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RESEARCH ARTICLE

Exploration of plant extracts and fungal antagonists against *Sclerotium rolfsii* causing sclerotium wilt of stevia

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ABSTRACT

Stevia is an important medicinal plant used as a low calorie sweetener. Sclerotium wilt caused by *Sclerotium rolfsii* is an important disease and is a major constraint in stevia cultivation. Evaluation of the biocontrol agents indicated that maximum inhibition of mycelial growth of *S. rolfsii* (78.51%) was noticed in *Trichoderma harzianum* (Dharwad isolate). Among botanicals tested, Durantha was highly effective at 10 per cent (75.14) followed by Glyrecidia (72.14%).

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INTRODUCTION

Stevia (*Stevia rebaudiana* Bertoni) is a herbaceous perennial plant of the Asteraceae, native to Paraguay where it grows in sandy soils near streams (Katayama *et al.,* 1976). The leaves of this plant are 30 times sweeter than sugar with zero calories where as pure extract is 300 times sweeter than sugar. The main active ingredient, stevioside, is 100 to 300 times as sweet as sucrose. The glycosides in its incredible sweetness, makes it unique among the nearly 300 species of stevia plants. Stevia is likely to become a major source of high potency sweetener for the growing natural food market in the future. Fungicidal sprays are generally recommended for the control of this disease. But extensive use of chemicals leads to serious environmental problems; development of resistance and it may also affect the quality of the crop as many people consume it. Therefore, it becomes necessary to look for economically better and safer means of disease control.

MATERIAL AND METHODS

***In vitro* evaluation of plant extracts :**

Fresh plant materials were collected and washed first in tap water and then in distilled water. Hundred grams of fresh sample was chopped and then crushed in a surface sterilized pestle and mortar by adding 100 ml sterile water (1:1 w/v). The extract was filtered through two layers of muslin cloth. Finally filtrate thus obtained was used as stock solution.

To study the antifungal mechanism of plant extracts, the poisoned food technique was used (Nene and Thapliyal, 1982). Five and ten ml of stock solution was mixed with 95 and 90 ml of sterilized molten potato dextrose agar(PDA) medium, respectively so as to get 5 and 10 per cent concentration. The medium was thoroughly shaken for uniform mixing of extract. Twenty ml of medium was poured into sterile Petriplates. Mycelial discs (five mm) of *Sclerotium rolfsii* were cut out by sterile cork borer and one such disc was placed at the centre of each agar plate. Totally 14 plant extracts were evaluated at two concentrations. Controls were also maintained by growing the pathogen on PDA plates. Then such plates were incubated at 27±1^{0}C temperature and radial growth was taken when maximum growth occurred in control plates. The efficacies of plant extracts were expressed as per cent inhibition, which was calculated by using the formula suggested by Vincent (1947) :

$$I = \frac{[C - T]}{C} \times 100$$

where,

I = Per cent inhibition

C = Radial growth in control
T = Radial growth in treatment

***In vitro* evaluation of biocontrol agents :**

Seven biocontrol agents such as *Trichoderma harzianum, Trichoderma harzianum* (Dharwad isolate), *Trichoderma koningii, Trichoderma virens, Trichoderma viride, Pseudomonas fluorescens* and *Bacillus subtilis* were tested against *Sclerotium rolfsii.* Both biocontrol agents and test fungus were cultured on potato dextrose agar in order to get fresh and active growth of fungus. The cultures of antagonistic microorganisms used in the present study were obtained from the Project Directorate of Biological Control (PDBC) Bangalore, Karnataka state.

Twenty ml of sterilised and cooled potato dextrose agar was poured into sterile Petriplates and allowed to solidify. For evaluation of fungal biocontrol agents, mycelial discs of test fungus was inoculated at one end of the Petriplate and antagonistic fungus was placed opposite to it on the other end. In case of evaluation of bacterial antagonist, the bacterium was streaked at middle of the Petriplates and mycelial discs of the fungus was placed at the centre. The plates were incubated at 27±1^{0}C and zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organism. The colony diameter of pathogen in control plate was also recorded. The per cent inhibition of the growth of the pathogen was calculated by using the formula given by Vincent (1947).

