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Bio efficacy of fungicides, biocontrol agents and botanicals against brown spot (*Cercospora longipes* Butler) of sugarcane

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Abstract

Sugarcane (*Saccharum officinarum* L.) is an important cash and industrial crop of India. Brown spot of sugarcane is one of miner disease which sporadically has become frequent occurring disease in some parts of northern Karnataka. Pathogenicity of brown spot disease was proved by mycelial bit inoculation method. Further identified the brown spot (*Cercospora longipes* Butler) pathogen with produced hyaline conidiophore in clusters and conidia were hyaline, two to many celled, needle shaped and produced shy sporulation. *In vitro* evaluation fungicides, plant extract and bioagents revealed that hexaconazole 5% EC, propiconazole 25 EC and tebuconazole 250 EC at 0.05 per cent concentration showed cent per cent inhibition of mycelial growth of *C. longipes* in systemic fungicides, mancozeb @ 0.2 and 0.3 per cent concentration showed cent per cent inhibition in non-systemic fungicides, cabendazim 12% + mancozeb 63%, hexaconazole 18% + zineb 68%, captan 70% + hexaconazole 5% @ all the concentration and Carboxin 37.55% + thiram 37.55% @ 0.1 and 0.2 per cent concentration showed 100 per cent inhibition in combi products, leaf extracts of *Azadiracta indica* (10%) and *Trichoderma virens* were most effective against brown spot.

Keywords: *Cercospora longipes*, 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. $2n = 70-140$) 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 brown spot diseases except reporting of the diseases and description of symptoms.

Butler and Singh as early as in 1934 reported brown spot (*Cercospora longipes* Butler) of sugarcane. Symptoms appear as large number of minute, reddish, rust like spots on both the surfaces of leaves. As infection progresses, spots coalesce and complete distortion of leaves takes place. From the literature it is seen that, *Cercospora* sp. usually does not grow readily on artificial media and so far only a few species have been successfully isolated (Jamadar, 1988)^[7]. Thus, isolation on lab media and proving pathogenicity of *Cercospora* is very necessary to confirm the association of sugarcane with pathogen and it helps for further studies on

epidemiology and management aspects. Management of plant diseases done by different chemical and non-chemical methods. Brown 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 brown spot of sugarcane includes isolation of *Cercospora* pathogen, proving pathogenicity of *Cercospora* 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 brown spot disease, need to focus on biocontrol agents and botanicals under laboratory condition is also necessary for non-chemical management of brown spot disease. Overall basic research helps to studies on epidemiological research and integrated diseases management practices for brown spot disease of sugarcane. Considering the aforementioned facts, a research programme was formulated.

Material and Methods

Isolation, identification and proving pathogenicity of pathogen

Sugarcane leaves infected with brown 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 *Cercospora longipes* was identified based on its morphological and cultural characters like any other members of genus *Cercospora*. Later the pathogenicity for brown 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 0.1%, 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 *Cercospora longipes* for radial growth inhibition using poison food technique. 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) ^[16]. After recording all the observations they were tabulated and analysed statistically for further interpretation.

The antagonistic effects of five bioagents were evaluated against *Cercospora longipes* for radial growth inhibition using dual culture technique. 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) ^[16]. The antifungal mechanism of seven plant extract were evaluated against *Cercospora longipes* 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 °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) ^[16] formula.

Results

The isolation of *Cercospora longipes* from the infected leaves was made as described in standard tissue isolation technique. The *Cercospora longipes* was identified based on its morphological and cultural characters like any other members of genus *Cercospora*. The colonies of *C. longipes* were uniformly dense on potato dextrose agar. The colonies appeared whitish colored, rough colony with circular to irregular sharp edges. The medium beneath the colony became dark brownish with yellow margin and cracking of colony at center appeared. Hyphae of the fungus were hyaline and septate. The fungus grew very slowly on PDA medium reaching a maximum growth of 60-75 mm and there was negligible sporulation in the medium. The culture was identified based on mycelial characters like knotted mycelium and spore morphology and the spore size. The conidia were hyaline, two to many celled, needle shaped, slightly broader at one end and pointed at the other end and conidiophores were in cluster. Based on these characters the pathogen was identified as *C. longipes*. The pathogenicity of the *C. longipes* was conformed once again through Koch's postulation and the symptoms of brown spot started developing on inoculated plants 7 to 8 days after mycelial bit inoculation. Sporulation of *C. longipes* also noticed in inoculated plant with mycelial bit after section of reisolated plant tissues and it produced cluster of conidiophores with shy conidia.

