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Bio-control based integrated disease management of basal stem rot disease of coconut

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Basal stem rot, caused by *Ganoderma applanatum* and *G. lucidum,* is the most devastating disease accounting to severe yield loss in southern states of India. The pathogen first infects the root system where early detection of the disease based on morphological symptoms is impossible. Exudation of reddish brown viscous fluid from the basal portions of the stem is the first visible symptom of the disease in the affected palm followed by wilting of leaflets, production of sporophores, shrivelling of the stem and death of the tree.

The pathogen persists for longer periods in soil by producing various resting stages such as melanised mycelium, basidiospores and pseudosclerotia (Susanto *et al*., 2005). Among the chemicals, Tridemorph provides certain degree of control, but it adversely affects beneficial soil microorganisms.

The alternate approach to chemical control is the use of biological control agents or the use of resistant germplasm. Resistant germplasm to a particular disease, if available, provides better management of the soil borne diseases avoiding the hazardous chemical use and minimizing the cost of cultivation. Bio-control agents are another alternative for managing the destructive soil borne diseases of perennial crops such as basal stem rot disease of coconut (Srinivasulu *et al*., 2008) and oil palm (Nur Ain Izzati and Abdullah, 2008). They are largely non-phytotoxic, systematic and environment friendly in nature.

Trichoderma spp. have gained wide acceptance as effective bio-control agents against several phyto-pathogens. They have been successfully used for management of pathogens of various field crops and perennial crops such as oil palm (Nur Ain Izzati and Abdullah, 2008), peach (Schnabel *et al.,* 2011) and forest trees (Schubert *et al*., 2008). In addition to its cellulose and chitinase activity that disintegrates the cell wall of phytopathogens (Almeida *et al*., 2007), *Trichoderma* species synthesizes a variety of antibiotics, such as gliotoxin, viridine, trichodermin, *etc.* and also stimulate plant native defence mechanisms (Hibar *et al*., 2007) as well as plant growth and development. *T. viride* is the most suitable alternative for basal stem rot disease of coconut. Srinivasulu *et al.* (2008) reported that the fungal bio-agents *T. viride, T. harzianum* and *T. hamatum* were effective against the coconut pathogens, *G. applanatum, G. lucidum* and *Thielaviopsis paradoxa.*

In the present study, utilization of *T. viride* (50 g) along with neem cake (5 kg) was assessed on large scale in an area of 5 acres at two villages, Antarvedi and Kesanapalli of East Godavari district of Andhra Pradesh against basal stem rot disease during March 2012 to August 2013.

Native *T. viride* culture available at Plant Pathology Division of Horticultural Research

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Station, Ambajipet was used in the study. *T. viride* was sub-cultured and allowed to grow on Petri dishes containing potato dextrose agar medium for a period of seven days. One week old fungal mat from PDA plate was then inoculated into potato dextrose broth in conical flask or fermentor and incubated at 28^0C temperature for another 15 days. The mycelial mat thus obtained was checked for the spore load of $6x10^8$ concentration. It was then homogenized and mixed with talc powder at 1:2 ratios for talc based experiments. Carboxy methyl cellulose at the rate of 5 g per kg was added to the talc powder and the mixture was allowed to dry in shade at room temperature.

The treatment, 50 g of *T. viride* along with 5 kg of neem cake at yearly interval which was found to be the most effective against basal stem rot disease of coconut in the preliminary field trial from August 2010 to March 2012 combined with the integrated disease management practices was implemented in field demonstrations. The above said mixture at the rate of one kg $palm^{-1}$ $year^{-1}$ was applied to all the healthy palms in the land holding as a prophylactic measure as the pathogen initially infects the root system where the farmer is unable to notice the symptoms in the initial stages.

The bio-control based integrated disease management measures against basal stem rot disease of coconut involved the following steps: drip or basin method of irrigation, frequent watering or irrigation especially during summer months, avoiding injury or damage to roots, raising and ploughing *in situ* green manure crops, uprooting and destruction of diseased and dead palms along with the roots, isolation of diseased palms from healthy ones by digging isolation trenches of 1m depth and 0.5 m width, application of 50 g of *T. viride* in combination of 5 kg of neem cake to the diseased palms once in every year and application of the above said mixture at the rate of 1 kg to all the healthy palms.

