Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(4): 304-307
Received: 29-06-2020
Accepted: 30-07-2020

Nanjundaswamy JC
Department of Plant Pathology
College of Agriculture,
University of Agricultural
Sciences, Dharwad, India

ST Naik
Department of Plant Pathology
College of Agriculture,
University of Agricultural
Sciences, Dharwad, India

Nandan M
Department of Plant Pathology,
College of Agriculture,
University of Agricultural
Sciences, Bangalore, India

Corresponding Author:
Nanjundaswamy JC
Department of Plant Pathology
College of Agriculture,
University of Agricultural
Sciences, Dharwad, India

Efficacy of fungicides, biocontrol agents and botanicals against ring spot disease (*Leptosphaeria sacchari* Van de Brenda) of sugarcane.

Nanjundaswamy JC, ST Naik and Nandan M

Abstract
Sugarcane (*Saccharum officinarum* L) is an important cash and industrial crop of India. Ring spot (*Leptosphaeria sacchari* Van de Brenda) of sugarcane is one of miner disease which sporadically has become frequent occurring disease in some parts of Northern Karnataka. Pathogenicity of ring spot disease was proved by mycelial bit inoculation method. Further identified by produced mycelium septate, hyaline, sexually ascospores were ellipsoid, ends broadly obtuse, hyaline, three celled with second cell slightly swollen with a measured of 16.13 to 27.09 µm in length and 3.99-8.00 µm in breadth and asexually conidia were hyaline, pale brown in mass, ellipsoid to subfusoid, guttulate, one- celled with a measured of 5.67 to 9.66 µm in length and 3.99-5.88 µm in breadth. *In vitro* evaluation fungicides, plant extract and bioagents revealed that hexaconazole 5% EC (0.1%) , propiconazole 25% EC (0.15%) ,carbendazim 50% WP (0.15%) and thiophanate methyl 75% WP(0.15%) in systemic fungicides, Copper oxychloride 50% WP (0.2%), mancozeb (0.3%) in non-systemic fungicides, trycyclozole 18% + mancozeb 62% WP (0.05%), cabendazim 12% + mancozeb 63%, hexaconazole 18% + zineb 68% and captan 70% + hexaconazole 5% in combi products were most effective and leaf extract of *Eucalyptus* sp and *Azadirachta indica* @ 10 per cent concentration and *Trichoderma viride* and *T. harzianum* were most effective against *L. sacchari.*

Keywords: Leptosphaeria sacchari, Bio Efficacy, fungicides, plant extract and bioagents.

Introduction
Sugarcane (*Saccharum officinarum* L) is an important cash and industrial crop of India. It belongs to the genus *Saccharum* (*Saccharum* spp. 2*n* =70–140) of the family Poaceae. Because of sweetness of stalk juice, it is domesticated from perennial grass species and cultivated for its stalks, which accumulate sucrose. The crop is grown in tropical and subtropical regions of the world. It contributes 60 per cent of the raw sugar produced world-wide, the remaining 40 per cent coming from sugar beet.

Sugarcane is considered as long durated crop and lazgman's crop its production is affected by different 'pests problems. It suffers from many diseases caused by fungi, bacteria, viruses, nematodes and also abiotic stresses. Among the fungal diseases, several pathogenic and saprophytic foliar fungal diseases *viz.*, rust, smut, yellow spot, ring spot and brown spot have been reported in sugarcane plant in several parts of the world. Because of increased area of cultivation and continuous spread of same crop. Hither to causing losses both qualitatively and quantitatively. Minor diseases have reached the proportion of covering larger area. Thus ring spot and brown spot diseases which sporadically have become frequent occurring diseases in some parts of northern Karnataka. This has made to draw attention of sugarcane scientists to initiate some work. Survey of literature suggested that there is no much systematic work carried out on various aspects on ring spot diseases except reporting of the diseases and description of symptoms.