In vitro evaluation of fungicides against *C. longipes*

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 hexaconazole, propiconazole and tebuconazole at all the concentration which found significantly superior over rest of the treatments. The least inhibition of mycelial growth was observed in thiophonate methyl (28.72%) at 0.05 per cent. Irrespective of concentrations of fungicides tested, the hexaconazole, propiconazole and tebuconazole recorded maximum mean per cent mycelial inhibition (100%) followed by carbendazim (63.56%) and least per cent mycelial inhibition was recorded in thiophonate methyl (37.61%). Among the non-systemic fungicides, complete mycelial inhibition was recorded in mancozeb (100%) at 0.2 per cent and 0.3 per cent concentrations which found significantly superior over rest of the treatments and it was followed by mancozeb (90.26%) at 0.3 per cent. The least inhibition of mycelial growth was observed in captan (48.21%) at 0.1 per cent. Irrespective of concentrations of fungicides tested, the treatment involving mancozeb recorded maximum mean per cent mycelial inhibition (96.75%) and least per cent mycelial inhibition was recorded in captan (57.26%) at 0.1 per cent have been presented in Table 2.

Among the four combi-fungicides, complete mycelial inhibition was recorded in treatment with cabendazim 12% + mancozeb 63%, hexaconazole 18% + zineb 68% and captan 70% + hexaconazole 5% at all the concentration at all the three concentrations and Carboxin 37.55% + thiram 37.55% at 0.1 and 0.2 per cent concentration. which was found significantly superior over all other treatments and the least per cent inhibition of mycelial growth was observed which are tricyclozole 18% + mancozeb 62% WP (68.03%)

Irrespective of concentration of fungicides tested, the treatment involving cabendazim 12% + mancozeb 63%, hexaconazole 18% + zineb 68% and captan 70% +

hexaconazole 5% recorded maximum mean per cent mycelial inhibition (100%) which was found to be on par with Carboxin 37.55% + Thiram 37.55% (96.58%) and least per

cent mycelial inhibition was recorded in tricyclozole 18% + mancozeb 62% WP (77.49%) at 0.1 per cent have been presented in Table 3.

Table 1: *In vitro* evaluation of systemic fungicides against *C. longipes*

Systemic fungicide	Per cent inhibition of radial growth over control			Mean
	Concentration (%)			
	0.05%	0.1%	0.15%	
Carbendazim	59.66(50.55)*	64.96(53.68)	66.15(54.40)	63.59(52.88)
Hexaconazole	100.00(89.96)	100 (89.96)	100.00(89.96)	96.29(83.47)
Thiophanate methyl	28.72 (32.39)	37.95(38.01)	46.15 (42.78)	37.61(37.73)
Propiconazole	100.0 (89.96)	100.00(89.96)	100.00(89.96)	100.00(89.96)
Tebuconazole	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00(89.96)
Mean	77.68 (70.57)	80.58 (72.32)	82.46 (79.00)	
	S.E m±		`C.D at 1%	
Fungicides (F)	0.096		0.37	
Concentrations (C)	0.075		0.29	
F x C	0.167		0.65	

Figures in parentheses indicate Arcsine transformed values

Table 2: *In vitro* evaluation of non-systemic fungicides against *C. longipes*

Non systemic fungicide	Per cent inhibition of radial growth over control			Mean
	Concentration (%)			
	0.1%	0.2%	0.3%	
Copper oxy chloride	84.96(67.15)*	86.15(68.13)	88.37(70.04)	86.50(68.44)
Chlorothalonil	56.41 (48.66)	59.83(50.65)	88.37(54.71)	60.97(51.34)
Captan	48.21 (43.95)	57.78(49.45)	65.81(54.20)	57.26(49.20)
Mancozeb	90.26 (71.78)	100.00(89.96)	100 (89.96)	96.75(83.90)
Mean	69.96 (57.89)	75.94(64.55)	80.21(67.23)	
	S.Em±		C.D at 1%	
Fungicides (F)	0.135		0.53	
Concentrations (C)	0.117		0.46	
F x C	0.234		0.92	

Figures in parentheses indicate Arcsine transformed values

Table 3: *In vitro* evaluation of combi products against *C. longipes*.

