease\ severity = \frac{Sum\ of\ disease\ rating\ in\ each\ plot}{Total\ stands\ sampled\ x\ Maximum\ rating\ scale}\ x\ 100$$

Statistical analysis

All data collected were subjected to Analysis of variance (ANOVA) using Minitab version 17 software. Means were separated using Tukey test.

Results

Percentage inhibition of mycelia growth of *C. paradoxa* by the *Trichoderma* spp evaluated

In the *in-vitro* study, the highest percentage mycelia growth inhibitions of 80.37 and 86.04 at 48 hours and 72 hours pre-inoculation time intervals respectively were recorded for *T. koningii*. The two values were significantly higher than the others. The values recorded for *T. harzianum* and *T. viride* were not significantly different at both inoculation intervals. Table 1.

Effects of treatments on cane germination

Percentage germination increased progressively from the 3rd to the 5th week after planting for all the treatments. The best germination percentage (80.00) was however recorded for T6. at 5 weeks after planting. This value was significantly higher than the others. The lowest percentage germination of 30.00 was recorded for T2 at the same period. This was significantly lower than the others, except T3. Table 2.

Germination failure in each treatment

Significantly lowest germination failure was recorded for T5 and T6. The highest germination failure of 70.00% was recorded tor T2, while T3 gave 63.33%. The two values were not significantly different, but were significantly higher than the others. Fig 1.

Effect of treatments on seedling establishment

The highest value for seedling establishment with statistical significance (98.00%) was recorded for T6. This was followed closely by T5 with 94.12%. The lowest value of 62.50% was obtained from T2. Fig 2.

Effect of treatments on disease incidence

Table 3. Shows the values obtained for disease incidence in all treatments evaluated. T4 gave significantly highest value for disease incidence at 2 months after planting. At 3 and 4 months after planting however, T3 with 30.17% and 63.88 respectively was significantly higher than the others. By the 5th and 6th months after planting, T2 showed disease incidence values of 18.74% and 16.43% respectively. These values were significantly the highest for each month. Worthy of note also was the fact that disease symptoms manifested continuously in T2 up to the 6th month after planting, even though it had stopped in the other treatments.

Effect of treatments on disease severity

T2 gave significantly highest value for diseases severity at 2 months after planting. T1 and T2 were not significantly different at 4 and 5 months after planting, but at 6 months after planting, the highest value of disease severity, 11.04% was recorded for T2. This value was significantly different from the others. It is important to point out that disease severity continued till the 6th months after planting in T2, even though it has stopped in the other treatments. No definite pattern was noticed in disease severity as fluctuations in values were was the rule in all the treatments for the 6 months for which data were collected. Table 3.

Table 1: Percentage inhibition of mycelia growth by the bio-control agents evaluated

Bio-control agents	Inoculation time intervals		
	SIM	48 HRS	72 HRS
T. harzianum	55.80 a	66.02 a	68.80 b
T. viridii	45.49 b	58.03 b	73.06 b
T. koningii	40.00 b	80.37 a	86.04 a

Note. Means with the same letter on each column are not significantly different at 5% level of probability.

Legend

T1 = Positive control. (Cane setts sprayed with sterile distilled water only)

T2 = Negative control. (Cane setts sprayed with 10^7 spore suspension of *C. paradoxa* only)

T3 = Therapeutic application of 10^5 conidia/ml suspension of *T. koningii*

T4 = Therapeutic application of 10^7 conidia/ml suspension of *T. koningii*

T5 = Prophylactic application of 10^5 conidia/ml suspension of *T. koningii*

T6 = Prophylactic application of 10^7 conidia/ml suspension of *T. koningii*

Table 2. Effect of treatments on cane germination (%)

Treatments	Weeks after planting		
	3	4	5
T 1	43.33 b	51.66 b	58.33 c
T 2	10.00 c	23.33 c	30.00 d
T 3	16.66 c	23.33 c	36.66 d
T 4	40.00 b	55.00 b	63.33 bc
T 5	56.66 a	73.33 a	75.00 ab
T 6	43.33 b	73.33 a	80.00 a

Note. Means with the same letter on each column are not significantly different at 5% level of probability.