The ring spot disease (*Leptosphaeria sacchari* Van de Brenda*)* of sugarcane was first reported in Florida by Krueger (1892). Later, Van Brenda (1892) [12] described the details of same disease. (The fungus *Leptosphaeria sacchari* belongs to Phylum -*Ascomycota*; Class -*Dothieomycetes;* Order -*Pleosporales;* Family-*Leptosphaeriaceae*; perfect stage was identified as genus *Phyllosticta sacchari*) subsequently it was reported from many sugarcane growing regions of the world. The ring spot occurred severely on susceptible varieties in Barbados, Porto rico, Cuba and in several places in Florida. According to Agnihotri (1982) the typical ring spot disease appears very frequently on susceptible cane varieties in December and January and symptoms first appear as minute bronze brown flecks which gently enlarge to become elongated flecks with yellow edges. As the infection progresses, lesions become elongated and oval-shaped varying from 4-18 mm in length. The characteristic feature of the

ring spot lesion is the straw colored centre with a well-defined reddish-brown margin.

Management of plant diseases done by different chemical and non-chemical methods. Ring spot disease is as a miner and novel emerging disease in the recent years which sporadically has become frequent occurring disease in major sugarcane growing area of northern Karnataka. Hence, there is a scope for initiate the fascinating field of research on noval ring spot of sugarcane includes isolation of *Leptosphaeria* spp. pathogen, proving pathogenicity of *L. sacchari* is very necessary to confirm the association of sugarcane with pathogen, identification by morphological and cultural studies necessary for advance research, need to studies on bio efficacy of different systemic, non-systemic, combi-product fungicides for chemical management of ring spot disease, need to focus on biocontrol agents and botanicals under laboratory condition is also necessary for non-chemical management of ring spot disease. Overall basic research helps to studies on epidemiological research and integrated diseases management practices for ring spot disease of sugarcane. Considering the aforementional facts, a research programme was formulated.

Material and Methods

Isolation, identification and proving pathogenicity of pathogen

Sugarcane leaves infected with ring spot were collected from different places (*viz.*, Belagavi, Bagalkote, Dharwad and Uttar Kannada) during survey and used for isolation of the pathogen. Their isolation was done by following standard tissue isolation technique. The *L. sacchari* was identified based on its morphological and cultural characters like any other members of genus *Cercospora*. Later the pathogenicity for ring spot pathogen was proved through Koch's Postulation.

In vitro evaluation of fungicides, bio agents and botanicals

The bio efficacy of five systemic fungicides (at the concentration of 0.05%, 0.1% and 0.15%) four non-systemic fungicides (at the concentration of 01%, 0.2% and 0.3%) and five combi-products (at the concentration of 0.05%, 0.1% and 0.2%) were assayed *in vitro* against *L. sacchari* for radial growth inhibition using poison food technique (Dennis and Webster, 1971) [4]. A control plate having only the test pathogen was also kept for comparison. Three replications were maintained for each isolate. Per cent inhibition of mycelial growth over control was calculated by using the formula given by Vincent (1947) [13]. After recording all the observations they were tabulated and analysed statistically for further interpretation.

The antagonistic effects of five bioagents were evaluated against *L. sacchari* for radial growth inhibition using dual culture technique (Dennis and Webster, 1971) [4]. A control plate having only the test pathogen was also kept for comparison. Three replications were maintained for each isolate. The per cent inhibition of the growth of the pathogen was calculated by the formula given by Vincent (1947) [13].

The antifungal mechanism of seven plant extract were evaluated against *L. sacchari* by using the poisoned food technique (Nene and Thapliyal, 1973) [10]. Controls were also maintained by growing the pathogens on PDA plates. Then such plates were incubated at 27±1 ^{0}C and radial growth was taken when maximum growth was observed in control plate. The efficacy of plant product or botanicals was expressed as per cent inhibition of radial growth over the control which was calculated by using Vincent (1947) [13] formula.

