Evaluation of *Trichoderma harzianum* Rifai treatment in comparison with fungicides for the management of stem canker of cocoa caused by *Phytophthora palmivora* (Butl.)Butl.

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A recent survey of cocoa growing areas in India revealed that cocoa stem canker caused by *Phytophthora* is a serious problem. With the expansion of cocoa growing and the increase in canker incidence it became imperative to develop ecofriendly and economically viable disease management strategies. Field trials were laid out for two years to develop disease management strategies for stem canker of cocoa. There were six treatments with three fungicides *viz.*, mancozeb, copper oxychloride and phosphorous acid, a biocontrol agent, *Trichoderma harzianum* and cultural practices alone. The treatments were applied only once during December. Though the different treatments except control did not vary significantly, phosphorus acid treatment was found to be better than all other treatments in controlling the disease. The number of active lesions 5 months after the treatment with phosphorous acid and *T.harzianum* did not vary much. Phosphorous acid (Akomin 40 @ 5ml/L. water) was applied to the wound caused after removing the bark from the canker lesion as well as spraying the whole plant. Coir pith cake seeded with *T.harzianum* (activated by soaking in water) was applied to the area where only 25cm^2 bark surface layer was removed. In addition, five *T.harzianum* cakes soaked in water were kept at equidistance in the soil around cocoa plants at a distance of 15 cm from the base of the stem and covered with dried leaves of arecanut and cocoa as mulch. Luxurious growth of *T.harzianam* covered the entire cake after 48 h and had spread to the infected internal tissue. Removal of infected tissues from the canker (cultural practices alone) without any other treatment was also found to be effective in reducing lesion size to some extent. But the number of active lesions after 5 months of this treatment was much higher than all other treatments. Though all the treatments were on par and significantly different from the control, treatment with *T.harzianum* is expected to be more effective in the long run as the number of active lesions after treatment was less and because this biocontrol agent can survive and multiply under the congenial microclimatic conditions in the garden, thereby suppressing the *Phytophthora* population in the cropping system.

Keywords: Cocoa, Stem canker, *Phytophthora*, *Trichoderma*

Cocoa (*Theobroma cacao* L.) is grown mainly in the southern states of India, *viz.*, Kerala, Karnataka, Tamil Nadu and Andhra Pradesh and covers 31885 ha, yielding 10560 metric tonnes of cocoa annually. With the recent expansion in cocoa cultivation and the increasing age of existing plantations, the incidences of diseases are also on the increase. *Phytophthora* diseases, especially black pod and stem canker, are the major causes of economic loss to cocoa growers. In India, stem canker of cocoa caused by *Phytophthora palmivora* was first reported in 1978 (ChandraMohanan 1978, 1080-1082). Since then the disease has been found to be increasing year after year. A recent survey of cocoa plantations in the southern states of India revealed that stem canker occurs in all 4 states with the highest incidence in Andhra Pradesh. It was observed in about 58 % of the gardens surveyed in the four states (Peter and ChandraMohanan 2010, 65-66 & 2011, 44-50). Though stem canker of cocoa is a lethal disease, very little work has been done on the management of the disease. Considering the severity and importance of the disease an attempt was made to develop an eco-friendly disease management practice.

Materials and Methods

To develop proper management practices for stem canker disease of cocoa, field trials were laid out in the Dhakshina Kannada district of Karnataka State, one of the major cocoa growing areas in India where stem canker of

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cocoa was first reported. Separate field trials were laid out during 2009 -10 and 2010-11 in cocoa- arecanut (*Areca catechu* L.) mixed gardens where cocoa is grown at a spacing of 2.7 x 5.4 m as a mixed crop between arecanut palms. These gardens were irrigated at intervals of 5-7 days during dry periods. Stem canker lesions were generally observed after the south - west monsoon (June to September). Field and plant hygienic practices such as removal of fallen branches and pods and proper pruning were carried out in all the plots during May-June in both years. In addition, black pods and mummified pods were also removed and buried outside the garden in November. Treatments were applied only once during December. The following treatments were assessed:

- Removal of all visible infected tissue from canker lesions
- Removal of infected tissue + wound dressing, as well as spraying the whole plant with phosphorous acid (Akomin 40 @ 5 ml/ L water).
- Removal of infected tissue + wound dressing with mancozeb (Indofil M 45 @5g/ L water)
- Removal of infected tissue + wound dressing with copper oxychloride (Blitox 50 WP 0.5 %).
- Removal of outer layer of bark from 25 cm^2 area of the lesion and keeping T. harzianum seeded coir pith cake (5 cm diameter) presoaked in water on wound.
- Control (sprayed the whole plant with water).

Each treatment was replicated four times with three plants per replication in a randomised complete block design.