Large scale demonstrations were conducted at two locations, Kesanapalli and Antarvedi villages of East Godavari District of Andhra Pradesh. Number of palms infected with the disease and disease spread in centimetres on the coconut trunk were recorded before the treatment imposition and at quarterly intervals after the treatment imposition. Diseased palms were selected in such a way that the treatment included palms with early, middle and advanced stages of disease. In addition, leaf number and nut yield before and after treatments from each palm at both the locations were recorded at quarterly intervals from March 2012 to August 2013.

The proportion of diseased palms before and after treatments was compared to see the effect of treatment on spread of the disease. Let p_1 and p_2 are the proportion of diseased palms before and after the treatment, then the test statistics is given by $z = (p_1 - p_2) / SE$, where $SE = sqrt\{ p * (1 - p) * [(1/n_1) + (1/n_2)] \}$, $p = (p_1 * n_1 + p_2 * n_2) / (n_1 + n_2)$, where n_1 and n_2 are the total number of palms before and after the treatment. For large values of n_1 and n_2, z follows a standard normal distribution and therefore z-test is used to compare the proportion of diseased palms in the pre and post treatment.The above test was also used to compare the palms under the control treatment. The mean spread of the disease (in cm) of affected palms of the pre-treatment was compared with the post treatment mean value using the paired t-test to see the effect of treatment on the spread of the disease.

Of the 16 treatments tested against basal stem rot disease in the preliminary field trial from August 2010 to March 2012 (involving *T. viride, Pseudomonas fluorescens*, neem cake as single or combination, as basal application or root feeding), a combination of 50 g of talc formulation of *T. viride* and 5 kg of neem cake was found effective in containing the spread of the disease (data not shown). This package was demonstrated in an area of 5 acres at two villages.

The coconut field at Antarvedi village was more infected (PDI of 35.0) with the basal stem rot disease when compared to the other garden at Kesanapalli village (PDI of 20.9). In Antarvedi, out of 297 palms, 104 palms were found infected with the disease with varied levels of disease incidence. Treatment imposition at the rate of 50 g of *T. viride* combined with 5 kg neem cake was applied to these 104 palms. The above said mixture at the rate of one kg per palm was applied to the remaining 193 healthy palms.The PDI before treatment application in April 2012 was 35.0 and it was reduced to 10.4 by the end of August 2013. Out of 104 diseased palms, only 31 remained diseased after one year of treatment and the other palms recovered from the disease (Fig. 1 & 2A). In control palms at Antarvedi,

Fig. 1. Reddish brown oozing symptom at the base of basal stem rot infected palm before treatment application

the initial PDI of 22 per cent was increased to 34 per cent by the end of August 2013 (Tables1 & 2).

In Kesanapalli, out of 230 palms, 48 palms were found infected with the disease with varied levels of disease incidence. Treatment imposition at the rate of 50 g of *T. viride* combined with 5 kg neem cake was applied to these 48 palms. The above said mixture at the rate of 1 kg per palm was applied to the remaining 182 healthy palms. The PDI before treatment application in April 2012 was 20.9 and it was reduced to 13.0 by the end of August 2013 (Fig. 2B). Out of 48 diseased palms, only 30 palms remained diseased after one year of treatment and the other 18 palms recovered from the disease. In control palms at Kesanapalli, the initial PDI of 18 per cent was increased to 34 per cent by the end of August 2013 (Tables 1 & 2). Yield performance of the palms in control remained the same over the treatment period.

The large scale demonstration showed significant disease reduction in implemented villages. Field effectiveness of *Trichoderma* spp. both as seed treatment and soil application was

Fig. 2. Drying of the reddish brown oozing symptom at the base of the treated palm at A) Antarvedi village and B) Kesanapalli village

Table 1. Per cent disease incidence of basal stem rot disease before and after 15 months of treatment imposition at Antarvedi and Kesanapalli villages

Name of the village	Before treatment (April 2012)			After treatment (August 2013)			Z -value
	Total no. of palms	Diseased palms	Proportion P_1	Total no. of palms	Diseased palms	Proportion P_2	
Antarvedi	297	104	0.35	297	31	0.10	4.23 **
Control	100	22	0.22	100	34	0.34	-1.89 *
Kesanapalli	230	48	0.21	230	30	0.13	1.02
Control	100	18	0.18	100	34	0.34	-2.58 **

