Occurrence of black rot in *Jatropha curcas* L. plantations in India caused by *Botryosphaeria dothidea*

Ch. Srinivasa Rao, M. Pavani Kumari, Suhas P. Wani* and S. Marimuthu

International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502 324, India

A new disease in *Jatropha curcas* L. plants was observed during the summer season in 2009 and 2010 in plantations in several Indian states, including Andhra Pradesh, Assam, Chhattisgarh and Madhya Pradesh. The outbreak of the disease coincided with the leaf-dropping (dormant) stage of the crop, which sets in with the increasing moisture stress. Affected *J. curcas* plants showed drying along with shrivelling, and discoloration of the stem with sticky reddish-brown exudation at the base of the plants. Black lesions (soft and rotting) on the stem under the bark and cambium layer were also observed. From the affected stem parts, fungal cultures were isolated and tested for their pathogenicity on pot-grown plants. The fungal isolates caused symptoms on *J. curcas* leaves and petioles (black spots, 1–3 mm in diameter). Then they spread to the stems causing shrivelling and gummosis of hard-wood stems, and finally led to the death of the infected plant. The causal fungus was identified as *Botryosphaeria dothidea* using microscopic observations of hyphae and spores, and internally transcribed spacers (ITS) sequencing technique. In addition, four other fungal isolates were also isolated from the affected tissues, which were identified as *Macrophomina phaceolina*, *Phomosis longicolla*, *Fusarium oxysporum* and *Alternaria alternata* using the ITS sequencing technique. The role of these fungal cultures, i.e. whether they grow as saprophytes on the affected dead tissues or have any role in causing the black rot disease, needs further study. Spraying *J. curcas* plants showing early symptoms of this disease with Bavistin (carbendazim 50% WP) at the rate of 2 g l^{-1} water controlled the spread of the symptoms and led to the recovery of plants with new leaf growth after the rains.

Keywords: Black rot, *Botryosphaeria dothidea*, drought-affected plants, dry season, *Jatropha curcas*.

JATROPHA CURCAS L. (physic nut), a large shrub reaching to a height of 5 m, belongs to the family Euphorbiaceae[1]. Although native to Central America, *J. curcas* is widely distributed in tropical and sub-tropical regions of Africa and Asia[2,3]. *J. curcas* is drought-tolerant, having a unique adaptation mechanism for moisture-stressed situations, during which it sheds all leaves to avoid transpiration and remains dormant – and then gives rise to new flushes once the moisture is recouped in the soil. The seeds of *J. curcas* contain 27–40% non-edible oil[4]. Due to the presence of toxic components like phorbol esters and diterpenoids, the *Jatropha* oil is non-edible[5–8]. In recent years, an increased interest in the cultivation of *Jatropha* as a source of biodiesel in several countries is being observed[9,10].

Jatropha is considered as a wonder plant due to its drought tolerance, unpalatability to animals and high oil content in its seeds. However, not much is known about its agronomic yield potential, agronomic practices, pests and disease occurrence and management[11]. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has been exploring the use of *Jatropha* for rehabilitating the marginal lands and has planted block plantations in Velchal and Kothlapur villages in the Ranga Reddy District, Andhra Pradesh, India and also in its research farm at the ICRISAT centre in Patancheru (17°36′N, 78°16′E; 545 m altitude)[4]. A large-scale mortality of 3–4-yr-old *Jatropha* plants in Ranga Reddy and Kurnool districts, Andhra Pradesh (Figure 1) was observed during June 2009 and 2010, which was initially thought to be due to the failure of the pre-monsoon rains during March–April 2009. Thirty eight per cent mortality of plants was recorded in a 140 ha block plantation in the Velchal village. In another 76 ha block plantation at Rollapadu and Cherukucherla villages in Midthur Mandal, Kurnool District, 80% of the plants were affected and whole plantation was lost. Close examination of the dead plants showed the symptoms of shrivelling and discoloration of stems at the base of the plants. Black lesions (rotting) under the bark and cambium regions of the stem (Figure 2) were observed, confirming the occurrence of disease, besides the impact of the drought. During April–May 2010, the affected *Jatropha* plants showed reddish-brown gummy exudation from the infected (shrivelling)

Figure 1. Severe mortality of *Jatropha curcas* occurred due to infection caused by *Botryosphaeria dothidea* in Rollapadu village, Kurnool District, Andhra Pradesh, India, in August 2009.

*For correspondence. (e-mail: s.wani@cgiar.org)

parts of the stem, leading to the death of the plant (Figure 3). Occurrence and spread of this disease was mostly observed in summer months, when there was a severe water stress and drought conditions prevailed. There have been similar reports of the occurrence of such symptoms in commercial *Jatropha* plantations in Assam, Chhattisgarh and Madhya Pradesh (M. N. Pawar, pers. commun.), and affected samples were also used for this study.