RESULTS AND DISCUSSION

The results of the present investigation along with relevant discussion have been presented in the following sub heads :

***In vitro* evaluation of plant extracts :**

It is evident from the data (Table 1) that among 18 plant extracts evaluated, *Durantha repens* L. showed maximum inhibition of mycelial growth (72.54%) and was on par with *Glyrecidia* (70.62%) and both were significantly superior over all other plant extracts. Among the two concentrations, the leaf extracts at 10 per cent were significantly superior to five per cent. In the interaction between plant extract and concentration, *Durantha repens* showed significant increase in inhibition of mycelial growth at 10 per cent concentration (75.14%) compared to 5 per cent concentration (69.92%) and was at par with *Glyrecidia* at 10 per cent concentration (72.74

Table 1 : Inhibition of mycelial growth of *Scerotium rolfsii* by different plant extracts

Sr. No.	Plant extracts	Per cent inhibition of mycelial growth Concentration (%)		Mean
		5	10	
1.	Adathoda	16.03 (23.62)	22.11 (28.06)	19.07 (25.84)
2.	*Bougainvillea spectabilis*	27.78 (31.82)	44.63 (41.94)	36.20 (36.88)
3.	Calotropis	13.33 (21.43)	16.77 (24.19)	15.05 22.808
4.	*Cassia fistula* L.	10.37 (18.79)	12.03 (20.30)	12.05 (20.28)
5.	*Durantha repens*	69.92 (56.77)	75.14 (60.13)	72.54 (59.65)
6.	*Eucalyptus globes*	33.77 (35.55)	44.81 (42.04)	39.29 (38.80)
7.	*Euphorbium odoratum*	19.00 (25.85)	27.84 (31.86)	23.42 (28.85)
8.	*Allium sativum*	24.74 (29.84)	31.77 (34.32)	28.25 (32.08)
9.	*Glyrecidia maculata*	68.51 (55.95)	72.74 (58.55)	70.62 (65.31)
10.	*Jatropa carcas*	25.33 (30.23)	33.66 (35.48)	29.50 (32.85)
11.	*Prosopis juliflora*	10.00 (18.44)	13.73 (21.76)	11.01 (19.37)
12	*Lantana camera*	36.66 (37.28)	45.81 (42.61)	41.24 (39.95)
13.	*Azadirachta indica*	38.66 (38.47)	47.62 (43.66)	43.14 (41.06)
14.	*Allium cepa*	13.66 (21.70)	17.88 (25.02)	15.77(23.36)
15.	*Parthenium hysterophorus*	46.00 (42.72)	50.74 (45.44)	48.37 (44.08)
16.	*Pongamia glabra*	30.66 (33.64)	38.62 (38.44)	34.64 (36.04)
17.	*Tridax procumbens*	29.33 (32.81)	33.70 (35.50)	31.51 (34.15)
18.	*Ocimum sanctum*	18.00 (25.11)	23.70 (29.14)	20.85 (27.12)
	Mean	29.16 (31.99)	36.30 (36.58)	–
		Plant extract (P)	Concentration (C)	P x C
	S.Em±	0.18	0.05	0.24
	CD at 1%	0.67	0.18	0.89

*Figures in the parenthesis indicate angular transformed values

%). *Cassia fistula* L. showed least inhibition of mycelial growth (12.03%).The antifungal properties of plant extracts may be due to some antimicrobial substances present in the extract.

***In vitro* evaluation of bioagents :**

Among the nine bioagents evaluated against *S. rolfsii*, *Trichoderma harzianum* (Dharwad and PDBC isolates) inhibited maximum mycelial growth (78.51% and 76.29%), which was on par with *T. virens* (77.03%). Next best were *Trichoderma viride* Pers. (76.01%) followed by *Trichoderma koningii* (73.54%). However least inhibition was noticed in *B. subtilis* (40.99%) (Table 2).

Table 2 : Inhibition of mycelial growth of *Sclerotium rolfsii* by different biocontrol agents

Sr. No.	Biocontrol agents	Per cent inhibition of mycelial growth of *S.rolfsii*
1.	*Bacillus subtilis*	40.99 (39.76)
2.	*Pseudomonas fluorescens*	61.11 (51.41)
3.	*Trichoderma koningii*	73.54 (59.65)
4.	*Trichoderma virens*	77.03 (61.32)
5.	*Trichoderma viride*	76.01 (61.88)
6.	*Trichoderma harzianum* (Dharwad isolate)	78.51 (61.23)
7.	*Trichoderma harzianum*	76.29 (60.83)
	Mean	70.59 (65.28)
	S.Em±	0.52
	CD @ 1%	2.16

The results of dual culture technique on *S. rolfsii* revealed that all the five fungal antagonists significantly reduced the growth of *S. rolfsii* either by over growing or by exhibiting inhibition zones, except bacterial bioagent. *T. harzianum* is more effective antagonist which in addition to secreting antibiotic non-volatile compounds, also causes rapid death of the pathogen by mycoparasitism and lysis and could be therefore selected as a potential biocontrol agent against the pathogen (Robinson and Park,1966; Dennis and Webster, 1971).

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