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Major diseases of cashew (*Anacardium Occidentale* L.) Caused by fungi and their control in Odisha, India

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Abstract

Cashew is an important cash crop in India but the incidence of diseases incur considerable losses in cashew plantations leading to reduction both in terms of quality and quantity. The present investigation was to study the fungi associated with cashew plant in Odisha in view of the warm and humid climate. Isolation, identification, pathogenicity test and nutritional study of the test fungi as well as the control was undertaken *in vitro*. The result of the study revealed that seven species of fungi namely *Pestalotiopsis palmarum, Phyllosticta* sp., *Colletotrichum gloeosporioides*, *Botryodiplodia theobromae*, *Fusarium oxysporum*, *Rhizoctonia solani* and *Chaetomium brassiliense* were responsible for various diseases. *Pestalotiopsis palmarum* and *Phyllosticta* sp. were found to cause leaf spots; *Colletotrichum gloeosporioides* causing leaf spot, die back and gummosis of stem; *Botryodiplodia theobromae* causing inflorescence blight, die-back of twigs and stem gummosis; *Fusarium oxysporum* and *Rhizoctonia solani* causing seedling blight and root rot and *Chaetomium brassiliense* being responsible for causing storage rots in cashew nuts. Pathogenicity test revealed that all the test fungi were pathogenic to their respective host parts except stem gummosis. The results of the nutritional study on different solid media indicated that Malt Extract Agar medium supported the maximum mycelial growth of all the test fungi except *Chaetomium brassiliense* and *Phyllosticta* sp. *In-vitro* antifungal activity of some selected fungicides indicated that Bavistin could control all the pathogenic fungi by 100 %. Further studies can recommend suitable control measures for the farmers and commercial growers.

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Introduction

Anacardium occidentale L. belonging to the family Anacardiaceae is a native to Brazil and was introduced to India five centuries ago. Cashew is an important cash crop and India ranks second to Brazil in production (Shanthi and Vittal, 2012). In Odisha, cashew plantation started in 1954-55 by the Soil Conservation Department mainly as a cover crop. Later on, the State Forest Department and Odisha Forest Development Corporation were involved in the cashew plantation for rehabilitation of degraded forest lands. The diseases of cashew had been considered as minor importance in earlier days. Now some of them have been found to be serious to cause considerable losses in cashew plantations. A number of fungi are found to attack this field crop thereby leading to loss in productivity. *Lasiodiplodia* and *Fusarium* species were isolated from diseased inflorescences, *Lasiodiplodia* and *Pestalotia* species from infected twigs while *Fusarium* and *Pestalotia* species were associated with leaf blight of cashew (Adeniyi *et al.,* 2011). Inflorescence dieback is a serious disease of cashew caused by *Lasiodiplodia theobromae* (Teixeira, 1988). Twig dieback caused by *Lasiodiplodia theobromae* has remained a major factor limiting cashew production for decades in Nigeria (Olunloyo, 1983; Hammed and Adedeji, 2008). Other fungi named *Pestalotia heterocornis* Guba. is associated with leaf blight of cashew (Joshi, 2005). Suleiman (2010) isolated *Trichoderma viridae*, *Cephalosporium* sp. and *Aspergillus niger* from the diseased nuts of cashew. In southern Tanzania during August 2002 a new and damaging leaf and nut blight disease was observed on young tissues of cashew. From tropical regions, *Cryptosporiopsis* species have been isolated which is the first record of this genus attacking cashew (Vajna and Rozsnyay, 2006). A new and undescribed *Cryptosporiopsis* species was consistently isolated from the nut and leaf lesions of cashew (Sutton, 1980). Some species potentially toxigenic fungi such as *Alternaria alternata, Aspergillus clavatus*, *A. flavus*, *A. parasiticus*, *A. ochraceus*, *A. ustus*, *Penicillium citrinum* and *P. oxalicum* were frequently isolated from cashew kernels in Brazil (Freire and Kozakiewicz, 2005).

In view of little work being carried out on fungal diseases of cashew plant in Odisha conditions, the present study was undertaken to find out the major fungal pathogens, the diseases they cause including cashew nuts in Odisha and to control by using some fungicides.

Materials and methods

The present investigation was carried out in the P. G. Department of Botany, Utkal University, Bhubaneswar, Odisha, India. Odisha lies between the latitudes 17.78 °N and 22.73 °N, and between longitude as 81.37E and 87.53E. The state has an area of 155,707 km², which is 4.87% of total area of India, and a coastline of 450 km (Geography of Odisha, 2015). In summer, maximum temperature ranges between 35-40 °C and the low temperatures are usually between 12-14 °C. The average rainfall is 150 cm, experienced as the result of south west monsoon during July-September (Odisha Tourism, 2015).