Results

The isolation of *L. sacchari* from the infected leaves was made as described in standard tissue isolation technique. The *L. sacchari* was identified based on its morphological and cultural characters like septate mycelium and spore morphology. The ascospores were ellipsoid, ends broadly obtuse, hyaline, three celled with second cell slightly swollen and measured 18-25 x 3.5-6.0 mm size. The conidia were hyaline, pale brown in mass, ellipsoid to subfusoid, guttulate, one- celled and measured 9-14 x 3-5 mm size. Based on these characters the pathogen was identified as *L. sacchari.* The pathogen formed uniformly dense colonies with abundant mycelium, aerial growth, whitish colored, rough colony and concentric ring on potato dextrose agar. The medium beneath the colony became light brownish which showed high sporulation. The pathogenicity of the *L. sacchari* was conformed once again through Koch's postulation and the symptoms of ring spot started developing on inoculated plants 4 to 5 days after mycelial bit inoculation. Sporulation of *L. sacchari* also noticed in inoculated plant with mycelial bit after section of reisolated plant tissues and the growth of fungus was compared with original culture

In vitro evaluation of fungicides against *L. sacchari*

All the fungicides evaluated were significantly superior over the control with respect to per cent mycelial inhibition. Results have been presented in Table 1. Among the systemic fungicides, complete mycelial inhibition was recorded in carbendizim at 0.15 per cent, hexaconazole at 0.1 and 0.15 per cent and propiconazole and thiophanate methyl at 0.15 per cent concentration this was significantly superior over rest of the treatments. The least inhibition of was observed in thiophonate methyl (82.96%) at 0.05 per cent. Irrespective of concentration of fungicides tested, the hexaconazole recorded maximum mean per cent mycelial inhibition (96.29%) which was on par with propiconazole (94.04%) followed by carbendazim (92.40%).and least per cent mycelial inhibition was recorded in tebuconazole (89.11%). Among the non-systemic fungicides, complete mycelial inhibition was recorded in copper oxychloride (100%) at 0.2 per cent and 0.3 per cent concentrations. This was significantly superior over rest of the treatments and it was followed by mancozeb (82.05%) at 0.3 per cent. The least inhibition of mycelial growth was observed in captan (55.68%) at 0.1 per cent. Irrespective of concentrations of fungicides tested, the treatment involving copper oxychloride recorded maximum mean per cent mycelial inhibition (100%) followed by mancozeb (73.85%) and least per cent mycelial inhibition was recorded in captan (65.51%) have been presented in Table 2.

Among the four combi-fungicides, the complete mycelial inhibition was recorded in treatment with trycyclozole 18% + mancozeb 62% WP (100%) at all the three concentrations, cabendazim 12% + mancozeb 63% at 0.1 and 0.2 per cent, hexaconazole 18% + zineb 68% at 0.1 and 0.2 per cent and captan 70% + hexaconazole 5% at 0.1 and 0.2 per cent. which was found significantly superior over all other treatments and least per cent inhibition of mycelial growth was observed in carboxin 37.55% + thiram 37.55% (62.47%) at 0.05 per cent have been presented in Table 3.

Table 1: In vitro evaluation of systemic fungicides against L. sacchari

Systemic fungicide	Per cent inhibition of radial growth over control			Mean
	Concentration (%)			
	O.05%	O.1%	O.15%	
Carbendazim	85.92 (67.93)	91.29 (76.01)	100 (89.96)	92.40 (77.97)
Hexaconazole	88.88 (70.49)	100.0 (89.96)	100.00 (89.96)	96.29 (83.47)
Thiophanate methyl	82.96 (65.60)	88.88 (70.49)	100 (89.9)	90.61 (75.35)
Propiconazole	89.28 (70.85)	92.85 (74.45)	100 (90.00)	94.04 (78.43)
Tebuconazole	85.67 (67.77)	89.71 (71.26)	91.96 (73.47	89.11 (70.83)
Mean	86.69 (70.19)	88.037 (72.36)	94.07 (79.00)	
	S.Em±		C.D at 1%	
Fungicides (F)	0.99		3.90	
Concentrations (C)	0.72		2.80	
F x C	1.78		7.02	