Legend

T1 = Positive control. (Cane setts sprayed with sterile distilled water only)

T2 = negative control. (Cane setts sprayed with 10^7 spore suspension of *C. paradoxa* only)

T3 = therapeutic application of 10^5 conidia/ml suspension of *T. koningii*

T4 = therapeutic application of 10^7 conidia/ml suspension of *T. koningii*

T5 = prophylactic application of 10^5 conidia/ml suspension of *T. koningii*

T6 = prophylactic application of 10^7 conidia/ml suspension of *T. koningii*

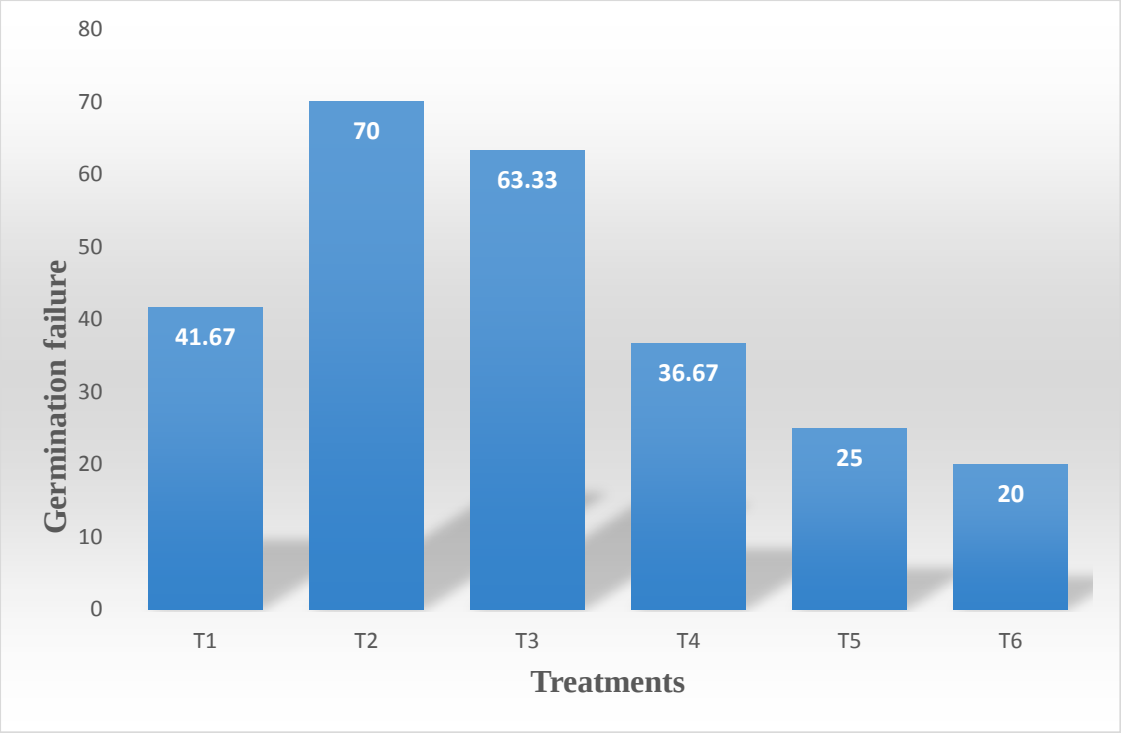

Fig 1: Percentage germination inhibition on sugarcane setts by *C. paradoxa*

Legend

T1 = Positive control. (Cane setts sprayed with sterile distilled water only)

T2 = Negative control. (Cane setts sprayed with 10^7 spore suspension of *C. paradoxa* only)

T3 = Therapeutic application of 10^5 conidia/ml suspension of *T. koningii*

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T6 = Prophylactic application of 10^7 conidia/ml suspension of *T. koningii*

