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Evaluation of aqueous extracts of some plants against Cauliflower Stalk Rot pathogen, *Sclerotinia sclerotiorum*

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Acqueous extracts of ten plants belonging to different families were tested *in vitro* at three different concentrations (5, 10 and 15%) against the cauliflower stalk rot pathogen *Sclerotinia sclerotiorum* in terms of radial growth, myceliogenic, carpogenic and ascospore germination. In all the *in vitro* experiments with plant extracts garlic bulb extract (GBE) recorded highest inhibition of radial growth, myceliogenic, carpogenic and ascospore germination of the fungus. GBE was followed by acqueous leaf extracts of *Azadirachta indica* or *Tagetes erecta* in one experiment or the other. All the other plant extracts significantly reduced the pathogen on the above parameters. GBE at 15% concentration completely inhibited radial growth of the pathogen which was at par with Carbendazim (Bavistin 50 WP @ 0.1%) treatment. On chromatographic analysis of GBE four major spots were noticed with Rf value of 0.81, 0.42, 0.21 and 0.12. Compounds with Rf value 0.81 was found to completely inhibit the growth of *S. sclerotiorum* while in other three compounds complete growth was observed 5 days after inoculation.

Key words: Aqueous plant extracts, cauliflower stalk rot, garlic bulb, *Sclerotinia sclerotiorum,* thin layer chromatography.

INTRODUCTION

Cauliflower (*Brassica oleracea* var. *botrytis* L. subvar. cauliflower DC) is the most popular vegetable among cole crops in India. India ranks second in production of cauliflower after China. However, in terms of productivity India find its rank towards the bottom of the list. This is mainly due to various biotic and abiotic factors. Among the fungal diseases, stalk rot caused by *Sclerotinia sclerotiorum* (Lib) de Bary is one of the most destructive diseases of cauliflower. The stalk rot disease has restricted the production of cauliflower seed in northern India for more than 30 years. In recent years, losses from this disease are recorded as high as 30 per cent, with a few individual field losses exceeding 80 per cent in Solan, Kulu and Kashmir Valley. The average seed yield has also decreased from 625 to 250 kg per ha (Verma and Sharma, 1999). The future of the seed production industry is greatly threatened with the occurrence of the stalk rot disease.

The pathogen *Sclerotinia sclerotiorum* is a soil-inhabiting one and is considered as one of the most omnivorous and successful plant pathogens known to mankind. A number of fungicides are evaluated against this disease and found effective in reducing the damage on cauliflower crop. However, chemical control measures alone are not econimical and eco-friendly. Their residual toxicity, wide spectrum activity and the continuous use of these potentially hazardous chemicals is posing an increasing threat to environment. Moreover, pesticides contamination of the environment is harmful to wild life and to other non-target beneficial microorganisms. Hence, use of bio-products to manage plant diseases is gaining importance to the researchers and farming community for the cause of sustainable and organic agriculture. Considering the economic importance of cauliflower and reduction in its production due to stalk rot disease in India and growing demand or ofganically grown food in these days, this work has been undertaken with the objectives of finding out the suitable plant extract against the pathogen.

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MATERIALS AND METHODS

Preparation of acqueous extracts of plants

Acqueous extracts of ten plants (Table 1) were evaluated against *Sclerotinia sclerotiorum.* Fresh leavesof *Azadirachta indica, Ocimum sanctum, Vinca rosea, Withamia Somnifera, Ranunculus asiaticus, Moringa oleifera, Tagetes erecta, Polyalthia longifolia* and *Curcuma longa* and bulbs of *Allium sativum* were collected and washed properly with distilled water. Hundred grams of fresh and washed plant material (leaves and bulbs) were ground well with 100 ml (1:1 w/v) sterilized distilled water separately. The macerate was filtered through double layer muslin cloth. The extract thus obtained was considered as standard extract (100% stock).