Figures in parentheses indicate Arcsine transformed values

Table 2: In vitro evaluation of non-systemic fungicides against L. sacchari

Non systemic fungicide	Per cent inhibition of radial growth over control			Mean
	Concentration (%)			
	O.1%	O.2%	O.3%	
Copper oxy chloride	92.96 (74.59) *	100 (89.96)	100 (89.96)	97.65(84.84)
Mancozeb	66.79 (54.79)	72.47 (58.33)	81.98 (64.86)	73.74(59.32)
Chlorothalonil	57.78 (49.46)	65.68 (54.12)	75.19 (60.10)	66.21(54.56)
Captan	55.68 (48.24)	66.67 (54.71)	74.20(59.45)	65.51(54.13)
Mean	68.30 (56.77)	76.20 (64.28)	82.84(68.59)	
	S.Em±		C.D at 1%	
Fungicides (F)	0.120		0.47	
Concentrations (C)	0.104		0.41	
F x C	0.208		0.82	

Figures in parentheses indicate Arcsine transformed values

Table 3: In vitro evaluation of combi products against L. sacchari.

Combi products	Per cent inhibition of radial growth over control			
	Concentration (%)			
	O.O5%	O.1%	O.2%	Mean
Trycyclozole18% + Mancozeb 62% WP (Merger)	100.00 (89.96)	100.00 (89.96)	100.00 (76.34)	100. (89.96)
Carbendazim 12% + Mancozeb 63% WP (Saaf)	93.33 (75.01)	100.00 (89.96)	100.00 (89.96)	97.78 (84.98)
Carboxin 37.5% + Thiram 37.5% WP (Vitavax power)	62.47 (52.20)	82.72 (65.41)	87.28 (69.08)	77.49 (62.23)
Hexaconazole 4% + Zineb 68% WP (Avatar)	93.33 (75.01)	100.00 (89.96)	100.00 (89.96)	97.78 (84.98)
Captan 70% + Hexaconazole 5% WP (Taqat)	93.33 (75.01)	100.00 (89.96)	100.00 (89.96)	97.78 (84.98)
Mean	88.49 (73.44)	96.54 (85.05)	97.46 (85.79)	
	S.Em±		C.D at 1%	
Fungicides (F)	0.241		0.94	
Contrations (C)	0.186		0.73	
F x C	0.417		0.94	

Figures in parentheses indicate Arcsine transformed values

Table 4: In vitro evaluation of bioagents against L. sacchari.

Botanicals	Per cent inhibition of mycelial growth		
	Concentration (%)		
	5	10	Mean
Eucalyptus sp	10.86 (3.44)	26.67 (5.24)	18.77 (4.34)
Azadirachta indica	12.47 (3.67)	19.75 (4.56)	16.11 (4.11)
Chromolaena odorata	2.35 (1.81)	4.94 (2.43)	3.64 (2.12)
Parthenium hysterophorus	2.84 (1.95)	4.94 (2.44)	3.89 (2.19)
Pongamia pinnata L	0.25 (1.11)	4.20 (2.22)	2.22 (1.67)
Jatropha curcas	0.37 (1.17)	2.72 (1.89)	1.54 (1.53)
Cassia fistula L.	0.00 (1.00)	0.86 (1.00)	0.43 (1.00)
Mean	4.16 (2.02)	9.15(2.83)	
	S.E m ±	C.D at 1%	
Botanicals (B)	0.117	0.34	
Concentrations(C)	0.063	0.18	
B X C	0.166	0.48	

Figures in parentheses indicate Arcsine transformed values

In vitro evaluation of bio-agents against *L. sacchari*

There was a significant difference between the fungal and bacterial bio-agents on per cent inhibition of mycelial growth of *L. sacchari* have been presented in Table 4. In this case of fungal antagonists gave higher inhibition as compared to bacterial antagonists. Maximum per cent mycelial inhibition of *L. sacchari* was recorded in *Trichoderma viride* (82.41%) which was found significantly superior over rest of the treatments and it was followed by *T. harzianum* (70.67%). The least inhibition of mycelial growth was recorded in *Verticilium lacanicillium* (41.39%). In this case of bacterial bio-agents tried, *Bacillis subtilis* gave maximum inhibition of mycelial growth (44.51%). The least inhibition of mycelial growth was observed in *Pseudomonas fluorescens* (30.07%).