The cultures isolated from the stems of the plants showing symptoms of rot were used for identifying the potential pathogens associated with the affected areas. Microscopic examination of the affected plant tissues revealed the presence of scattered globose pycnidia near the margins of the black lesions. Conidia were ovulate, hyaline, aseptate, mostly single-celled and measured 20–32 × 12–15 μm. Bi-celled, brown, mature conidia were also observed (Figure 4). The organism was identified as *B. dothidea* based on the characteristics of the mature, bi-celled conidia and morphological observations using microscopy.

Disease-affected plant parts were collected from plantations in Andhra Pradesh and Chhattisgarh. Samples were cut into small pieces (~1 cm) and surface disinfected with 5% sodium hypochlorite solution for 1 min, rinsed thoroughly with sterile distilled water and cultured on potato dextrose agar (PDA) plates. The plates were incubated at 25°C and after 4–5 days colonies developed on the affected stem pieces. The isolate was identified as *B. dothidea* on the basis of the colony morphology *in vitro* on PDA[12] and hyphae characteristics studied using microscopy and confirmed by ITS sequencing using the universal fungal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). The PCR product was sequenced at Macrogen Inc., Seoul, South Korea. Colonies on PDA were grey to black in colour with regular margin immersed along with light grey aerial mycelium, and the mycelium was hyaline and septate. *B. dothidea* is a known pathogen causing black rot in many other plants, including tree species[13–15].

For proving pathogenicity, the standard Koch's postulates process was adopted. Four centimetre long pieces of healthy stems were taken and the top epidermal layer was scraped using a scalpel and inoculated with 0.05–0.2 ml *B. dothidea* spore suspension (10^6 CFU/ml concentration) and tested for pathogenicity. The inoculated stems were covered with plastic bags and incubated at 25°C with a

Figure 2. Shrivelled appearance of black rot-affected stem (left) and black lesions over the stem under the bark (right) caused by *B. dothidea*.

Figure 3. Exudation of reddish-brown gummy substance from the late infected stem (left) and discoloration of the affected stem (right) of *J. curcas* plants infected by *B. dothidea*.

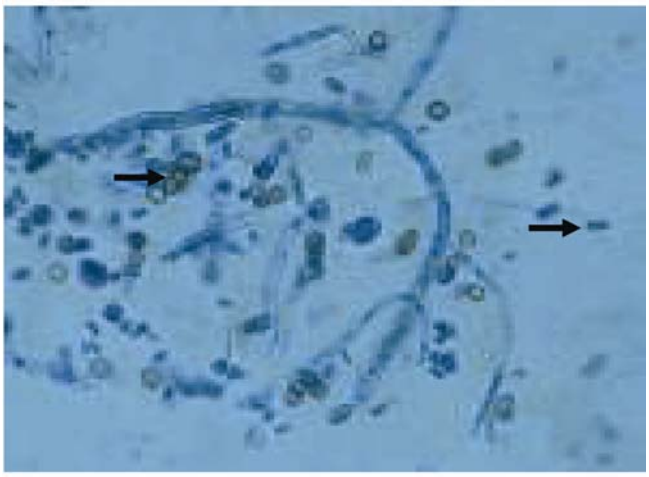

Figure 4. Microscopic view of mature, bi-celled, brown conidia of *B. dothidea*.

Figure 5. Developing light yellowish lesions in healthy leaves of *Jatropha* plant inoculated with spore suspension of *B. dothidea* after eight days of incubation.

Table 1. Most potential fungal isolates from diseased *J. curcas* plant parts by BLAST analysis in ITS region sequence analysis

Code name	Organism	Acc. No	Identities (%)
JF01	*Macrophomina phaseolina isolate* MP112	GU046903.1	98
JF02	*Phomopsis longicolla strain* SM9713	FJ009527.1	97
JF03	*Fusarium oxysporum isolate* 158CH/S	GU066709.1	100
JF04	*Alternaria alternata isolate* VC38	GQ169766.1	100
JF05	*Botryosphaeria dothidea*	AY626347.1	98

relative humidity of 80%. After 48 h of incubation, the stem pieces were transferred to petri dishes. The colonies were observed around the inoculated area of the stem pieces on the third day of incubation. The colonies were white in colour initially and then turned black on the fifth day of incubation. The bark around the inoculated area shrivelled and turned black on the eighth day of incubation. We also studied the pathogenicity by rubbing the infected (with spore suspension culture) stem pieces over the stems of healthy plants. Brown lesions were observed on leaves after eight days of incubation. The size of the lesions ranged from 1 to 3 mm in diameter and similar lesions were also noticed on the leaf petiole (Figure 5). The size of the lesions was enlarged in the infected leaf petiole on the 13th day of incubation. In another test, the entire plant was sprayed with a spore suspension (10^6 CFU/ml) and then covered with plastic bags to maintain humidity during incubation in a glasshouse. However, no symptoms were observed in the plants which were sprayed with the spore suspension in this manner. Koch's postulates were completed by isolating and identifying the culture from artificially infected *Jatropha* plants, and hence the culture was confirmed as *B. dothidea*. For controlling the disease, the affected plant parts were pruned-off and sprayed with the fungicide Bavistin (carbendazim 50% WP) at the rate of 2 g/l of water. After spraying the fungicide the recovery symptoms, i.e. growth of new sprouts from ground part of the stem and new foliage on the affected branches were observed.