Table 1 : Plant names with families used in the present study

Common name	Botanical Name	Familes
Neem	*Azadirachta indica*	Meliaceae
Garlic	*Allium sativum*	Liliaceae
Tulsi	*Ocimum sanctum*	Labiatae
Periwinkle	*Vinca rosea*	Apocynaceae
Ashwagandha	*Withamia somnifera*	Solanaceae
Buttercup	*Ranunculus asiaticus*	Ranunculaceae
Drumstick	*Moringa oleifera*	Moringaceae
Marigold	*Tagetes erecta*	Asteraceae
Debdaru	*Polyalthia longifolia*	Annonaceae
Haldi	*Curcuma longa*	Zingiberaceae

Effect of plant extracts on radial growth of S. sclerotiorum in vitro

Poisoned food technique was adopted for evaluating the plant extracts against *Sclerotinia sclerotiorum* at 5, 10, 15 per cent concentration *in vitro.* The medium without plant extracts served as the control. Medium with plant extracts were autoclaved at 121°C for 20 minutes. The 5 mm disc of 4 days old culture was cut out through the flame sterilized cork borer and transferred in the center of Petri plates. After inoculation the plates were incubated at 25°C for 7 days. The observations were recorded after 4 days and 7 days of inoculation. The percent inhibition was determined with help of mean colony diameter and calculated by using following formula : Per cent inhibition = (X-Y)/X × 100; Where, X = colony diameter in control, Y = colony diameter in treated medium.

Effect of plant extracts on myceliogenic germination of S. Sclerotiorum

The effect of plant extracts on myceliogenic germination of sclerotia was studied by keeping surface sterilized sclerotia in plant extract solutions of three different concentrations (5, 10 and 15%) for 2. Twenty five sclerotia were kept for each treatment and replicated three times. Sclerotia were removed with the help of fine forceps and placed on sterilized blotting paper to remove excess of plant extract solutions and then placed on PDA plates. For positive control, same numbers of sclerotia were dipped in Bavistin (Carbendazim) solution (0.1%) for the same period and negative control was maintained by dipping sclerotia in sterilized distilled water only. Petri plates were incubated at 25°C for 7 days and data on sclerotial germination were recorded.

Effect of plant extracts on carpogenic germination of S. sclerotiorum

The effect of plant extracts on carpogenic germination of sclerotia was studied by treating the sclerotia with plant extracts as mentioned above and then placed inside a 100 ml sterile conical flask with 10 ml of sterile distilled water. For positive control, same number of sclerotia were dipped in Bavistin solution (0.1%) for the same period and negative control was maintained by dipping sclerotia in sterilized distilled water only. Conical flasks were plugged and incubated at 18°C for 60 days and data on carpogenic germination of sclerotia were recorded.

Effect of plant extracts on ascospore germination of S. sclerotiorum

To study the effect of plant extracts on ascospore germination of *S. sclerotiorum,* one drop of each of ascospore suspension and different concentrations (5, 10 and 15%) of various plant extracts were put separately into cavity slides under aseptic conditions. Three replications of each treatment were maintained. The slides were placed in Petri plates, lined with moist blotter paper to serve as moist chambers. For control, the spores were added to sterilized water. Per cent germination of spores was recorded after 24 of incubation at 25°C. The data on spore germination were recorded on the basis of observation on 500 ascospores and tabulated.

Table 2 : Effect of plant extracts on radial growth of cauliflower stalk rot pathogen *Sclerotinia sclerotiorum in vitro*

Plant Extracts	Radial growth (mm)*								
	Five percent concentration		Mean	Ten percent concentration		Mean	Fifteen percent concentration		Mean
	4 days after	7 days after		4 days after	7 days after		4 days after	7 days after	
Azadirachta indica	35 (5.96)	72 (8.51)	7.23	30 (5.52)	52 (7.24)	6.38	20 (4.52)	31 (5.61)	5.06
Allium sativum	10 (3.23)	25 (5.05)	4.14	9 (3.08)	15 ((3.93)	3.51	0 (0.70)	0 (0.70)	0.70
Curcuma longa	40 (6.36)	80 (8.97)	7.67	35 (5.95)	65 (8.09)	7.03	30 (5.52)	49 (7.03)	6.27
Ocimum snactum	38 (6.20)	75 (8.68)	7.44	30 (5.52)	58 (7.58)	6.55	25 (5.04)	45 (6.74)	5.89
Vinca rosea	44 (6.67)	80 (8.97)	7.82	39 (6.28)	62 (7.90)	7.09	34 (5.87)	50 (7.10)	6.49
Withamia somnifera	28 (5.33)	65 (8.09)	6.71	20 (4.52)	35 (5.95)	5.24	15 (3.93)	25 (5.05)	4.49
Ranunculs asiaticus	40 (6.36)	75 (8.68)	7.52	35 (5.95)	60 (7.77)	6.86	26 (5.15)	45 (6.74)	5.94
Moringa oleifera	43 (6.59)	90 (9.49)	8.04	40 (6.33)	72 (8.51)	7.42	39 (6.28)	60 (7.77)	7.03
Tagetes erecta	39 (6.28)	75 (8.68)	7.48	35 (5.95)	60 (7.75)	6.85	25 (5.05)	40 (6.36)	5.70
Polyalthia longifolia	48 (6.96)	90 (9.51)	8.23	42 (6.51)	75 (8.68)	7.60	43 (6.57)	62 (7.90)	7.23
Carbendazim (0.1%)	0 (0.70)	0 (0.70)	0.7	0 (0.70)	0 (0.70)	0.70	0 (0.70)	0 (0.70)	0.70
control50 (7.10)	90 (9.51)	8.31	50 (7.10)	90 (9.51)	8.31	50 (7.10)	90 (9.51)	8.31	