In vitro evaluation of Botanicals against *L. sacchari*

There was very less inhibition of mycelial growth in all the botanicals tested. Among the seven botanicals tested at two concentrations (5% and 10%) the maximum mycelial inhibition was recorded in treatment involving *Eucalyptus* sp (26.67%) at 10 per cent concentration which was significantly superior over rest of the treatments this was followed by *Azadirachta indica* (19.75%) at 10 per cent. There was no

inhibition in *Cassia fistula* at 5 per cent (00%). Irrespective of concentrations of plant extracts tested, the treatment involving *Eucalyptus* sp recorded maximum mean per cent mycelial inhibition (18.77%) followed by *Azadirachta indica* (16.11%) and minimum mean mycelial inhibition was recorded in *Cassia fistula* (0.43%).

Discussion

The ring spot of sugarcane is foliar disease which was first reported by Van Brenda in Florida (UAS) by Krueger (1892). Later, Van de Brenda (1892) [12] described the details of this disease. Survey of literature suggested there is no much systematic work on various aspects on ring spot sugarcane disease except reporting and description of symptoms. The information available on ring spot disease and causal pathogens is very scanty. The identification of the *L. sacchari* was made on the basis of important morphological characters such as; mycelial, colour, septation and ascospore and conidial characters. The morphological descriptions of different structures was given by Brenda (1892) [12] which were compared with the fungus obtained in pure form and thus identity of the pathogen was confirmed as *L. sacchari*. The pathogenicity of the pathogens was conformed once again through Koch's postulation by artificial inoculation of mycelial bit and symptoms started developing on inoculated plants 7 to 8 days after inoculation. such pathogenicity studies are also done by other workers and similar symptoms were described by Brenda (1892) [12] in case of sugarcane, Humpherson and Jones (1983) [7] in case of *L. maculans* on cabbage, Nicolla *et al.* (2006) [11] in case of *L. maculans* on *Brassica juncea.*

Among the systemic fungicides, hexaconazole at 0.1 and 0.15 per cent and propiconazole, carbendizim and thiophanate methyl at 0.15 per cent concentration showed complete inhibition of mycelial growth of *L. sacchari*. In non-systemic fungicides, copper oxychloride showed 100 per cent inhibition of mycelial growth at 0.2 and 0.3 per cent concentration and mancozeb showed 81.98 per cent inhibition at 0.3 per cent concentration. In combi products, trycyclozole 18% + mancozeb 62% WP at all the concentrations, cabendazim 12% + mancozeb 63%, hexaconazole 18% + zineb 68% and captan 70% + hexaconazole 5% at 0.1 and 0.2 per cent concentration were found to be 100 per cent inhibition. In the non-systemic fungicides, copper oxychloride and mancozeb gave effective level of inhibition of mycelium, in the systemic fungicide, tryazoles give effective level of inhibition. Thus, it appear that in the combi products whenever tryazoles and mancozebs are in combination there is higher level of inhibition. Though such studies are not done against *L. sacchari*, but the study carried out in other species of *Leptosphaeria* also indicated similar type of findings like tryazoles playing important role in inhibiting the fungus in laboratory and also under field conditions.this was evidented by Huang *et al.* (2011) [6] investigated the effects of the fungicide Punch C (flusilazole plus carbendazim) on growth of *L. maculans* and *L. biglobosa* in oilseed rape and in controlled-environment experiments.