T. harzianum culture was mixed with sterilized coir pith, compressed and dried to prepare a cake formulation (www.ipindia.nic.in- Ref.: *The Patent Office Journal*, No. 50, 2011). Before application the fungus was activated by soaking in water and the cake was tied to the wound from where only the outer layer of the bark was removed from the lesion. To keep the *Trichoderma* seeded cake in position it was covered and tied with a thin layer of arecanut leaf sheath. In addition, 5 seeded cakes were kept in the soil around the cocoa plants at a distance of 15 cm from the base of trunk and covered with dried leaves of arecanut and cocoa as mulch. The colony forming units (CFUs) of *T.harzianum* in the canker lesion tissue were determined after one month of the treatment.

Pre-treatment disease incidence was recorded by measuring the size of external and internal lesions. After measuring the area of external lesions in all the treatments except *Trichoderma* and control treatments, the external layer of bark from each lesion was scraped out to expose the reddish brown infected internal layer of bark and to measure the internal lesion area. Based on visual observation of the discoloured area, the infected tissue was removed as far as possible after measuring the internal lesion size. The lesion size in the internal tissues of the bark in *Trichoderma* treatments and control plots was measured by scraping out the outer layer of bark from an area of 0.5 to 1.5 cm^2, depending on the lesion size from the periphery of the external lesion at two points opposite each other length and breadth-wise (4 points) in smaller lesions and from 8 equidistant points around the periphery of bigger lesions. Thus the inner layer of bark was exposed at 4-8 places in the periphery of external lesion to observe the advancing margin of the internal lesion. To find out the effect of treatments, post-treatment lesion size was measured after 5 months. For this the outer layer of bark was removed from around the treated wound in all treatments except the control and the area of the active lesion whenever observed was measured. In the control plot the sizes of external and internal lesions were measured and recorded as post-treatment disease incidence. The effect of the treatments was determined by comparing the lesion size in the inner bark tissues before and after treatments. Post-treatment data of internal lesion spread were analysed statistically. Pair-wise comparison of treatments was done using Duncan's Multiple Range Test (Duncan 1955, 1-42). The lesions which were active after 5 months of the treatment (determined

by visual observation of the spread of the lesion) were also recorded.

Results and discussion

Canker lesions on the cocoa stem can be detected only after the rainy season. This may be due to the difficulty in differentiating a lesion in its initial stages from the dark colour of the bark of cocoa. Stem canker appeared on different parts of the tree including jorquette and fan branches. The symptoms on the surface of bark can be detected only by close examination. The size and shape of external lesions as well as internal symptoms on bark varied. External symptoms appeared as a grayish-brown water soaked lesion with a broad dark brown to black margin. A reddish brown liquid oozing out from such lesions dried and formed a rusty deposit (Gregory 1974). Based on studies on the symptoms of the disease occurring in the Westcoast of India, different kinds of external symptoms have been reported. A dark brown, round to oval discoloration of the bark formed as a result of the exudation of reddish brown liquid from the point of infection was consistent symptom. Sometimes the lesions were water soaked and grayish brown. In severe cases, canker lesions coalesced to form larger lesions. Cankers at the collar region were bigger and spread faster. The collar infection appeared as a dark brown irregular water-soaked lesion with reddish brown liquid oozing out. The collar infection then spread to the taproot and main trunk

When the outer bark of a canker-infected portion of the stem was removed, the tissues beneath always showed a characteristic reddish brown color. Lesions in the internal tissues coalesced which led to extensive rotting. The infection spread from the cortical tissues to the vascular tissues and reached the wood. Wood infection appeared as a grayish-brown to black discolouration with black streaks. When canker girdled the stem, dieback occurred and the tree died. The spread of infection in the internal tissues of the bark was faster than the spread on the surface of bark (Rao and ChandraMohanan, 1993, 153-158 and 1995, 35-37). *P.palmivora* is the only species reported so far from India as the causal organism of stem canker of cocoa.