*Mean of three replications Figures in parentheses are square root transformed values

	CD at 5%	SEm	CD at 5%	SEm	CD at 5%	SEm
Plant extract	0.10	0.03	0.09	0.03	0.80	0.28
Concentration	0.07	0.02	0.03	0.01	0.06	0.02
Plant extract x concentration	0.24	0.08	0.11	0.03	0.21	0.07

Chromatographic Analysis of Garlic Bulb Extract (GBE)

To analyze GBE chromatographically, garlic bulb (1 kg) was soaked in methanol for 48 after peeling off the skin and cutting into pieces. The extract was filtered with double layer muslin cloth. the methanol extract was partitioned with hexane. Two fractions (methanol extract was hexane extract) thus obtained were concentrated in rotary evaporator. The concentrated residues were tested against *S. sclerotiorum* by paper disc method using ascospore solution. Out of the two fractions, the fraction which showed the maximum zone of inhibition against *S. sclerotiorum* was dissolved in acetone and chromatographed on (Thin Layer Chromatography) TLC plates (silica gel 60 F_{254} Merck) by developing in hexane solvent system and visualizing by iodine vapour. The Rf (Retention Factor values of the compounds) separated on TLC plates were calculated using the following formula : Rf value = Distance traveled by the analyte from origin/Distance traveled by the solvent front from origin.

These compounds were further purified using prepatory TLC (silica gel 60 F_{254} Merck) by developing in hexane solvent system and visualizing by iodine vapour. The bands from preparatory TLC plate were scraped and separated using acetone. By preparatory TLC four compounds were separated which were tested against *S. sclerotiorum* by paper disc method using ascospore solution to find out the most antifungal compound.

RESULTS AND DISCUSSION

The antifungal activity data of different aqueous plant extracts against *S. sclerotiorum* are presented in Table 2. All the plant extracts significantly reduced the radial growth of the test fungus at all concentrations (5, 10 and 15%). 15% concentration of all the plant extracts were found significantly superior as compared to other two concentration in inhibiting radial growth of *S. sclerotiorum.* Among the plant extracts tested, bulb extract of *Allium sativum* was found to be the most effective in checking the radial growth of the fungus as it completely inhibited the radial growth of *S. sclerotiorum* at 15% concentration (Fig. 1) as compared to mean radial growth of 8.31 mm in control. At 15% concentration *A. sativum* proved to be as good as Carbendazim (Bavistin 50 WP @ 0.1%) which also completely inhibited radial growth of the fungus when tested by