Among fungal bioagents tried, *T. viride* (82.41%), *T. harzianum* (70.67%) and *T. virens* (64.35) were found to inhibit mycelial growth of *L. sacchari* in that order. Present studies recorded significant antibiosis or mycoparasitism by species of *Trichoderma* as compared to bacterial antagonist's *viz., P. fluorescens* and *B. subtilis*. It may be due to mycoparasitism mechanism as reported by Mukherjee *et al.* (2000). These studies receive support by the reports of Rajesh and Dilantha Fernando (2006) in case of *L. maculans* in different locations. In plant extracts tested, *Eucalyptus* sp showed an inhibition of 26.67 per cent of the growth of fungus at 10 per cent concentration and others gave lesser inhibition (*Azadirachta indica* leaf extract (19.75%) at 10 per cent). Similar results were obtained by Wulff (2012) [14] in case of sorghum caused by *Leptosphaeria sacchari*, *Fusarium* spp, *Cochliobolus lunatus* and *Cladosporium spp.* in sub-Saharan Africa

Table 5: *In vitro* evaluation of bioagents against *L. sacchari.*

Bio agents	Per cent inhibition of mycelial growth(mm)
Bacillus subtiles	44.51 (41.83)
Pseudomonas fluorescens	30.07 (33.24)
Trichoderma harzianum	70.67 (57.19)
Trichoderma viride	82.41 (65.18)
Trichoderma virens	64.35 (53.32)
Verticilium lecani	41.39 (40.03)
S.Em ±	0.40
CD at 1%	1.44

References

1. Agnihotri JP. *Diseases of Sugarcane*, Oxford and IBH Publ. Co. New Delhi (India), 1983, 172.
2. Anonymous, 2018a, http://www.indiastat.com
3. Anonymous, 2018b, Final estimation of area, production and yield of principle crops in Karnataka for the year. Directorate of Economics and Statistics, Bangalore, 2014, 118.
4. Dennis C, Webster J. Antagonistic properties of species groups of *Trichoderma*. II. Production of volatile antibiotics. *Transact. Brit. Mycol. Soc.* 1971; 57:41-48.
5. Dilantha F, Crahay JN, Dernoeden PH, O'nell NP. Growth and pathogenicity of *Leptosphaeria korrae* in bermodagrass. Pl. Dis. 2006; 72(11):945-949.
6. Huang YJ, Hood JR, Eckert MR, Stonarda JF, Cools HJ, King GJ, *et al.* Effects of fungicide on growth of *Leptosphaeria maculans* and *L. biglobosa* in relation to development of phoma stem canker on oilseed rape (Brassica napus). Pl. Path. 2011; 60:607–620.
7. Humpherson, Jones FM. Pathogenicity studies on isolates of *Leptosphaeria maculans* from brassica seed production crops in south-east England. Ann. Appl. Bid. 1983; 103:37-44.
8. Krueger W. Ring spot of sugarcane. Sugarcane Dis. World. 1892; 2:3-58.
9. Mukherjee N. Antifungal activities of some oils and detergents II. Effect of germtubes growth, sclerotia plant pathogen. *Zpflkranth Pflactutz*. 1976; 83:305-308.
10. Nene YL, Thapliyal PN. *Fungicide in Plant Diseases Control,* (Third Edition; Oxford and IBH publishing Co. Pvt. Ltd., New Delhi, 1973, 325.
11. Nicolla T, Neree D, Martin B, Krishnapillai S. Enhanced pathogenicity of *Leptosphaeria maculans* pycnidiospores from paired co-inoculation of *Brassica napus* cotyledons with ascospores. Ann. Bot. 2006; 97:1151-1156.
12. Van de Brenda, Ring spot disease of sugarcane, Sugar News, 1892, 172.
13. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature.* 1947; 150:850.
14. Wulff EG, Zida, E, Torp J, Lund OS. *Yucca schidigera* extract: A potential biofungicide against seed borne pathogens of sorghum. Pl. Path. 2012; 61:331-338.