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First Report of Incidence of Stem Rot of Cowpea Caused by *Pythium aphanidermatum* from Kerala, India and its Management

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Abstract

Cowpea (Vigna unguiculata* sub.sp. *sesquipedalis* (L.) Verdcourt.*) has come to occupy a prime position among the vegetable crops raised in Kerala in coverage and popular preference. During November 2012, high incidence of stem rot symptoms were observed in cowpea grown in open field conditions at the Department of Olericulture, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, India. The disease was identified to be Pythium stem rot caused by* Pythium aphanidermatum*. The pathogen also showed symptoms in snake gourd, chilli, tomato and amaranthus, when artificially inoculated. Management experiments revealed that application of metalaxyl MZ and azoxystrobin recorded the lowest incidence of the disease in term of Percentage Disease Incidence, followed by fungicide copper oxychloride and fish amino acid. KAU released bioagents* Trichoderma harzianum *and* P. fluorescens*, and panchagavyam were also able to suppress the disease. It was also found that all growth and yield parameters except plant dry weight were maximum with the plants treated with the systemic fungicide azoxystrobin.

Key words: Cowpea, stem rot, Pythium aphanidermatum, biocontrol agents, organic preparations, fungicides, integrated disease management.

I. INTRODUCTION

Cowpea (*Vigna unguiculata* sub.sp. *sesquipedalis* (L.) Verdcourt) is one of the most important food legumes, which serves as a vital source of protein in the diet of the people of developing countries. The tender green pods used as vegetable are rich in protein, minerals, vitamins and dietary fibre (Ibrahim et al., 2010). Pythium stem rot caused by *Pythium aphanidermatum* (Edson) Fitzp. which infects cowpea at all stages of the plant growth is an important soil borne disease of cowpea that was first reported from Nigeria with an incidence of 0.94 % seed decay and 11% stem rot (Onuorah, 1973). Here we report for the first time, the incidence of Pythium stem rot disease of cowpea from Kerala, India, and its management strategy with chemical and biological agents.

II. MATERIALS AND METHODS

A. Isolation and pathogenicity

Infected cowpea plants showing stem rot disease symptom were collected from the field and diseased tissues from stem portion were surface sterilized with 0.1% mercuric chloride, aseptically transferred in to potato dextrose agar (PDA) medium supplemented with amoxicillin (200mg/l) and incubated for 24 hours at 29± 1 0C. The morphological and cultural characters of the fungus were studied for the identification of isolated pathogen (Al-Sheikh and Abdelzaher, 2012). The pathogenicity of the isolate was established on cowpea plants by sowing surface sterilised cowpea seeds in autoclaved soil in plastic pots and inoculating with mycelial suspension of the pathogen immediately after sowing the seeds. The pots were kept in a green house (29±1 0C) with regular watering.

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B. Host range study

A preliminary study on the host range of the pathogen in other vegetable crops grown in the field near the experimental site was also conducted. Plants showing similar stem rot and

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wilting symptoms were collected and isolation of the pathogen was done from the infected plants.

C. Field management study

For the integrated management study, a field experiment was conducted at College of Agriculture, Vellayani, Thiruvanathapuram, Kerala from August 2013 to November 2013. Two organic preparations *viz.* fish amino acid and panchagavyam; two talc based formulation of biocontrol agents, *Pseudomonas fluorescens* and *Trichoderma harzianum.*; five chemical fungicides such as metalaxyl MZ, azoxystrobin, iprovalicarb + propineb, copper hydroxide and copper oxychloride were included as treatments. Fish amino acid was prepared by mixing one kilogram of sardine fish (*Sardina pilchardus*) with one kilogram of jaggery in a plastic container and keeping it covered with paper under shade for 25 days. After 25 days, the content was filtered through muslin cloth and used at a concentration of 5 % (v/v) solution (Weinert et al., 2014). For preparing panchagavyam, seven kg of cow dung and one kg of cow ghee were mixed thoroughly in a plastic container. The mixture was thoroughly agitated twice daily for further three days. After three days, 10 litres of cow urine and 10 litres of water were added in to the above mixture. The prepared mixture was kept for 15 days with regular mixing both in the morning and evening hours. After 15 days, three litres of cow milk, two litres of cow curd, three litres of tender coconut water, three kg of jaggery and 12 numbers of banana (variety Poovan) were added in the above mixture and kept of 12 days with regular mixing twice a day and used at a concentration of 5 % (v/v) solution (KAU, 2009).