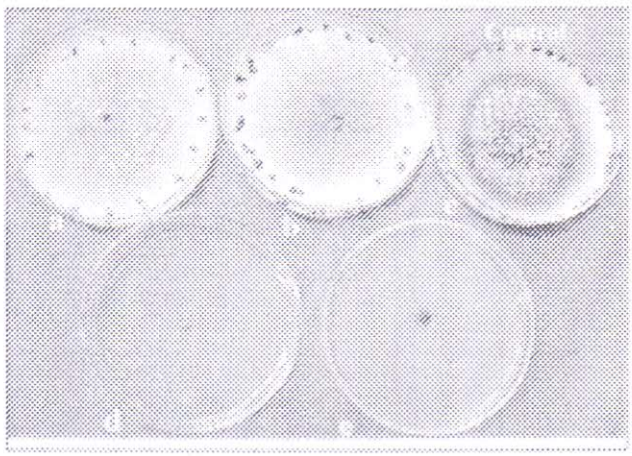

Fig 1. Growth of *S. sclerotiorum* on PDA poisoned with 15 aqueous extract of (a) *Moringa oleifera* (b) *Polyalthia longifolia* (c) Control (d) Bavistin (0.1%) (e) *Allium sativum* 10 days after inoculation

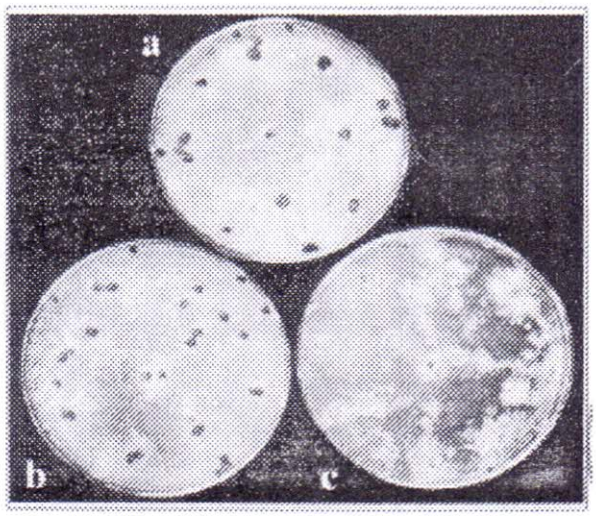

Fig 2. Comparison of size of *S. sclerotiorum* on PDA plates poisoned with aqueous plant extracts (5%) (a) Control (b) *Tagetes erecta* (c) *Allium sativum*

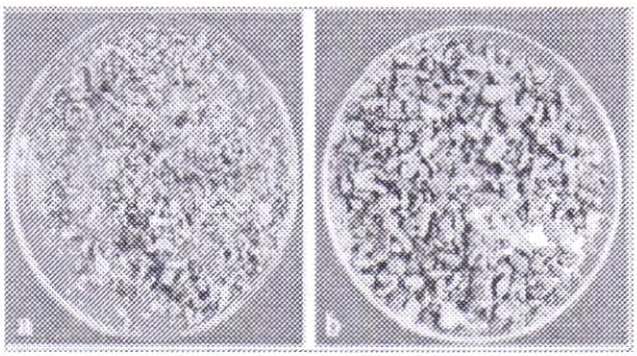

Fig 3. (a) Sclerotia of *S. sclerotiorum* harvested from PDA plates poisoned with 5% aqueous extract of *A. sativum* (b) Control plate