The data on post-treatment disease incidence in both years indicated that there was considerable reduction in lesion size in all the treatments. Though size of lesion in different treatments except in control did not vary significantly, removal of infected tissue and treating with phosphorous acid was found to be better than all other treatments in controlling the enlargement of canker lesion. The size of lesion expansion was the lowest in this treatment after 5 months. The area of lesion spread in the internal tissues after the treatments varied from 2.14 to 4.83 cm^2 and 2.60 to 4.91 cm^2 during 2009 and 2010 respectively (Table 1). Of the 12 plants treated with phosphorous acid, only 25 and 33.33 % of lesions were found to be active after 5 months during 2009-10 and 2010-11 respectively. The number of active lesions in *Trichoderma* and copper oxychloride- treated plants was also less (33.33 %) than the other two treatments. The number of plants with active lesions after 5 months of phosphorous acid and *Trichoderma* treatments did not vary much. Removal of infected tissue without any other treatment was also found to be effective in reducing the lesion size to some extent. But, in this treatment 42 and 67 % of the plants in 2009-10 and 2010-11 respectively showed active lesions after 5 months. When the plants were treated with mancozeb, 42 % of the plants exhibited active lesions in both years. Although removal of infected tissue without any other treatment was found to be effective to some extent, the chances of revival of the active canker tissue are more, which may lead to higher disease incidence. In the *Trichoderma* treatment there was dark- green thick, fluffy growth of *T.harzianum* covering the wet formulation 48 h after applications. *T.harzianum* was also found spreading from the cake formulation to the rotten internal bark and growing in the lesion. The CFUs of *T.harzianum* in the treated tissue after one month were found to be 16.83×10^6 / g tissue. It is expected that the *Trichoderma* treatment will be more effective if the treatment is continued annually so that there will be an increase in the *Trichoderma* population in the garden and on cocoa plants. As cocoa is mainly grown as a mixed crop in the existing coconut and arecanut gardens in India and the gardens are frequently

irrigated during the dry season, the microclimate in the cropping system may favour the survival and multiplication of *Trichoderma*. Moreover, in the *Trichoderma* treatment, only external layer of bark is removed from an area of 25 cm^2. Hence, this treatment is less labour-intensive compared to all other treatments.

The results of the phosphorous acid treatment and an earlier report (Guest et al. 1994, 479-492) indicate that this treatment can be further modified to make it more effective in the management of stem canker of cocoa. However, like Indonesian farmers who are reluctant to adopt trunk injection of phosphorous acid because of multiple wounding (McMahon and Purwantara 2004), Indian cocoa growers are also not in favour of injection. Non-availability of suitable stem injectors in the local market is also a constriant in recommending injection to farmers. Based on detailed studies on the effect of potassium phosphonate treatment on stem canker, McMahon et al. (2010, 170-175) reported a clear decrease in stem canker disease and average severity in the treatment with phosphonate after 4 months of the initial treatments. They also found that incidence decreased to negligible levels in phosphonate injected trees, indicating that cankers were decreasing substantially with the phosphonate treatment. The present study also revealed the effectiveness of phosphorous acid in reducing the severity and incidence of stem canker of cocoa caused by *P.palmivora*. But the *Trichoderma* treatment will be cheaper than the potassium phosphonate treatment, and offers more advantages such as the build up of *Trichoderma* population in the garden which, in turn, is expected to reduce not only canker incidence but also all other *Phytophthora* diseases such as black pod, chupon wilt, foliar infection and twig dieback, as well as soil phase of *Phytophthora*.

Table 1: Effect of *Trichoderma harzianum* Rifai treatment in comparison with fungicides for the management of stem canker of cocoa caused by *Phytophthora palmivora* (Butl.) Butl.

Sl. No	Treatments	2009-10			2010-11			Grouping based on DMRT* *
		Pre- treatment incidence		Post-treatment	Pre- treatment incidence		Post - treatment	
		External lesion size (cm^2)	Internal lesion size (cm^2)	Size of lesion spread (cm^2) internal	External lesion size (cm^2)	Internal lesion size (cm^2)	Size of lesion spread (cm^2) internal	
1	Removal of infected tissue (RIT)	52.44	62.19	4.83	50.83	59.88	4.91	a
2	RIT + Potassium phosphonate (0.5 %)	77.58	84.75	2.14	48.18	57.13	2.60	a
3	RIT + Mancozeb (0.5 %)	50.00	58.26	2.71	46.07	55.13	2.93	a
4	RIT + Copper oxychloride (0.5%)	41.38	51.25	2.93	44.87	54.55	2.66	a
5	Trichoderma cake	40.49	48.72	3.38	51.18	60.55	2.79	a
6	Control*	63.82	70.85	81.28	47.67	57.59	67.70	b

* Size of external lesion (Post treatment): 71.62 cm^2 (2010); 52.84 cm^2 (2011)

** Values of post-treatment lesion size followed by the same letter do not differ significantly (p=0.05) in Duncan's Multiple Range Test (DMRT)

Based on the results of the investigation, integrated disease management strategies have been developed for the economic management of stem canker disease of cocoa such as removal and destruction of canker affected dead plants, frequent removal and destruction of *Phytophthora*- infected pods, proper pruning of cocoa plants including the removal of *Phytophthora*- infected chupon and twigs and treatment of canker lesions with either phosphorus acid or *Trichoderma harzianum*.In general, fungicide treatment is not necessary if all other management practices including removal and destuction of infected tissues preferably in the initial stages of canker are systematically adopted. However *Trichoderma* treatment can be recommended as one of the components in the IDM as it has several advantages.

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