The treatments were imposed starting with two weeks after transplanting and continued at the interval of fifteen days till harvest. The design followed was RBD with five blocks, and cowpea variety used was Vellayani Jyothika. The treatments were given as either soil drenching alone or as soil drenching and foliar spray as detailed in Table 1. Absolute control without any treatment was also included. Observations were made daily and the disease occurrence in the field recorded. The percentage disease incidence was calculated by using the formula given by Wheeler (1969).

$$\text{Percentage disease incidence} = \frac{\text{Number of plants dead}}{\text{Total number of plants}} \times 100$$

Growth and yield parameters such as number of leaves, plant weight, plant height, root length, root weight and fresh pod yield were recorded. Data obtained were subjected to statistical analysis following two-way ANOVA.

III. RESULTS AND DISCUSSION

A. Isolation and pathogenicity

Cottony white aerial mycelial growth of the fungus was observed on PDA on isolation of the pathogen from the tissues.The width of the fungal hyphae ranged from 3.1 to 6.8 µm. The sporangia consisted of terminal complexes of swollen hyphal branches of varying length (Fig. 1). Oogonia were terminal, globose, smooth, with a diameter of 18 to 21.6 µm (av. 19.7 µm) (Fig. 2). Intercalary antheridia that are broadly sac-shaped, 9.4 to 13.6 µm long and 7.5 to 10.4 µm wide, one per oogonium, monoclinous or diclinous were observed. Oospores were aplerotic, with a diameter of 14.1 to 19.5 µm (av. 16.4 µm), having a wall thickness of 1 to 2 µm (Fig. 2). The fungus was identified as *Pythium aphanidermatum* (Edson) Fitzp. (Fig. 3) on the basis of cultural and morphological characters (Al-Sheikh and Abdelzaher, 2012; Van der Plaats-Niterink, 1981).The culture was deposited at the Agharkar Research Institute, Pune (Accession No. DA-01). During pathogenicity test, both pre and post-emergence damping off

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symptoms started appearing after 2-3 days of inoculation. The pathogen was successfully re-isolated, from the infected plants confirming the pathogenicity.

In the field, natural infestation by the fungus in mature plants showed symptoms of wilting of the entire leaves (Fig. 5) followed by wet rotting, appearance of water soaked lesions at basal stem portion and spreading of the lesion to aerial parts, as well as to the roots (Fig. 4). The infected plant succumbed one or two days after the appearance of initial symptoms. White cottony outgrowth of the pathogen appeared on affected stem at condition of high relative humidity (Fig. 6). A perusal of literature indicates that this is the first report of *P. aphanidermatum* causing stem rot of cowpea in India.

B. Host range study

P. aphanidermatum was recovered from stem of seedlings and mature snake gourd (*Trichosanthes anguina*), seedlings of chilli (*Capsicum annuum*), tomato (*Lycopersicon esculentum*) and red amaranthus (*Amaranthus tricolor*) with similar rotting symptom. These four crops also showed stem rot symptom in the *in vitro* inoculation with same pathogen. This suggested that the pathogen is having a wide host range and could pose serious threat to other vegetable crops as well. Rahimian and Banihashemi (1979) have reported the occurrence of *P. aphanidermatum* on cucurbits causing root rot from Iran. Muthukumar et al (2011) reported post emergence damping off of chilli from India and Suleiman (2010) has reported the fungus causing root rot on tomato from Nigeria.