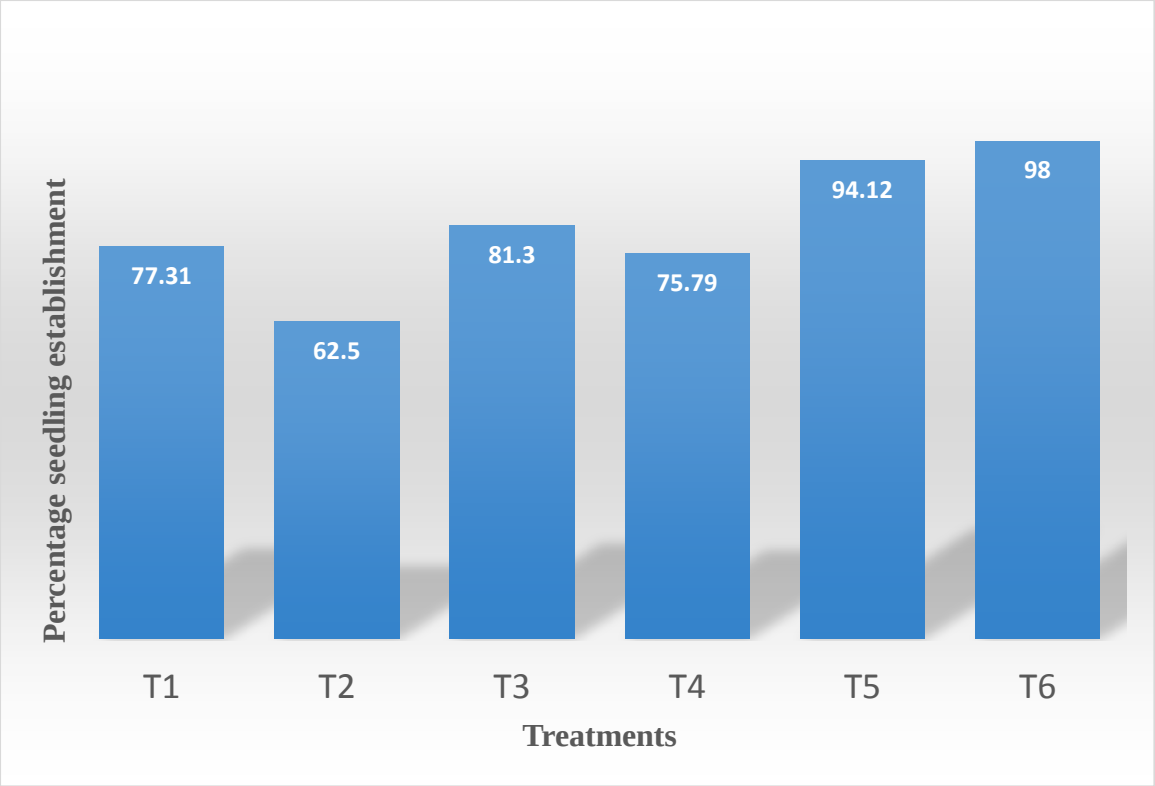

Fig 2: percentage seedling establishment

Legend

T1 = Positive control. (Cane setts sprayed with sterile distilled water only)

T2 = Negative control. (Cane setts sprayed with 10^7 spore suspension of *C. paradoxa* only)

T3 = Therapeutic application of 10^5 conidia/ml suspension of *T. koningii*

T4 = Therapeutic application of 10^7 conidia/ml suspension of *T. koningii*

T5 = Prophylactic application of 10^5 conidia/ml suspension of *T. koningii*

T6 = Prophylactic application of 10^7 conidia/ml suspension of *T. koningii*

Table 3: Effect of treatments on disease incidence

Treatments	Months after planting				
	2	3	4	5	6
T1	23.38 b	7.64 d	5.39 c	2.96 bc	0.00 d
T2	10.37 d	19.73 b	33.48 b	18.74 a	16.43 a
T3	17.45 c	30.71 a	63. 88 a	4.16 b	0.00 b
T4	49.33 a	0.00 f	4.86 c	1.38 cd	0.00 b
T5	13.82 cd	2.38 e	6.72 c	0.00 d	0.00 b
T6	16.89 c	17. 49 c	0.00 d	0.00 d	0.00 b

Note. Means with the same letter on each column are not significantly different at 5% level of probability.