Even though five pathogenic fungi were isolated from the disease-affected plant tissue, our laboratory experiment revealed that the symptoms that appeared on artificially inoculated *Jatropha* plants with *B. dothidea* culture were similar to those in plants affected in the fields, and hence it was concluded that *B. dothidea* is the main causative agent of mortality in *Jatropha* plants. To our knowledge, this is the first report of the pathogen *B. dothidea* causing black rot and severe damage to *Jatropha* in block plantations.

1. Divakara, B. N., Upadhyaya, H. D., Wani, S. P. and Laxmipathi Gowda, C. L., Biology and genetic improvement of *J. curcas* L., a review. *Appl. Energy*, 2010, **87**, 732–742.
2. Wani, S. P., Osman, M., Emmanuel, D'Silva and Sreedevi, T. K., Improved livelihoods and environmental protection through biofuel plantation in Asia. *Asian Biotechnol. Dev. Rev*., 2006, **8**, 11–29.
3. GEXSI LLP, Global Market Study on *J. curcas*, Final Report – Abstract. Prepared for the World Wide Fund for Nature, The Global Exchange for Social Investment, London/Berlin, 8 May 2008.
4. Wani, S. P., Sreedevi, T. K., Marimithu, S., Kesava Rao, A. V. R. and Vineela, C., Harnessing potential of *J. curcas* plantations for improving livelihoods and rehabilitating degraded lands. In Proceedings of 6th International Biofuels Conference, Winrock International, New Delhi, 2009, pp. 256–273.
5. Makkar, H. P. S., Backer, K., Sporer, F. and Wink, M., Studies on nutritive potential and toxic constituents of different provenances of *J. curcas*. *J. Agri. Food Chem*., 1997, **45**, 3152–3157.
6. Adolf, W., Opferkuch, H. J. and Hecker, E., Irritant phorbol derivatives from four *J. curcas* species. *Phytochemistry*, 1984, **23**(1), 129–132.
7. Rakdhit, K. D., Darukeshwara, J., Raj, K. R., Narasimhamurthy, K., Saibaba, P. and Bhagya, S., Toxicity studies of detoxified *J. curcas* meal (*J. curcas*) in rats. *Food Chem. Toxicol*., 2008, **46**, 3621–3625.
8. King, J. A., Wei, He., Cuevas, J. A., Ramiaramanana, D. and Graham, I. A., Potential of *J. curcas curcas* as a source of renewable oil and animal feed. *J. Exp. Bot. Rev*., 2009, **60**(10), 2897–2905.
9. Becker, K., Francis, G. and Edinger, R., A concept for simultaneous wasteland reclamation, fuel production and socio-economic development in degraded lands in India: need, potential and perspectives of *J. curcas* plantations, biodiesel from *J. curcas* plantations. *Nat. Resour. Forum*, 2005, **29**, 2–24.
10. Wani, S. P., Sahrawat, K. L., Sreedevi, T. K., Bhattacharyya, T. and Sreenivasa Rao, Ch., Carbon sequestration in the semi-arid tropics for improving livelihoods. *Int. J. Environ. Stud*., 2007, **64**, 719–727.
11. FAO, *J. curcas*: a small holder bioenergy crop. The potential for pro-poor development. Food and Agriculture Organization of the United Nations, Rome. *Integrated Crop Management*, 2010, **8**, 114.
12. Boerema, G. H., De Gruyter, J., Noordeloos, M. E. and Hamers, M. E. C., *Phoma Identification Manual. Differentiation of Specific and Infraspecific Taxa in Culture*, CABI Publishing, UK, 2004.
13. Boerema, G. H., Contributions towards a monograph of *Phoma* (Celomycetes) – II. Section Peyronellae. *Persoonia*, 1993, **15**, 197–221.
14. Blechtova, A., Ability of some micromycetes to cause necroses of apple tree bark *in vitro*. *Ochrana Rostlin*, 1995, **31**, 265–276.
15. Pinter, C., Fischl, G., Kadlicsko, S., Danko, J., Gara, M. and Mako, S., Pathogens of walnut in Hungary. In Proceedings of the 52nd International Symposium on Crop Protection, Gent, Belgium, 9 May 2000, **65**, 673–676.

ACKNOWLEDGEMENTS. We thank the National Oil Seeds and Vegetable Oils Development Board, Government of India, and the Government of Andhra Pradesh, Rain Shadow Area Development Department for financial support. We also thank Dr S. Gopalakrishnan for his suggestions and comments on this manuscript.

Received 11 October 2010; accepted 24 March 2011