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

A. Snehalatharani*, N.B.V. Chalapathi Rao, G. Ramanandam, H.P. Maheswarappa¹, C.T. Jose¹ and E. Padma

Horticultural Research Station, AICRP on Palms, Dr.Y.S.R. Horticultural University, Ambajipeta-533 214 Andhra Pradesh, India

¹ ICAR-Central Plantation Crops Research Institute, Kasaragod-671124, Kerala, India

(Manuscript Received: 09-07-15, Revised: 21-12-15, Accepted: 19-01-16)

Keywords: Basal stem rot, bio-control, coconut, Ganoderma wilt, Trichoderma viride

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 phytopathogens. 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 Kesana palli 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

*Corresponding Author: snehalatharani@gmail.com
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°C temperature for another 15 days. The mycelial mat thus obtained was checked for the spore load of $6 \times 10^8$ 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, Kesana palli 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 Kesana palli 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,
the initial PDI of 22 per cent was increased to 34 per cent by the end of August 2013 (Tables 1 & 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
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 of palms | Total no. of palms | Diseased palms | Proportion of palms |
| 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

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 |
|----------------------------------|----------------------|
| Palms with completely dried symptom on the stem | Antarvedi: 70.2, Kesanapalli: 41.7 |
| Palms with reduced disease spread on the stem | Antarvedi: 6.7, Kesanapalli: 37.5 |
| Palms with no further disease spread on the stem | Antarvedi: 9.6, Kesanapalli: 12.5 |
| Palms with increased disease spread on the stem | Antarvedi: 13.5, Kesanapalli: 8.3 |

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...
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.

Acknowledgements

The authors gratefully acknowledge the Project Co-ordinator, AICRP on Palms and the Chairman, Coconut Development Board, Kochi, Kerala, India for funding the research. The authors also want to thank the colleagues for their support and useful discussion.

References

Almeida, F., Cerqueira, F., Silva, R., Uhoa, C. and Lima, A. 2007. Mycoparasitism studies of Trichoderma harzianum strains against Rhizoctonia solani; evaluation of coiling and hydrolytic enzyme production. Microbiology 153: 1734-1742.

Harman, G.E., Howell, C.R., Viterbo, A., Chet, I. and Lorito, M. 2004. Trichoderma species-opportunistic, avirulent plant symbionts. Nature Reviews Microbiology 2: 43-56.

Hibar, K., Daami-Remadi, M. and El Mahjoub, M. 2007. Induction of resistance in tomato plants against Fusarium oxysporum f. sp. radicis-lycopersici by Trichoderma spp. Tunisian Journal of Plant Protection 2: 47-58.

Nur Ain Izzati, M.Z. and Abdullah, F. 2008. Disease suppression in Ganoderma-infected oil palm seedlings treated with Trichoderma harzianum. Plant Protection Science 44: 101-107.

Rosado, I., Rey, M., Codon, A., Gonavites, J., Moreno-Mateos, M.A. and Benitez, T. 2007. QID74 cell wall protein of Trichoderma harzianum is involved in cell protection and adherence to hydrophobic surfaces. Fungal Genetics and Biology 44: 950-964.

Schnabel, G., Rollins, A.P. and Henderson, G.W. 2011. Field evaluation of Trichoderma spp. for control of Armillaria root rot of peach. Plant Health Progress doi:10.1094/PHP-2011-1129-01-RS.

Schubert, M., Fink, S. and Schwarz, F. 2008. Field experiments to evaluate the application of Trichoderma strain (T-15603.1) for biological control of wood decay fungi in trees. Arboricultural Journal 31: 249-268.

Srinivasulu, B., Nagamalleswari, Y., Aruna, K., Lakshmi, M.V., Kumar, K.V.K. and KrishnaSri, M. 2008. Scanning electron microscopy confirmation of mycoparasitism exhibited by Trichoderma spp. on coconut pathogens, G. applanatum, G. lucidum and T. paradoxa. Current Trends in Biotechnology and Pharmacy 2: 562-567.

Susanto, A., Sudharto, P.S. and Purba, R.Y. 2005. Enhancing biological control of basal stem rot disease (Ganoderma boninense) in oil palm plantations. Mycopathologia 159: 153-157.