Legend

T1 = Positive control. (Cane setts sprayed with sterile distilled water only)

T2 = Negative control. (Cane setts sprayed with 10^7 spore suspension of *C. paradoxa* only)

T3 = Therapeutic application of 10^5 conidia/ml suspension of *T. koningii*

T4 = Therapeutic application of 10^7 conidia/ml suspension of *T. koningii*

T5 = Prophylactic application of 10^5 conidia/ml suspension of *T. koningii*

T6 = Prophylactic application of 10^7 conidia/ml suspension of *T. koningii*

Table 4: Effect of treatments on disease severity

Treatments	Months after planting				
	2	3	4	5	6
T1	14.44 b	4.68 d	19.67 a	6.74 a	0.00 b
T2	19.40 a	7.02 c	21.07 a	7.02 a	11.04 a
T3	9.72 c	12.66 a	17.59 ab	0.83 b	0.00 b
T4	8.40 c	9.96 b	12.93 b	0.30 b	0.00 b
T5	5.77 c	1.95 e	13.41 b	0.00 b	0.00 b
T6	6.77 c	11.72 ab	0.00 c	0.00 b	0.00 b

Note. Means with the same letter on each column are not significantly different at 5% level of probability.

Legend

T1 = Positive control. (Cane setts sprayed with sterile distilled water only)

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T6 = Prophylactic application of 10^7 conidia/ml suspension of *T. koningii*

Discussion

Since Weindling (1932) discovered the antagonistic ability of *Trichoderma* spp about 84 years ago, extensive research work have been done on different species of the organism. Phytopathologists have found them to be partner in progress when it comes to biological control of plants diseases *in-vitro* and *in-vivo*. The severity of bean disease caused by *Sclerotium rolfsii* reduced significantly with the use of *T. harzianum.* (Elad, *et al.*, 1980). Also, Martinez-Alvarez, *et al*., (2012) reported a high degree of success in the control of *Fusarium circinatum* (the pitch canker pathogen) with the use of *T. viride in-vivo* and *in-vitro*. It was as expected therefore, that the three *Trichoderma* spp evaluated in this study exhibited between 58% - 86% inhibition on the mycelia growth of *C. paradoxa in-vitro* when they were inoculated at least 48 hours ahead of the pathogen. This may be due to the fact that

the biocontrol agents have become well established in the culture medium, making it better able to compete and exhibit antagonistic properties over the pathogen.

In the *in-vivo* study, *T. koningii* brought about improved sugarcane germination and seedling establishment when used prophylactically. Sonja, *et al.*, (2013) reported significant increase in seed germination seedling establishment and vigour of soybean with the coating of the seeds with three *Trichoderma* spp. A similar result was reported for rice when it was treated with using seven different isolates of Trichoderma. (Febri, et al., 2014). All isolate evaluated were found to increase rice germination significantly.

Enhancement of germination by *Trichoderma* spp has been reported to be due to cellulose degradation and production of phytohormones amongst others. (Febri, *et al*., 2014).

Improved growth, reduced disease incidence and severity were the other effect of *T. koningii* observed during this study. These have also been reported by previous worker. Talukder, *et al*., (2007) reported that *T. harzianum* brought about improved germination, growth and yield of sugarcane.

On a general note, the mode of action of *Trichoderma* spp is through the production of volatile antibiotics with the ability to supress pathogen growth. Other methods include mycoparasitism and competition. (Chet, 1987). Induced resistance of the host plant has also been identified. (Sharma, *et al.*, 2012).

Results from this study showed that the application of *T. koningii* at 10^7 conidia/ml brought about improved performance of sugarcane. The treatment is therefore recommended for use in the control of pineapple disease. Further research work can be carried out to unravel the particular mode action of *T. koningii* against *C. paradoxa*, and to determine the effects of *T. koningii* on sugarcane growth parameters and yield.

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