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Symptomatology, variability and management of Zebra disease of Sisal in Western Orissa

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The genus *Agave sisalana,* commonly known as sisal is valued for its hardest known vegetable fibre and as ornamentals in horticultural lawn and kitchen garden. Both *A. sisalana* and Hybrid 1 (Leela) is affected by zebra disease. Isolations from infected plant samples consistently yielded *Phytophthora nicotianae* var. *parasitica.* In *A. sisalana* only leaf spot was detected. In hybrid sisal leaf spot, bole and spike rot phases of the disease was prevalent. In the present investigation, an attempt has been made to study the symptomatology of the disease, isolation of the casual organism, enumeration of losses under preparatory nursery and field conditions, disease management and variability in respect of the pathogen and host.

Key words: Sisal, *Agave sisalana,* Bamra hybrid (Leela), Zebra disease, *Phytophthora nicotianae var, parasitica.*

INTRODUCTION

Sisal fibre which is one of the most important raw materials for the country's rope industry is derived from the leaves of *Agave* species, chiefly *Agave sisalana.* Sisal in general grows better in fertile soils and also in well drained limestone soils mostly under tropical and sub-tropical conditions. Frost coupled with rains also cause damage to plants but temperature above 10°C provides better growth (Osborne and Singh, 1980). There are many species of Agaves but few are cultivated for the sake of their fibre (Lock, 1969). Other economically important species of Agaves are *Agave fourcroydes* (henequen), *A cantala, A. amaniensis* (blue sisal) and *A. angustifolia.* Man has domesticated sisal for its valued fibre, as an ornamental in kitchen garden and fence crop to protect agricultural lands from grazing cattles and wild animals. Sisal fibre is used in the manufacture of ropes, twines, cordages, carpets, specialty paper and fancy handicrafts. Sisal sap is utilized in the preparation of hecogenin, a precursor for the preparation of medicinal steroids. The countries leading in the production and commercial exploitation of sisal are Kenya, Brazil, Mexico and China. In India, sisal is mainly grown in western part of Orissa state. Inspite of sisal being hardy, xerophytic and semi-perennial in nature, it suffers from bole rot and zebra disease caused by *Aspergillus niger* and *Phytophthora* sp. respectively (Lock, 1969; Roy and Singh, 1999). More than 50% of the hybrid sisal cv. Bamra hybrid 1 (Leela) in adult plants (2 year old plantation) and > 80% plants in preparatory nursery were affected by zebra disease. The disease is endemic in nature and losses caused by this disease in western Orissa range from 10-20%. Keeping in view the endemic nature of the disease it has been felt worthwhile to i) isolate the organism(s) responsible for the disease, ii) proving pathogenicity test *in vivo* iii) symptomatology of the disease in nursery and field crop, iv) loss estimate under field conditions, v) management of the disease and vi) variability among isolates of the pathogen.

MATERIALS AND METHODS

A field and pot experiment was carried out during 1998-99 at Sisal Research Station, Bamra, Distict Sambalpur, Orissa to study variability, enumeration of losses and management of zebra disease of sisal. Isolation of the pathogen from infected samples and symptomatology of the disease was carried out from general plantation at Sisal Research station as well as from farmer's field in and around Bamra.

The infected leaf samples were cut into small bits, treated in 5% sodium hypochlorite for 3-4 minutes and washed in sterile distilled water. The bits were plated onto Potato Dextrose Agar medium amended with vancomycin (80 μl^{-1}), polymixin B-sulphate (80μl^{-1}).

The fungi isolated from hybrid sisal variety Bamra hybrid 1 (Leela) was multiplied in Potato Dextrose broth for one week followed by filtration and collection of fungal mass in pre-sterilized Whatman No. 1 filter paper. 5 g of the mycelium on fresh weight basis was ground in pestle to which 500 ml of water was added gradually. Six months old suckers of *A. sisalana* and cv. Bamra hybrid 1 (Leela) raised in pots were used for pathogenicity test *in vivo*. The plants kept in the open air were sprayed inoculated preceded by 2-3 inches linear, gentle incision over leaf lamina with sharp needle. The plants preceded by 2-3 inches linear, gentle incision over leaf lamina with sharp needle. The plants were covered with polythene bags to conserve moisture. The plants were mist sprayed with steile distilled water for two successive days and polythene bags were removed after 72 h. From two years old plantatiion, plants of *A. sisalana* and cv. Bamra Hybrid 1 (Leela) was selected for pathogenicity test *in vivo* during rainy season. A longitudinal cut at 2" depth was made with a sterile sharp knife at the base of the spike and fungal suspension measuring 5 ml was dropped with sterile pipette at the cut portion. The injured site was immediately covered with cotton dipped in sterilized water. The plants were water sprayed for three successive days.