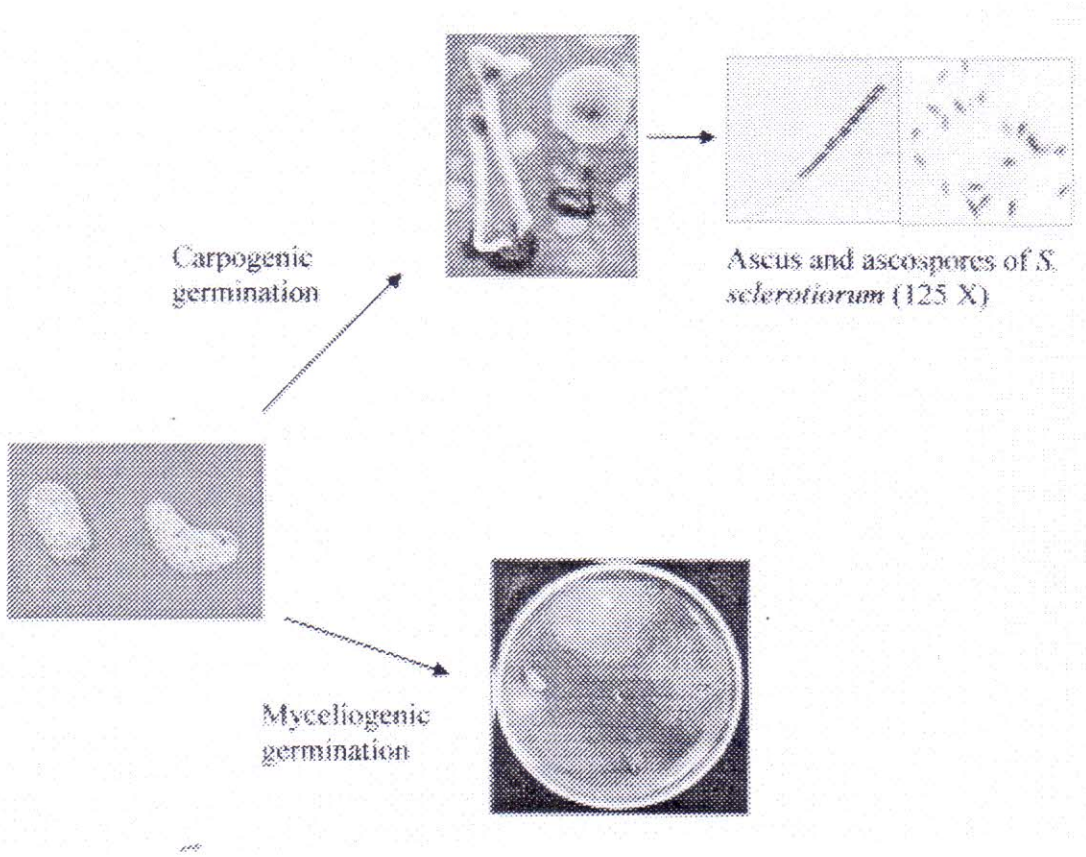

Fig 4. Two types of germination of sclerotia of *S. sclerotiorum*

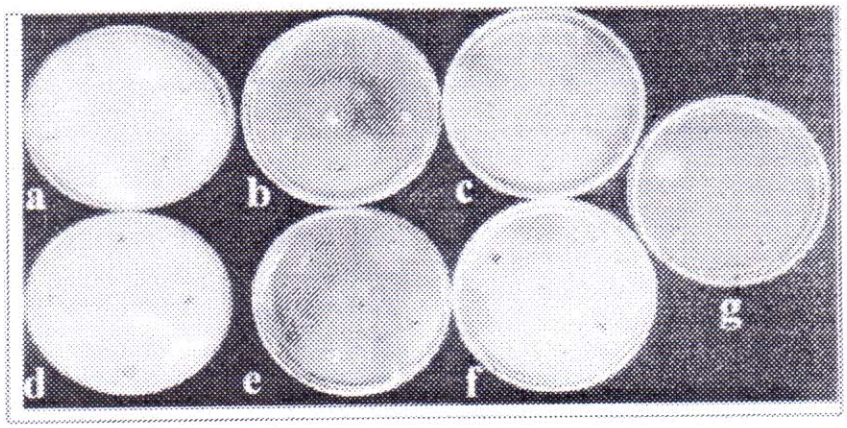

Fig 5. Effect of aqueous plant extracts (15%) on myceliogenic germination of sclerotia of *S. sclerotiorum* (a) Control (b) *T. erecta* (c) *P. longifolia* (d) *M. oleifera* (e) *A. indica* (f) *C. longa* (g) *A. sativum* 7 days after inoculation

poisoned food technique. At 15% concentration *A. sativum* bulb extract was followed by *Withamia somnifera* and *Azadirachta indica* which reduced the radial growth of the fungus to 25 mm and 31 mm repectively as compared to control (90 mm) 7 days after inoculation. The least effective in reducing the radial growth of *S. sclerotiorum* was *Polyathia longifolia* which allowed 62 mm of radial growth of the pathogen at 15% concentration 7 days after inoculation as compared to 90 mm in control. At 5% concentration of *A. sativum* and *A. indica* considerable reduction in size of sclerotia formed of PDA medium observed (Figs 2 and 3).

Two types of germination (Fig. 4) namely myceliogenic and carpogenic are possible in sclerotia of *S. sclerotiorum*. Plant extracts were evaluated *in vitro* against both kinds of germination which were achieved while carrying out this research work.