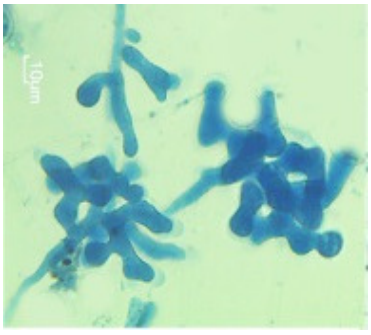

Figure 1. Lobed sporangium of P. aphanidermatum

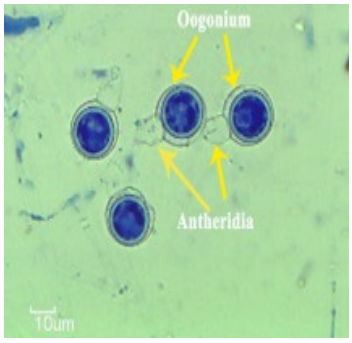

Figure 2. Antheridia, oogonia and oospores of P. aphanidermatum

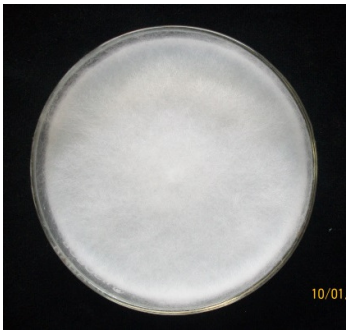

Figure 3. P. aphanidermatum culture plate

Figure 4. Stem rot symptom on cowpea

Figure 5. Wilting of entire leaves of infected plant

Figure 6. White cottony outgrowth of pathogen on affected stem

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C. Field management study

Application of metalaxyl MZ and azoxystrobin recorded the lowest incidence of the disease in terms of percentage disease incidence(4.32% and 4.32%respectively) followed by fungicide copper oxychloride (9.5%) and fish amino acid (9.5%). The highest percentage of disease incidence was observed in the untreated control (79.38%) (Table 1).Incidence of rhizome rot of ginger caused by *P. aphanidermatum* was low (5%) when this crop was sprayed with 0.3% Ridomil MZ (Singh et al., 2004). Reduction in pod rot of peanut caused by *Pythium* spp. has been reported by spraying with azoxystrobin (Thiessenet al., 2014).

Among the biometric characters, it was found that, the vine length and number of leaves per plant was found to be significantly higher in case of plant receiving the strobilurin fungicide azoxystrobin (Table 1). A similar growth promotional effect in rice by the application of azoxystrobin has been reported by Bhuvaneswari and Raju (2012).

Table 1. Field evaluation of selected organic preparations, biocontrol agents and chemical fungicides for the management of stem rot of cowpea and their effect on biometric characters of cowpea .

Treat No.	Treatments	Pod yield per plant(g)*	Vine length (cm)*	Plant fresh weight (g)*	Plant dry weight (g)*	Root length (cm)*	Root weight (g)*	No. of leaves per plant*	Percentage Disease Incidence (%)*	Disease reduction over control (%)
1.	Fish amino acid (5%) (SD+FP)	960.6	477.75^{b}	414.2	97.39	41.25	35.65	282^{b}	9.55 (18.00)^{ab}	76.92
2.	Talc based formulation of *Pseudomonas fluorescens*(2%)(SD+FP)	916.4	452.5^{b}	375.2	84.99	40.75	37.37	249.25^{b}	16.54 (24.00)^{abc}	69.23
3.	Metalaxyl MZ 68%WP (0.2%) (SD+FP)	933.7	458.75^{b}	365.2	84.91	33.10	35.97	265.00^{b}	4.32 (12.00)^{a}	84.61
4.	Azoxystrobin 23 % SC (0.15%) (SD+FP)	1003.2	557.75^{a}	439.2	101.05	42.92	40.69	374.00^{a}	4.32 (12.00)^{a}	84.61
5.	Copper hydroxide 77% WP (0.2%) (SD)	943.3	464.25^{b}	420.5	107.80	37.62	35.45	270.25^{b}	39.60 (39.00)^{c}	53.84
6.	Copper oxychlorideoxychloride 50% WP (0.2%) (SD)	914.8	466.00^{b}	424.0	103.84	37.07	35.71	257.25^{b}	9.55 (12.00)^{ab}	76.92
7.	Talc based formulation of *Trichoderma harzianum* (2%) (SD+FP)	923.4	444.75^{b}	367.5	85.67	39.26	36.96	257.00^{b}	12.84 (21.00)^{abc}	61.53
8.	Iprovalicarb 5.5%+ Propineb 61.25% (0.2%)(SD+FP)	911.4	432.25^{b}	359.5	86.01	37.78	35.45	260.00^{b}	34.54 (36.00)^{bc}	46.15
9.	Panchagavyam (5%) (SD+FP)	949.7	457.50^{b}	403.7	91.87	39.48	40.12	289.50^{b}	20.61 (27.00)^{abc}	61.53
10.	Control (without any treatment)	880.7	469.5^{b}	399.2	98.49	38.20	35.61	262.25^{b}	79.38 (63)^{d}	-
	CD (0.05)	NS	60.81	NS	NS	NS	NS	41.78	(18.98)	-

SD :Soil drenching, FS : Foliar spray
* Mean of 5 replication
Values in parenthesis are arcsine transformed
*Figures followed by same letter do not differ significantly according to two way ANOVA at P=0.05

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