

Survey and Prevalence of *Asperisporium Caricae*, Incitant of Black Leaf Spot of Papaya and Evaluation of Certain New Fungicidal

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Abstract: A roving survey was conducted in western mandals of Chittoor district for the incidence of black leaf spot of papaya caused by *Asperisporium caricae*. Formation of black pustules on the abaxial surface of the leaf are characteristic of this disease. The symptoms are scattered small spots, visible on both leaf surfaces. On the upper surface, the lesions are rounded or somewhat angular, 2-5 mm in diameter, pale yellow, with dark margins. Later the lesions become necrotic and whitish. On the lower surface, the lesions are covered with masses of fungal spores which appear as dark dots. The pustules can cover the whole lesion. The disease is more intense on the lower leaves. Sometimes the leaf lesions can cover an extensive area, causing yellowish and premature drop. The pustules also occur on fruit. Conidiophores closely packed together and covering the surface of the stroma, usually unbranched, hyaline to olivaceous brown, with several prominent conidial scars at the apex. Conidia solitary, ellipsoidal, pyriform or clavate, 1-septate, hyaline to mid pale brown, verrucose, 14–26 x 7–10 µm in diam. Among the fungicides tested, combi product Hexaconazole+ Zineb had shown 100% inhibition at 100 ppm under *in vitro* conditions.

1. INTRODUCTION

Papaya (*Carica papaya* L.), is a table fruit in most of the tropical countries cultivated mainly for fresh fruit consumption. The natural habitat of papaya lies in tropical, central and South America. Papaya flourishes in the frost - free and humid areas of the tropics and subtropics. It is regarded as an excellent source of ascorbic acid, a good source of carotene, riboflavin and a fair source of iron, calcium, thiamin, niacin, pantothenic acid, vitamin B - 6 and vitamin K. It has a high nutritive and medicinal value. Papain prepared from dried latex of its immature fruits is used in meat tenderizing, Seeds are also rich source of amino acids; scented oil was extracted, used in treatment of sickle cell disease and poisoning related disorders (Saran and Choudhary, 2013). It is consumed as a part of breakfast dessert and as fruit salad. It is also used in soft drinks, jams, ice-cream flavouring, crystallised fruit and also sold as canned cubes and juice in some countries. In India it is mostly consumed as a table fruit. Papaya has gained more importance owing to its high palatability, early fruiting and highest productivity per unit area and multifarious uses like food, medicine and industrial input. It is cultivated in the world in an area of 3.83 lakh ha with a production of 8.05 million tones. In India, it is cultivated in 73,000 ha with a production of 23.17 lakh tones (Singh et al., 2010). The area under papaya cultivation in India increased by 63% from 45.2 thousand ha. in 1991-92 to 73.7 thousand ha. in 2001-02 and the production increased from 8 lakh tones to 26 lakh tones. Papaya is mostly cultivated in the states of Andhra Pradesh, Karnataka, Gujarat, Orissa, West Bengal, Assam, Kerala, Madhya Pradesh and Maharashtra. In Andhra Pradesh, it is majorly grown in Cuddapah and Kurnool districts. Of late, cultivation of papaya has been gaining importance in Chittoor district because of high profitability.

Despite the success of the transgenic cultivars for virus control, several fungi continue to cause major disease problems and dramatically increase the cost of production, and reduce yield. A new papaya disease caused by the fungus *Asperisporium caricae* was found first on the island of Maui in February 2001, then on the island of Hawaii and Oahu, and by September on Kauai. The fungus causes extensive leaf spots and fruit spots and can render the fruit unsaleable. Hence, the present study was conducted to survey the incidence of disease in Western mandals of Chittoor district and to evaluate new fungicides under *in vitro* conditions against the test pathogen

2. MATERIALS AND METHODS

Roving survey was conducted in papaya growing areas in western mandals of Chittoor district and collected infected leaves and fruits. The infested leaves were used for isolation of test fungus *A.caricae* using tissue segment method. The laboratory experiments pertaining to the research work were conducted in the Department of Plant Pathology, Institute of Frontier Technology, Regional Agricultural Research Station, Tirupati, Chittoor district (A.P.).

The general laboratory techniques described by Dingra and Sinclair (1995), Rangaswami and Mahadevan (1999), Nene and Thapliyal (1993), Aneja (1993) were followed for preparation of media, sterilization, isolation and maintenance of fungal cultures with slight modifications wherever necessary. The pathogen was isolated from the black leaf spot affected leaves and fruits of infected plants by using tissue segment method (Rangaswami and Mahadevan, 1999) on potato dextrose agar (PDA) medium and identified the pathogen using mycological keys

In vitro studies were conducted to test efficacy of new fungicides comprises combination of systemic and contact fungicides viz; Tricyclazole +Mancozeb, Carbendazim + Mancozeb, Hexaconazole + Zineb and two systemic fungicides viz; Azoxystrobin, Difenconazole at 100, 250, 500 and 1000 ppm concentrations against the test fungus *A.caricae*. All the treatments were replicated thrice and suitable control was maintained. Statistical analysis of results was done using SPSS software.