The symptoms of zebra disease were recorded in preparatory nursery right from planting of bulbils and in adult plants at field, both for *A. sisalana* and cv. Bamra hybrid 1 (Leela) right from initiation to advanced stage of infection.

Field evaluation of foliage resistance to zebra disease was carried out in a total of 24 plots, 12 each under two year old plantation of *A. sisalana* and Bamra hybrid 1 (Leela). The area under individual plot measured 10 × 15 m with a popularion of 76 plants in double row plantation. A scale based on disease intensity range comprising 6 categories was developed [1 (0), 2 (0.5%), 3 (5.1-10%), 4 (10.1-25.0%), 5 (25.1-50.5%) and 6 (> 50%)] which was a modification of the scale given by Bjor (1988) on late blight of potato. PDI was computed based.

Efficacy of fungicides in the management of zebra was evaluated after the initiation of primary infection in secondary nursery with the onset of rainy season (initiating from second fortnight of June) in six months old bulbils of cvs. *A. sisalana* and Bamra hybrid 1 (Leela) collected from preparatory nursery. After the initiation of disease, the treatments for foliar management of the disease comprised of i) Ridomil MZ-72 (Metalaxyl 8% + Mancozeb 64% @ 0.2%), (ii) Dithane M-45 (mancozeb @ 0.2%), iii) Dithane M-45 followed by application of Ridomil MZ-72 after 15 days interval and iv) untreated check in randomized block design. A total of four replications served each treatment. The plot size in secondary nursery under each replication was 4 × 2 m with spacing in-between plants and rows of 25 × 50 cm. A total of 64 plants were accommodated in each replicated plot. After the application of fungicides, disease intensity was recorded after an interval of 7 days.

Studies on variability of the pathogen was carried out with the test isolate (PH_1) raised in Potato Dextrose Broth. From a week old growth of the pathogen, fungal mat was separated and 1 g of inoculum was finely grounded in porcelain mortar to which 50 ml of water was added gradually. Six month old suckers of *A. sisalana,* Bamra hybrid 1 (Leela), *A. amaniensis, A. angustifolia, A. meradorensis* and *A. cantala* were raised in pots keeping three replication under each type. The suckers were subjected to gentle abrasion over the leaf surface with sand paper followed by application of cotton swab dipped in 2 ml of the inoculum suspension at the site of injury. The plants were kept in open air and were mist sprayed with help of sprayer 2-3 times a day for a successive period of two days. After 48, 72, 120 and 168 h after incubation, lesion area (πr^2) was measured.

Variability among three isolates of *P. nicotianae,* two from Bamra hybrid 1 (Leela) and one from *A. sisalana* were studied for their comparative aggressiveness in the host system. Seven days old growth of the pathogen raised in Potato Dextrose broth was used for the present study. 5 g of mycelial mat was separated aseptically from growth of the pathogen in flasks, finely grounded in mortar and to which 100 ml of water was added gradually. Six months old suckers of *A. sisalana* and Bamra hybrid 1 (Leela) were subjected to injury by gentle abrasion over leaf surface with sand paper followed by application of cotton swab dipped in 2 ml of the inoculum suspension. The disease reaction was recorded (lesion area in πr^2) after 48, 72, 120 and 168 h of incubation.

RESULTS AND DISCUSSION

Isolation and pathogenicity test

Isolation from infected disease samples consistently yielded *Phytophthora nicotianae* Van Breda de Hann. It was confirmed from the description of the genus *Phytophthora* by Waterhouse (1970). Optimum temperature for the growth fo the fungus was found to be 25°C. The growth of the fungus was observed in temperature as low as 10-12°C and to a maximum of 34°C.