Collection of diseased samples

The infected leaves, twigs, roots of infected seedlings, inflorescence and bark of gummosis infected plants were randomly collected from different local gardens of Khordha, Puri, Balasore, Mayurbhanj, Jajpur and Cuttack districts of Odisha, India. To study the fungal rots of nuts, two years old cashew nuts samples were collected from different market places including store houses of Odisha.

The diseased samples were collected and kept separately in sterile polythene bags with properly labeled and brought to the Laboratory of Microbiology, P. G. Department of Botany of Utkal University, Bhubaneswar, Vani Vihar, Odisha and India for phyto pathological analysis.

Isolation and Identification of associated Fungi

The diseased plant samples of *Anacardium occidentale* L. were washed with tap water and surface sterilized with 0.1% mercuric chloride solution for 2-3 minutes. The samples were cut through by means of sterile knife. Slicing was done starting from the healthy portions. Pieces of 5 × 5 mm were cut and placed on potato dextrose agar (PDA) medium and incubated at room temperature for 24 to 35 hours.

Representative colony types were purified by sub-culturing on fresh PDA plates. Pure cultures were transferred to slants of PDA. Pure cultures of the isolates were grown singly on PDA for identification. The isolated fungi were identified based on the isolates colonial characteristics on culture plates and microscopic features in slide cultures. Using a sterile inoculating needle portion of each mycelial colony was aseptically taken and placed on a clean microscopic slide and teased in a drop of lacto-phenol cotton blue.

The isolates were identified by the help of the available literature and further authentication was made in the Department of Plant Pathology, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India.

Pathogenicity test

Healthy seedlings of about 7-8 months old were collected from local nursery to test the pathogenicity test on leaves, twigs and roots. For testing pathogenicity on inflorescence and nuts, apparently healthy nuts as well as inflorescence were collected from different local cashew farms. All the collected healthy plant parts were surface sterilized with 0.1 % mercuric chloride for 2-3 minutes. A sterilized needle was used to create wound on the healthy leaves, twigs, stems and nuts; and were inoculated with spore or mycelium of the respective fungus. A wound was made on and inoculated with only PDA to serve as control and all wrapped with parafilm. For the healthy inflorescences, spore suspensions of the culture isolates were sprayed on them and the unsprayed served as control. For healthy seedlings, the earthen pots containing sterilized soil was inoculated with desired fungus moistened with sterile water and covered with polythene bags. Each isolate was replicated three times. The cashew parts were observed for the development of disease symptoms.

Physiological study

A comparative nutritional study was conducted to know the effect of different solid nutritional media on the mycelial growth of seven fungal species causing diseases in cashew.

The test solid media were Malt Extract Agar Medium (MEA), Potato Dextrose Agar Medium (PDA), Richard's Agar Medium (RA) and Czapek's Sucrose Nitrate Agar (CSNA).

In-vitro evaluation of efficacy of selected fungicides

The effects of nine fungicides were tested for their ability to inhibit the mycelial growth of the test fungi at the rate of 0.1 % (1000 mg/1 liter). The test fungicides were Bavistin, Blitox-50, Macozeb, sulfex, Captaf, Radomil, Dhanucop, Captafal and Indofil. Each fungicide, at the rate of 0.1 % ingredient as incorporated in potato dextrose agar medium after sterilization, mixed thoroughly and plated in Petri plates. Medium without test chemicals was also plated so as to serve a control. Each plate was inoculated at the centre of the Petri plates with a mycelial disc of 2 mm in diameter taken from the periphery of seven day old colony of the desired fungus-fungicide combination. The colony diameter was measured in each case, in two planes, one at the right angle to the other, on the 10th day of treatment.

Results

Isolation of fungi from different infected plant parts of Anacardium occidentale L. and its pathogenicity

Total seven genera of fungi were found to be responsible for causing diseases in *Anacardium occidentale* L. plant. These are *Pestalotiopsis*, *Phyllosticta*, *Botryodiplodia*, *Colletotrichum*, *Fusarium*, *Rhizoctonia* and *Chaetomium*.