Combi products	Per cent inhibition of radial growth over control			
	Concentration (%)			
	0.05%	0.1%	0.2%	Mean
Trycyclozole18% + Mancozeb 62% WP (Merger)	68.03 (55.55)	75.73 (60.46)	88.72 (70.35)	77.49 (62.12)
Carbendazim 12% + Mancozeb 63% WP (Saaf)	100.00 (89.96)	100.00 (89.96)	100.0 (89.96)	100.0(84.98)
Carboxin 37.5% + Thiram 37.5% WP (Vitavax power)	89.74 (71.29)	100.00 (89.96)	100.0 (69.08)	96.58 (83.74)
Hexaconazole 4% + Zineb 68% WP (Avatar)	100.00 (89.96)	100.00 (89.96)	100.0 (89.96)	100.0 (84.98)
Captan 70% + Hexaconazole 5% WP (Taqtat)	100.0 (89.96)	100.0 (89.96)	100.0 (89.96)	100.0(84.98)
Mean	91.56 (79.35)	95.15 (84.06)	97.46 (86.04)	
	S.E m±		C.D at 1%	
Fungicides (F)	0.078		0.30	
Contrations (C)	0.061		0.24	
F x C	0.136		0.53	

Figures in parentheses indicate Arcsine transformed values

In vitro evaluation of bio-agents against *C. longipes*

There was a significant difference between the fungal and bacterial bio-agents on per cent inhibition of mycelial growth of *C. longipes* have been presented in Table 4. In this case also fungal antagonists gave higher inhibition as compared to bacterial antagonists. Maximum inhibition (77.82%) was noticed in *Trichoderma virens* followed by *T. harzianum*

(76.92%) and *T. viride* (76.15%) which were on par with each other. The least inhibition of mycelial growth was observed in *Verticillium lecanii* (46.92%) Between bacterial bio-agents tried *Pseudomonas fluorescens* gave maximum inhibition of mycelial growth (56.15%). The least inhibition of mycelial growth was observed in *Bacillis subtilis* to the extent of 49.81 per cent

Table 4: *In vitro* evaluation of bioagents against *C. longipes*.

Botanicals	Per cent inhibition of mycelial growth		
	Concentration (%)		
	5	10	Mean
<i>Azadirachta indica</i>	38.97 (38.61)	51.62 (45.91)	45.30 (42.26)
<i>Cassia fistula</i> L.	13.84 (21.74)	23.76 (29.16)	18.80 (25.45)
<i>Chromolaena odorata</i>	5.47 (13.41)	14.19 (22.04)	9.83 (17.73)
<i>Eucalyptus</i> sp	14.19 (22.11)	25.81 (30.52)	20.00 (26.32)
<i>Jatropha curcas</i>	28.03 (31.95)	51.62 (45.91)	39.83 (38.93)
<i>Parthenium hysterophorus</i>	37.44 (37.71)	45.81 (42.58)	41.62 (40.14)
<i>Pongamia pinnata</i> L	14.53 (22.37)	22.56 (28.34)	18.55 (25.35)
Mean	24.44 (29.14)	30.96 (32.63)	
	S.E m $\pm$	C.D at 1%	
Botanicals (B)	0.55	2.16	
Concentrations(C)	0.29	1.16	
B X C	0.78	3.06	

Figures in parentheses indicate Arcsine transformed values

In vitro evaluation of Botanicals against *C. longipes*

Results relating to the effects of plant extracts on per cent inhibition of *C. longipes* are presented in Table 5. 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 per cent mycelial inhibition was recorded in treatment involving *Azadirachta indica* (51.62%) and *Jatropha curcas* (51.62%) at 10 per cent concentrations which was found to be significantly superior over rest of the treatments this was followed by *Parthenium hysterophorus* (45.81%) at 10 per cent. The least inhibition of mycelial growth was observed in *Chromolaena odorata* at 5 per cent (5.47%). Irrespective of concentrations of plant extracts tested, the treatment involving *Azadirachta indica* recorded maximum mean per cent mycelial inhibition (45.30%) followed by *Parthenium hysterophorus* (41.61%) and minimum mean mycelial inhibition was recorded in *Chromolaena odorata* (37.61%).