In the plants kept in the open air for pathogenicity test, both *A. sisalana* and Bamra hybrid 1 (Leela) developed infection after 72 h after of incubation. Dark black, water soaked lesion with concentric wavy rings appeared in both sisal types which matched the symptoms developed under natural conditions. Leaf spots appeared at discrete points especially on lower leaves near to ground level followed by gradual withering. *P. nicotianae* was isolated consistently from infected samples. Bole and spike rot symptoms were expressed after three months of inoculation. Isolations at Sisal Research Station, Bamra, Orissa (India) from infected leaf and spike of zebra disease yielded *Phytophthora nicotianae.*

Association of *Phytophthora nicotianae, Phytophthora aracae* and *Phytophthora palmivora* from zebra disease of sisal has been reported from East Africa (Ann. Rept., Sisal Res. Station, Mlingano, Tanganyika 1963-64; 1965-66; Wienk, 1967; Peregrine, 1969). In India prevalence of *P. nicotianae var. parasitica* only with zebra disease of sisal has been recorded.

SYMPTOMATOLOGY

Symptoms in nursery

The early symptoms of the disease in nursery were characterized by small water soaked lesions on the leaf lamina which rapidly enlarge when the atmospheric RH exceeds 90% during rainy season. Subsequently, the centre darkened often with gummy exudates. In some plants, water soaked lesions at different portions of the leaf coalesced to form patch. The infected portions of the leaf usually exhibited transparency. The linear length of the primary lesion ranged from 0.1-0.5 cm. The older leaves touching the moist soil were highly susceptible to infection. The rate of build-up of the disease was very fast in hybrid sisal. The zebra disease symptoms in nursery plant of hybrid sisal had been presented in Fig. 1.

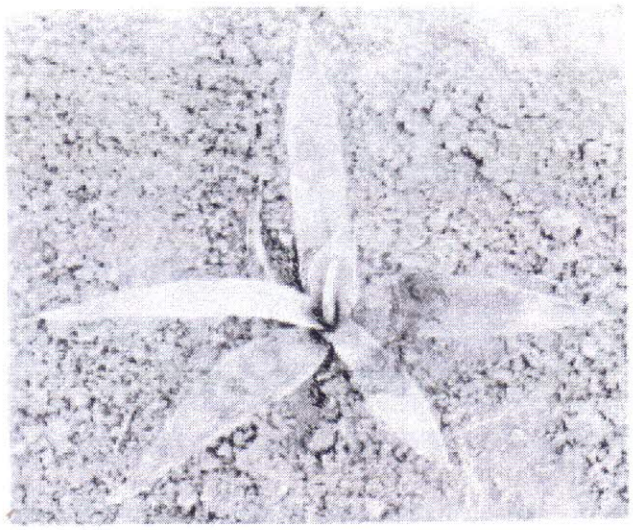

Fig 1. Zebra disease symptoms in nursery plant of Agave hybrid

Symptoms in adult plants

In adult plants, infection generally initiated from older leaves near the ground level. Zebra leaf spot appeared as small lesions on the lamina which rapidly enlarged, developing alternate concentric rings of dark purple and green, with light greenish yellow margin in the types, *A. sisalana* and Bamra hybrid 1. At the centre of these spots formation of gummy

exudates was often observed. Small spots coalesced to form larger spots and gummy exudates solidified giving crystalline brown appearance. Zebra disease symptoms in adult plant of *A. sisalana* had been presented in Fig. 2.