The data on the incidence of fungi on different diseased plant parts of *Anacardium occidentale* L. revealed that three genera of fungi such as *Pestalotiopsis, Colletotrichum* and *Phyllosticta* were encountered with 59 diseased leaf samples showing leaf-spots, *Botryodiplodia* and *Colletotrichum* from 37 diseased samples showing die-back and 21 diseased samples showing stem gummosis; two namely *Fusarium* and *Rhizoctonia* from 28 diseased samples showing seedling blight and one each namely *Botryodiplodia* collected from 17 diseased samples of inflorescence and *Chaetomium* from 7 diseased samples of nuts in varying frequencies in different localities. The pathogenicity test revealed that all the fungal isolates were pathogenic to their respective host plant parts (Table 1).

Table 1. Incidence of fungi in diseased samples of *Anacardium occidentale* L. plant parts.

Diseases	Fungal isolates		Frequency of incidence (%)
Leaf-spot	*1.*	*Pestalotiopsis palmarum*	50.84
	2.	*Colletotrichum gloeosporioides*	33.89
	3.	*Phyllosticta* sp.	15.25
Die-back	1.	*Botryodiplodia theobromae*	70.27
	2.	*Colletotrichum gloeosporioides*	29.72
Inflorescence-blight	*Botryodiplodia theobromae*		100
Stem gummosis	1.	*Botryodiplodia theobromae*	52.38
	2.	*Colletotrichum gloeosporioides*	47.61
Seedling blight/ root rot	1.	*Fusarium oxysporum*	28.57
	2.	*Rhizoctonia solani*	71.42
Nuts infection	*Chaetomium brassiliense*		100

Physiological studies

Studies on effect of five different solid media on the mycelial growth of seven isolated fungal species revealed that there was a significant variation among the media on mycelial growth. In a comparative study, it was found that Malt Extract Agar Medium supported the maximum mycelial growth for *Botryodiplodia theobromae*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Pestalotiopsis palmarum* and *Rhizoctonia solani* whereas potato Dextrose Agar Medium favored the maximum mycelia growth of *Phyllosticta* sp. and *Chaetomium brassiliense*. It might be seen from the Table 2 that there was significant differences among the media tested for the mycelial growth of *Fusarium oxysporum* (Table 2).

Table 2. Effect of four solid media on the growth of test fungi in mm.

Test fungi	PDA	MEA	RA	CSNA
Botryodiplodia theobromae	79.45 ± 1.05	82.17 ± 1.51	64 ± 0.81	67.35± 1.06
Colletotrichum gloeosporioides	51.26 ± 0.64	72.3 ± 1.47	54.4 ± 1.41	59.24 ± 0.98
Pestalotiopsis palmarum	56.43 ± 1.22	64.04 ± 0.81	54.4 ± 1.23	51.57 ± 1.22
Phyllosticta sp.	60.42 ± 1.97	58.53 ± 1.13	48.01 ± 1.63	58.5 ± 0.4
Fusarium oxysporum	51.1 ± 1.9	64 ± 1.63	59.62 ±2.04	57.56 ± 1.22
Rhizoctonia solani	63.14 ± 0.95	64.5 ± 1.22	57 ± 0.81	54.53 ± 1.22
Chaetomium brassiliense	58.61 ± 1.23	55.56 ± 1.67	52.1 ± 1.71	50.36 ± 1.84

In-vitro evaluation of fungicides

There is a significant difference among the test fungicides in inhibiting the mycelial growth of isolated fungi causing leaf-spot, die-back, inflorescence-blight, stem gummosis, seedling blight/root rot and nuts infection of cashew.

Out of nine tested fungicide, Bavistin completely inhibited the mycelial growth of all test fungi and it was found to be superior to the remaining fungicides. Captafal was also found to be quite effective next to Bavistin followed by Captaf and Radomil in inhibiting the mycelial growth (Table 3).

Table 3. Inhibition of mycelia growth of test fungi by 9 test-fungicides.