* Significance at p=0.05; ** significance at p=0.01

Table 2. Linear spread (in cms) of basal stem rot disease on diseased palms before and after 15 months of treatment imposition

Village	No. of diseased palms	Mean spread in cm		t-value
		April 2012	August 2013	
Antarvedi	104	68.9	27.7	9.267**
Kesanapalli	48	70.1	47.2	4.889**

** significance at p=0.01

earlier reported in annual crops such as bean, tomato, peanut, rice, lettuce, sugarbeet, pea, brinjal, tobacco, sesame and okra and in perennial crops such as oil palm, peach and forest trees (Nur Ain Izzati and Abdullah, 2008; Schubert *et al*., 2008; Schnabel *et al.,* 2011).

The linear spread of the disease was recorded from all the palms at both the demonstration sites. After 15 months of treatment imposition, many of the treated palms at both the demonstration villages showed dried bleeding symptom on the stem. Some palms showed reduced disease spread whereas other palms showed no further spread of the disease. Hence, the number of palms showing dried bleeding symptom out of the treated palms were calculated and percent recovery of the palms was estimated. Further, the palms showing reduced linear disease spread or palms showing no further spread were calculated out of the treated palms in the villages.

Per cent recovery from disease at Antarvedi and Kesanapalli was recorded as 70.2 and 41.7, respectively. In addition, 7 out of 104 palms at Antarvedi and 18 out of 48 palms at Kesanapalli showed reduced disease spread on the stem (Table 3). Ten out of 104 palms at Antarvedi and 6 out of 48 palms at Kesanapalli showed no further spread of the disease symptom on the stem. Fourteen out of 104 palms at Antarvedi and four out of 48 palms at Kesanapalli showed increase in disease development (Table 3).

Around 66 per cent palms at Antarvedi and 43 per cent palms at Kesanapalli showed increased leaf foliage after 15 months of treatment imposition. The average pre-treatment yield of 415 and 300 nuts per acre at Antarvedi and Kesanapalli was increased to 900 and 635 nuts per acre at Antarvedi and Kesanapalli during August 2013 (Table 4). Re-isolation studies for *T. viride* in the treated gardens confirmed the presence of bio-control agent at both the demonstration sites.

The study revealed that basal application of *T. viride* in combination with neem cake has a very good potential to reduce the basal stem rot disease of coconut. The response of the palms to the treatment depended on the stage of the disease development, good agronomic practices and availability of soil mycoflora. The treatment was

Table 3. Effect of bio-control based integrated disease management package against linear spread of basal stem rot disease at Antarvedi and Kesanapalli

Stage of the disease development	Percentage of palms	
	Antarvedi	Kesanapalli
Palms with completely dried symptom on the stem	70.2	41.7
Palms with reduced disease spread on the stem	6.7	37.5
Palms with no further disease spread on the stem	9.6	12.5
Palms with increased disease spread on the stem	13.5	8.3

Table 4. Yield performance of the gardens before and after demonstration

Village	Nut yield (Number of nuts per acre)			
	Mar' 12	Sep' 12	Mar' 13	Aug' 13
Antarvedi	415	509	718	900
Kesanapalli	300	344	525	635

more effective when the application was carried out at earlier stages of disease development *i.e.,* when the bleeding patches were within 30 cm from the base of the stem. The success of *T. viride* in this study can be attributed to its mycoparasitism, production of antibiotics and efficiency in promoting plant growth and inducing plant defence mechanisms which was reported earlier with other phytopathogens. Harman *et al*. (2004) reported that depending upon the strain, the *Trichoderma* can provide numerous advantages such as colonization of the rhizosphere (rhizosphere competence), allowing rapid establishment within the stable microbial communities in the rhizosphere, control of pathogenic and competitive/deleterious microflora by using a variety of mechanisms, improvement of the plant health and stimulation of root growth. According to Rosado *et al.* (2007), the main factor for ecological success of this genus is a combination of very active mycoparasitism mechanisms and an effective defensive strategy induced in the plants.

Of the two alternate approaches to chemical control *i.e.,* use of biological control agents or the use of resistant germplasm, bio-control with *T. viride* proved effective against basal stem rot disease of coconut. In the absence of resistant lines against this pathogen, the present work has its own significance for managing the most destructive disease of coconut, basal stem rot, and paves the way for biological control of phytopathogens in other horticultural crops, as well.

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