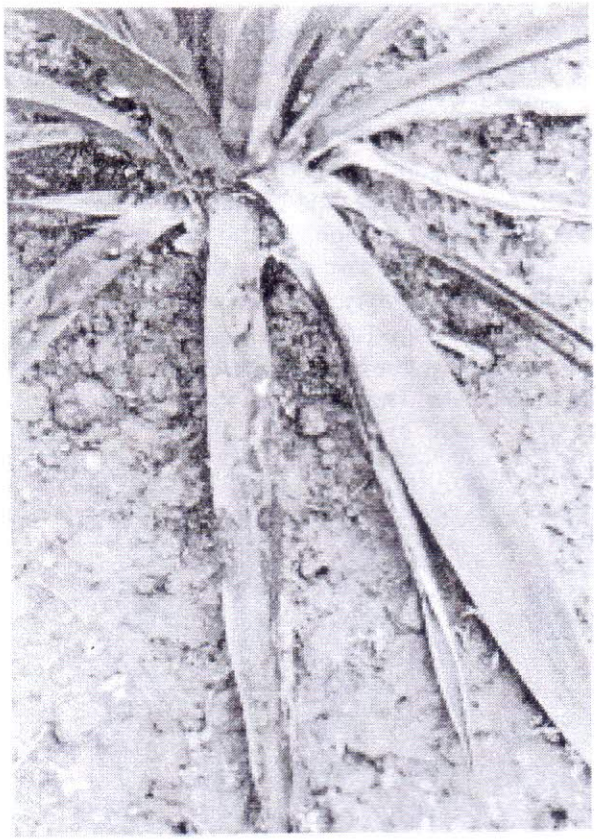

Fig 2. Zebra disease symptoms in adult plant of *A. sisalana*

Spike and bole rot phase of the disease had been recorded exclusively in Bamra hybrid 1 (Leela) which was an indication of advance stage of infection (Wienk, 1967; Peregrine, 1969). The infected spike in most of the cases bended at 45° angles or more and could be easily detached. Such bending of spike was as a result of advanced rotten state of the bole. Bole rot was preceded by spike rot. Bole rot was recognizable only at advanced stage when plants became pale yellow and leaves started rolling. This phase of the disease took time and gradually sets in after rains. If infected bole was cut into two halves, the pith was found rotten indicating colonization by the invading fungus. The color of the pith was found to be brown with blackish tinge. All the phases of the disease i.e. foliar spot/lesion spike and bole rot in sisal had been reported in sisal hybrid No. 11648 from East Africa (Clinton and Peregrine, 1963; Peregrine 1963). Observations at Sisal Research Station, Bamra indicated that only foliar symptoms were expressed in *A. sialana.* In Bamra hybrid 1 (Leela) foliage lesion, spike and bole rot phases of the disease were expressed. Any possible injury to the spike or in the plant as a whole in hybrid sisal as a result of field operation, grazing cattle's, inclement weather and hail storm, predisposed the plants to zebra infection. Necessity of breeding efforts to combine the resistance to zebra disease caused by *Phytophthora* spp. from *Agave decipens* had been suggested by Osborne and Singh, (1980).

Loss Estimate

Field reaction in *A. sisalana* and *Agave hybrid* to zebra disease had been depicted in Fig. 3. for the observations taken during the years, 1998 and 1999. The PDI during 1998 for *A. sisalana* and *A. hybrid* was 18.12 and 33.52 respectively. The degree of disease reaction in hybrid sisal was 46% higher to that of *a. sisalana* during 1998. During 1999 the difference in the disease reaction between *A. sisalana* (PDI 27.12) and hybrid sisal (PDI 70.23) was rose to 61.34%. The increase in disease reaction in *A. sisalana* and hybrid sisal from 1998 to 1999 was 33.15 and 52.27% respectively. The result was indicative of the fact that Agave hybrid was highly susceptible to zebra disease compared to *A. sisalana.*

Disease Management

The results on management of the disease in *A. sisalana* and Bamra hybrid 1 (Leela) under field condition had been presented in Table 1. Perusal of the results revealed that all the fungicides i.e. mancozeb 0.2% (Dithane M 45), metalaxyl 8% + mancozeb 64% formulation 0.2% (Ridomil MZ 72) and their combination in alternation were effective in reducing the infection of zebra disease. However, infection

Table 1 : Screening for zebra disease reaction in different sisal types

Sisal types	Observation dates/Lesion area (cm^2)			
	1	2	3	4
A. sisalana	0.08	1.88	15.11	22.47
Agave hybrid	1.58	4.33	20.42	47.45
A. amaniensis	1.43	4.33	15.72	27.17
A. angustifolia	4.91	6.38	11.71	30.17
A. meradorensis	2.69	5.51	12.80	29.45
A. cantala	3.22	7.06	32.40	81.27

was reduced significantly (7.07%) in Dithane M45 when aplied in alternation with Ridomil MZ 72. In *A. sisalana* (9.43%), the extent of disease reduction was significant compared to Bamra hybrid 1 (20.83%). At interaction level of fungicide x types, significant reduction in the disease was obtained in Dithane M 45 application in alternation with Ridomil MZ 72 (5.52%) which was at par with Ridomil MZ 72 (6.89%) in *A. sisalana*.