Fungicides	Percentage of inhibition of mycelial growth on 10th day						
	Botryodiplodia theobromae	*Colletotrichum gloeosporioides*	*Fusarium oxysporum*	*Pestalotiopsis palmarum*	*Phyllosticta* sp.	*Rhizoctonia solani*	*Chaetomium brassiliense*
Bavistin	100	100	100	100	100	100	100
Blitox-50	48.03 ± 0.81	66.5 ± 1.22	50.5 ± 1.22	67.23 ± 0.98	60.7 ± 0.94	63 ± 0.81	44.36 ± 1.96
Macozeb	25.36 ± 1.88	37.9 ± 0.73	29.43 ± 1.22	68.83 ± 0.69	26.25 ± 1.25	68.73 ± 1.06	44.06 ± 0.85
Sulfex	18.2 ± 1.2	27.36 ± 0.73	12.03 ± 1.67	36.96 ± 1.63	17.78 ± 1.44	46.03 ± 1.14	36.03 ± 1.63
Captaf	59.03 ± 0.81	67.03 ± 0.81	68.66 ± 1.34	87.46 ± 1.22	55.76 ± 0.61	76.36 ± 2.09	66.3 ± 0.97
Radomil	73.96 ± 1.63	58.46 ± 2.85	23.9 ± 1.55	63 ± 1.22	90.83 ± 1.47	57.8 ± 0.94	45.76 ± 1.47
Dhanucop	46.1 ± 0.82	63.13 ± 0.93	77.03 ±1.22	27.13 ± 0.93	58.66 ± 0.77	49.1 ±1.34	64.83 ± 1.1
Captafal	100	100	67.9 ±0.81	100	68.03 ±0.81	72.36 ±1.55	70.83 ± 0.69
Indofil	34.63 ± 0.89	17.13 ± 0.77	10.93 ±2	22.6 ± 1.3	8.2 ±0.86	12.46 ± 0.57	27.56 ± 1.1

Discussion

Among the fungal species isolated during the present investigation *Botryodiplodia* and *Colletotrichum* were found to be associated in majority of the samples under study. Earlier report on an unidentified species of *Cryptosporiopsis* sp. was found to be associated to cause leaf-spot of cashew (Sijaon *et al.*, 2005). *Botryodiplodia theobromae*, associated with die-back, inflorescence-blight and gummosis was observed during the present investigation. Association of this fungus with die-back was reported by Mishra, 1983 and Pattnaik *et al.,* 1987. Among all the isolates *Colletotrichum gleosporoides* associated with gummosis; *Fusarium oxysporum* and *Rhizoctonia solani* causing seedling blight; and *Chaetonium brassiliense* causing storage rot of cashew nut. *Pestalotiopsis palmarum* was isolated from leaf of cashew causing leaf blight. Different species of *Pestalotiopsis* (=*Pestalotia*) namely *P. conglomarata* (Polanco, 1973), *P. heterocormis* (Intini, 1987), *P. dichaeta*, *P. microspora*, *P. palmarum* (Sarbkay *et. al.,* 1978; Mishra, 1983; Adeniyi *et al.*, 2011) have been reported from different cashew growing countries in the world. *Colletotrichum gloeosporioides* isolated from leaf of Cashew causing leaf-spot, die-back of twigs and gummosis of stems. *C. gloeosporioides* causing leaf spot (Abraham and Padmakumar, 1980; Freire *et al.*, 2002) and die back of twigs (Singh *et.al.,* 1967; Nambiar, 1974) was reported earlier from India. But there was no report on the association of this fungus causing gummosis of stem, although an unidentified species of *Colletotrichum* was reported earlier (Mishra, 1983).

An unidentified species of *Phyllosticta* was found to be associated to cause leaf-spot of Cashew (Mishra, 1983); a report also revealed an unidentified species of *Phyllosticta* causes leaf-spots. *Botryodiplodia theobromae* was found to be associated with die-back, inflorescence-blight and gummosis during the present investigation. The pathogenicity of the fungus was well established with die-back and inflorescence-blight. Association of this fungus with die-back was reported by Mishra (1983) and also its association with inflorescence blight was reported (Mishra, 1983 and Pattnaik *et al.,* 1987). *Fusarium oxysporum* was isolated from infected roots of cashew seedlings causing seedling blight. The disease was characterized by the yellowing of leaves of seedlings. These seedlings showed rotting of these roots with infection starting from the root tips. Infected roots become black and soft. Vascular browning was obtained in infected seedlings when cut open longitudinally. *Rhizoctonia solani* was found to cause seedling-blight of cashew producing the similar types of symptoms as in case of symptoms produced by *F. oxysporum*. *Chaetomium brassiliense* was isolated from stored nuts of Cashew causing storage rot. The infected nuts showed brown to blakish coloured patches on the surface of nuts. Internal kernel showed dry rot type of symptoms with brownish discolouration of the tissues. Mishra (1983) recorded the best growth of *Botryodiplodia theobromae* and *Phyllosticta* sp. on Potato Dextrose Medium, *Colletotrichum* sp. on Malt Extract Agar Medium, *Pestalotia* sp. on Richard's Agar Medium.

Therefore, the detailed studies on the nutritional aspects on these fungi causing diseases of Cashew plants were studied.

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