3. EVALUATION OF FUNGICIDAL EFFICACY

Poisoned food technique was followed as described by Vyas (2002). Fifty ml double strength PDA was mixed with 50 ml of double concentrated fungicidal solution to obtain required final concentrations of 50,100,250, 500 and 1000 ppm. 20 ml of this medium was plated in 90 mm diam Petriplates. A 6 mm mycelial disc of 5 d old pathogen was inoculated at the centre and incubated at $28 \pm 2C$ for 10 d. A control was maintained without fungicide. Per cent reduction in radial growth of pathogen over control was calculated using the formula: $I = (C-T/C) 100$ Where, I = Pre cent reduction in growth of test pathogen, C = Radial growth of test pathogen (mm) in control , T = Radial growth of test pathogen (mm) in treatment

4. RESULTS AND DISCUSSION

The survey was conducted in western mandals of Chittoor district. The symptoms of this disease are irregular dark brown to black fungal spots on the lower leaf surface of older papaya leaves (Fig. 1). Appearance of black pustules on the abaxial surface of the leaf are characteristic of this disease. The first symptoms are scattered small spots, visible on both leaf surfaces. On the upper surface, the lesions are rounded or somewhat angular, 1-4 mm in diameter, pale yellow, with dark margins. Later the lesions become necrotic and whitish. On the lower surface, the lesions are covered with masses of fungal spores which appear as dark dots. The pustules can cover the whole lesion On the upper leaf surface, the infection causes slightly sunken tan spots to occur (Fig. 2). Black spots have also been observed on the surface of fruits, though not nearly as heavy as that found on the foliage. On fruits, the symptoms are more prominent that initially water soaked symptoms appeared later on conspicuous dark spots are evident. In advanced stage, there is oozing of gum is also seen from the fruit.

Papaya infested with black leaf spot: isolation of organism and identified as *Asperisporium caricae*



Fig1. Black spots on under surface of leaf



Fig2. Black spot on fruits

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Fig3. Pure Culture of *A.caricae*

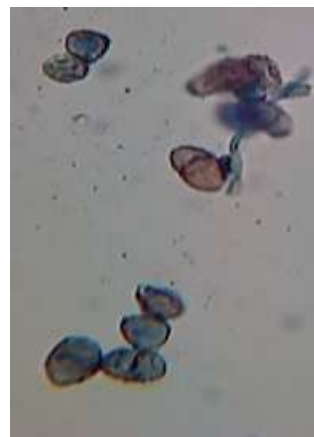


Fig4. Conidia of *A.caricae*

The fungus was isolated on PDA medium after incubation at 27° C for 7 days. Initially the mycelium was pale white later turned grey colour and covered the Petriplate within 7 days of incubation. Photomicrographs of fungus revealed that conidiophores are closely packed together with several prominent conidial scars at the apex. Conidia solitary, ellipsoidal, pyriform or clavate, 1-septate, hyaline to mid pale brown, verrucose.

The results presented in table 2 indicated that all the fungicides at all concentrations reduced mycelial growth of *A. caricae* when compared to control. Among the three combi products viz; Tricyclazole + Mancozeb, Carbendazim+ Mancozeb and Hexaconazole + Zineb, the combi product, Hexaconazole + Zineb was highly effective which recorded 100 % inhibition even at 100 ppm followed by Carbendazim + Mancozeb which could exhibit 100% inhibition at 250 ppm whereas Tricyclazole + Mancozeb recorded 100% inhibition at 500 ppm. Whereas, Difenconazole had recorded inhibition percentage of 56.35, 61.92, 63.03, 66.97 with 100 ppm, 250 ppm, 500 ppm and 1000 ppm respectively While the systemic fungicides viz; Azoxystrobin recorded inhibition percentage of 35.81,40.73, 41.64, 45.49 at 100 ppm, 250 ppm, 500 ppm and 1000 ppm respectively.