Table 2 : Pathogenic behaviour of *P. nicotianae* isolates in sisal types

Isolates	Lesion Area (cm^2)					
	Agave sisalana			Agave hybrid		
	1	2	3	1	2	3
PH^1	2.54	4.33	10.38	3.97	6.38	15.89
PH^2	0.71	1.65	2.76	2.54	2.95	5.72
PH^3	1.77	3.14	6.41	3.63	5.31	10.46

Table 3 : In vivo efficacy of fungicides against zebr disease in *A. sisalana* and Agave hybrid

Fungicides	Disease incidence (%)		
	A. sisalana	Agave hybrid (Leela)	Mean
Ridomil MZ-72	6.89	12.53	9.71
Dithane M 45	8.25	13.91	11.08
Dithane M45 + Ridomil MZ72	5.52	8.63	7.07
Untreated Check	17.08	48.27	32.67
Mean	9.43	20.83	
	SEm	CD at 5%	
Fungicide	0.52	1.59	
Variety	0.37	1.12	
Fungicide x Variety	*0.74*	*2.25*	

There were reports of metalaxyl resistance in nature against *Phytophthora infestans* causing late blight of potato (Dowley and Sullivan, 1981; Carter *et.al.*, 1982; Cohen and Reuveni, 1983; Cohen and Coffey, 1986). Application of Ridomil MZ 72 should be restricted to two sprays only. Application of metalaxyl + mancozeb formulations should be in alternation with contact fungicides like mancozeb (Dithane M 45) in the management strategy for zebra disease of sisal.

Variability in isolates

Pathogenic behaviour in *P. nicotianae var. parasitica* isolates against zebra disease of sisal in types *A. sisalana* and Bamra hybrid 1 (Leela) has been presented in Table 3. The results indicated that disease reaction was faster in isolate PH1 and PS3 as there was significant increase in lesion area from second to third observation. The rate of infection in isolate PH1 and PS3 was higher in Bamra hybrid 1 (Leela) than *A. sisalana*. The infection rate in isolate PH2 was lowest both in sisal types.

Disease reaction in different sisal types

The rate of disease reaction in different sisal types recorded after second, third, fifth and seventh day of

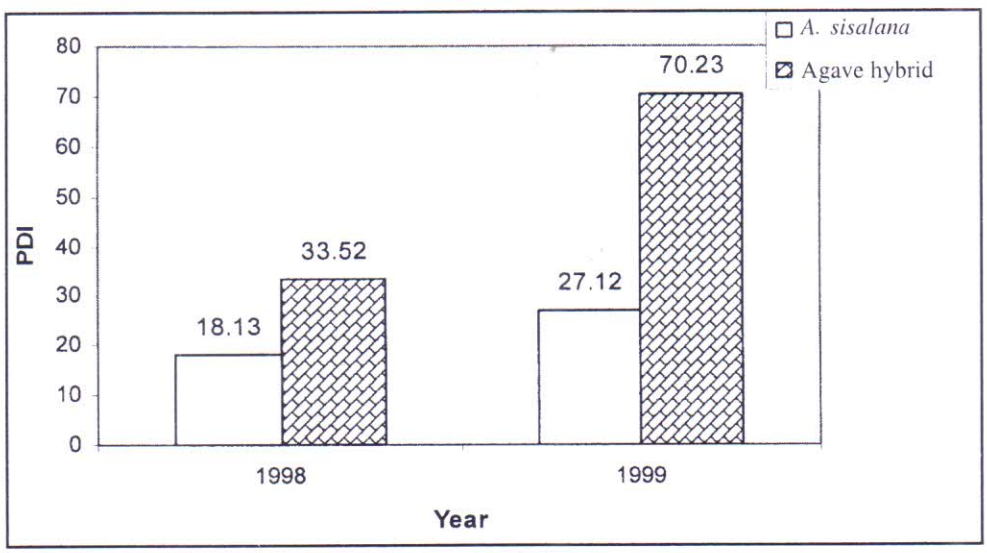

Fig 3. Field reaction of zebra disease in *A. sisalana* and Agave hybrid

inoculation with isolate PH1 of *P. nicotianae var. parasitica* has been presented in Fig. 3. From the perusal of results it indicated that rate of disease reaction recorded after fifth day of inoculation was fastest in *A. cantala* (81.27 cm^2) followed by Agave hybrid (47.45 cm^2) followed by *A. amaniensis* (12.16 cm^2), *A. meradorensis* (12.61 cm^2) and *A. angustifolia* (13.30 cm^2).

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