Table 5: *In vitro* evaluation of bioagents against *Cercospora longipes*

Bio agents	Per cent inhibition of mycelial growth(mm)
<i>Bacillus subtilis</i>	49.81 (34.82)
<i>Pseudomonas fluorescens</i>	56.15 (32.25)
<i>Trichoderma harzianum</i>	76.92 (22.78)
<i>Trichoderma viride</i>	76.15 (23.17)
<i>Trichoderma virens</i>	77.82 (22.30)
<i>Verticillium lecanii</i>	46.92 (35.95)
SEM $\pm$	0.15
CD at 1%	0.63

Discussion

The brown spot (*Cercospora longipes* Butler) of sugarcane is foliar disease which was first reported by Butler and Singh (1934). Survey of literature suggested there is no much systematic work on various aspects on brown spot sugarcane disease except reporting and description of symptoms. The information available on brown spot disease and causal pathogens is very scanty. The identity of the *C. longipes* was done by studying its morphological and growth characteristics and were compared with earlier reports. Colonies appeared uniformly dense, initially white in colour later turned to light brown colour. The identity of the fungus was confirmed by comparing the characters with earlier report given by Butler (1906) in case of sugarcane and Bhat *et al.* (2008) in case of pepper. Similarly pathogenicity study was taken up by

artificial inoculation with mycelial bit methods 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 Butler (1892) in case of sugarcane, Veena (2012) and Kavyashree (2014) [8] in case of *C. canescens* on greengram.

Among the systemic fungicides hexaconazole, propiconazole and tebuconazole at all the concentration showed 100 per cent inhibition of mycelial growth. Similar findings were reported by Satyaprashant (2004), Swamy (2010) [14] and Veena (2013) [15] who reported that, Hexaconazole @ 0.05% and combi-product, carbendazim + mancozeb @ 0.025% recorded complete inhibition of mycelial growth of *C. canescens*. In case of non-systemic fungicides, The 100 per cent mycelial inhibition was recorded in mancozeb at 0.2 and 0.3 per cent concentrations. These findings are in conformity with work of Hemachandra (2013) [6] who reported that copper oxichloride, mancozeb, thiram (0.25%) and chlorothalonil (0.2%) were effective with 100 per cent inhibition against *C. beticola*. In case of four combi products, cabendazim 12% + mancozeb 63%, hexaconazole 18% + zineb 68% and captan 70% + hexaconazole 5% at all the concentrations and carboxin 37.55% + thiram 37.55% at 0.1 and 0.2 per cent concentration showed 100 per cent inhibition of mycelia growth. These findings are in conformity with work of Swamy (2010) [14] in case of *Cercospora capsici* in chilli.

Among fungal bioagents tried, *Trichoderma virens* (77.82%) *T. harzianum* (76.92%) and *T. viride* (76.15%) were found to inhibit mycelial growth of *C. longipes* in that order. However, *P. fluorescens* (56.15%) as a bacterial antagonist was found less effective. Higher efficacy by fungal antagonists may be due to antibiosis mechanism as reported by Brain (1951) [4] in *Trichoderma* spp. or mycoparasitism mechanism as reported by Mukherjee *et al.* (2000). Similar results are reported by Siddaramaiah (1986) [13] in case of leaf spot of mulberry caused by *C. moricola*. Prashanth (2004) [11] in case of *C. kikuchii* and Veena (2012) in case of leaf spot of greengram for the higher efficacy of fungal antagonists. *A. indica* (51.62%) and *Jatropha curcas* (51.62%) at 10 per cent concentration showed highest inhibition and indicating plant extracts may be better for *C. longipes*. These findings are in conformity with work of Asaduzzaman (2013) [3] reported that, garlic tablet solution of 1:1 w/v concentration against *C. capsici* was found promising in inhibiting radial mycelia growth in laboratory conditions. Hemachandra (2013) [6] and Smamy (2010) also found mycelial inhibition of *Cercospora* spp. in sugarbeet and chilli crops by plant extracts

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