Table1. Survey for Incidence of Black Leaf Spot of Papaya in Chittoor District

S.No	Mandal	Location/Area	Morphological & cultural characters of <i>A.caricae</i>
1.	Kalikiri	Jamma palli	Irregular, dark brown to black fungal spots measuring typically 1/16" to 1/4" on the lower leaf surface. Conidia solitary, ellipsoidal, pyriform or clavate, 1-septate, hyaline to mid pale brown, verrucose, 14–26 x 7–10 µm
		Agraharam	On the upper surface, the lesions are rounded or somewhat angular, 2-5 mm in diameter, pale yellow, with dark margins Fungal spots are irregular, dark brown to dark measuring 1/12" to 1/3" on lower surface, conidia pyriform or obclavate , 10-23 to 4-7 µm.
		Noonevandlapalli	Spots are round to irregular, grey coloured measuring 1/17" to 1/6" on lower surface, conidia pyriform or obclavate , 14-28 to 7-12 µm
		Palamanda	Spots are irregular to round, black measuring 1/10" to 1/4" on lower surface, conidia pyriform, 12 -26 to 5-8 µm
2.	Somala	KondamarriUppar apalli	Fungal spots are irregular, jet black measuring 1/10" to 1/7" on lower surface, conidia pyriform or obclavate , 13-28 to 8-10 µm
		Chowdepalli	Fungal spots are round to irregular, dark brown measuring 1/12" to 1/3" on lower surface, conidia pyriform or obclavate , 8-20 to 5 -8 µm
		Nanjampeta	On the upper surface, the lesions are angular, 3-7 mm in diameter, pale yellow, with dark margins. Fungal spots are round to irregular, dark brown to dark measuring 1/10" to 1/7" on lower surface, conidia pyriform, 8 -18 to 2-4 µm
3.	Piler	Yarravari palem	Fungal spots are round to irregular, dark brown to dark measuring 1/12" to 1/3" on lower surface, conidia pyriform or obclavate , 10-23 to 4-7 µm
		Kallur	Fungal spots are round to irregular, dark brown to dark measuring

			1/16" to 1/8" on lower surface, conidia pyriform or obclavate , 15-30to 6-8 µm
		Bodireddigari palli	Fungal spots are irregular, dark brown to dark measuring 1/10" to 1/3" on lower surface, conidia pyriform or obclavate , 10-23to 4-7 µm
4	Damala charuvu	Chattivari palem	Fungal spots are irregular to round, dark brown measuring 1/9" to 1/4" on lower surface, conidia pyriform or obclavate , 9 -18 to 4-7 µm
		Diguvuru	Fungal spots are irregular, dark brown to dark measuring 1/12" to 1/3" on lower surface, conidia pyriform or obclavate , 10-23 to 5 -8 µm
		Ulasalavari palli	Fungal spots are irregular to round, dark brown to dark measuring 1/16" to 1/6" on lower surface, conidia pyriform or obclavate , 14-28 to 8-10 µm
5	Sodam	Jandrapeta	Fungal spots are irregular, dark black measuring 1/12" to 1/3" on lower surface, conidia pyriform, 8 -25 to 5-8 µm
6	Pakala	Irala	Fungal spots are round, dark brown measuring 1/18" to 1/4" on lower surface, conidia pyriform or obclavate , 12 -27 to 7-12 µm
7	Bhakara peta	Nallapareddi gari palli	Fungal spots are irregular, dark brown to dark measuring 1/16" to 1/5" on lower surface, conidia pyriform or obclavate , 16-32 to 8-12 µm

Table2. *In vitro* evaluation of certain new fungicides against *Asperisporium caricae*

S.No.	Fungicide	Per cent Inhibition over Control			
		Concentration (ppm)			
		100	250	500	1000
1	Azoxystrobin ^a	35.81	40.73	41.64	45.49
2	Difenconazole ^b	56.35	61.92	63.03	66.97
3	Tricyclazole + Mancozeb ^c	80.88	86.51	90.0	90.0
4	Carbendazim + Mancozeb ^d	85.96	90.0	90.0	90.0
5	Hexaconazole + Zineb ^e	90.0	90.0	90.0	90.0
6	Control ^f	0.0	0.0	0.0	0.0

- According to Dunnetts, ** indicates significant difference at 1% level with control
- Same alphabet indicates insignificant difference (DMRT)

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Dr.M.Reddi Kumar, working as a Senior Scientist (Plant Pathology), Institute of Frontier Technology, Regional Agricultural Research Station, Tirupati. He handled two major research projects on Biological Control of Root-Knot Nematode, *Meloidogyne incognita*, sponsored by AP- Netherlands Biotech Project, Hyderabad and another Project on Ecofriendly Management of Aflatoxins in Maize sanctioned by UGC, New Delhi as a Principal Investigator. He authored two text books entitled 1. Recent Trends in Rapid Detection of Plant Pathogens and 2. Biological Control of Plant Pathogens, Weeds and Phytoparasitic Nematodes. He had received Sri Veerapaneni Narasimham Gold Medal for Best Research Scientist in the discipline of Plant Pathology awarded by Acharya N.G.Agricultural Agricultural University, Hyderabad for the year 2010. He published 35 research papers in various reputed National and International journals. He acted as major Chairman for 9 M.Sc(Ag) students.