The data regarding the effect of plant extracts on myceliogenic germination of sclerotia of *S. sclerotiorum* presented in Table 3 revealed that all the plant extracts significantly reduced the myceliogenic germination of sclerotia of the test fungus in all the three concentrations (5, 10 and 15%). However, 15% concentration was most effective as compared to the other two concentrations (5 and 10%). Bulb extract of *A. sativum* was found to be most effective in reducing the myceliogenic germination (6.0%) as compared to contrl (100%). *A sativum* (at 15%) was only next to carbendazim (Bavistin 50 WP @ 0.1%) in effectiveness which resulted in complete inhibition of myceliogenic germination of sclerotia (Fig. 5). At 15% concentration *A. sativum* was followed by *T. erecta* and *A. indica* which reduced the myceliogenic germination to 42.3% and 52.0% respectively from 100% in control. The effectiveness of *O. sanctum* (54.0%) and *R. asiaticus* (56.0%) was at par. The least effective in reducing myceliogenic germination were *C. longa* (76.0%) and *P. longifolia* (79.3%) which were statistically non-significant.

The data presented in Table 4 clearly indicated that all the plant extracts caused a significant reduction in the carpogenic germination of sclerotia of *S. sclerotiorum* relative to control at all three concentrations (5, 10 and 15%). However, 15% concentration was found to be superior to two other concentrations in respect of all the plant extracts. Bulb extract of *A. sativum* at 15% concentration was found to be most effective than other plant extracts in reducing carpogenicgermination of sclerotia from 92% in control to 13% which is at par with Carbendazim (Bavitin 50 WP @ 0.1%) treatment. This was followed by *T. erecta, A. indica* and *W. somnifera* which resulted in per cent carpogenic germination of sclerotia ranging from 49% to 59%. *M. oleifera* was the least effective on reducing carpogenic germina-

Table 3 : Effect of plant extracts on myceliogenic germination of sclerotia of *S. sclerotiorum*

Plants	Sclerotia germination % at different concentration*		
	5	10	15
Azadirachta indica	70.6 (57.2)	64.7 (53.5)	52.0 (46.1)
Allium sativum	25.0 (29.9)	12.0 (20.2)	6.0 (14.0)
Curcuma longa	98.3 (82.8)	86.3 (68.4)	76.0 (60.7)
Ocimum sanctum	96.7 (80.2)	77.0 (6.14)	54.0 (47.3)
Vinca rosea	96.0 (78.7)	82.0 (64.9)	64.7 (53.5)
Withamia somnifera	89.3 (71.0)	69.7 (56.6)	59.0 (50.2)
Ranunculus asiaticus	78.7 (62.6)	65.0 (53.8)	56.0 (48.4)
Moringa oleifera	98.7 (83.4)	84.7 (67.1)	72.0 (58.1)
Tagetes erecta	74.0 (59.4)	60.0 (50.8)	42.3 (40.6)
Polyalthia longifolia	97.7 (81.6)	83.7 (66.2)	79.3 (63.0)
Carbendazim (0.1%)	0 (4.0)	0 (4.0)	0 (4.0)
Control	100 (85.9)	100 (85.9)	100 (85.9)
Figures in parenthses are arc sin transformed values			
*Mean of three replications			
CD (at 5%)	5.23	4.37	3.84
SEm	1.79	1.49	1.31

Table 4 : Effect of plant extracts on carpogenic germination of sclerotia of *S. sclerotiorum*

Plants extracts	% Carpogenic germination of sclerotia*		
	5% concentration	10% concentration	15% concentration
Azadirachta indica	78 (62.0)	65 (53.7)	54 (47.3)
Allium sativum	24 (29.3)	19 (25.8)	13 (20.2)
Curcuma longa	88 (69.7)	76 (60.7)	65 (53.7)
Ocimum sanctum	92 (73.6)	84 (66.5)	71 (57.4)
Vinca rosea	84 (66.4)	79 (62.7)	63 (52.5)
Withamia somnifera	86 (55.5)	68 (55.5)	59 (50.4)
Ranunculus asiaticus	90 (71.6)	81 (64.1)	67 (54.9)
Moringa oleifera	92 (73.8)	90 (71.7)	89 (70.7)
Tagetes erecta	80 (63.5)	63 (52.5)	49 (44.2)
Polyalthia longifolia	92 (74.0)	87 (68.9)	82 (64.9)
Carbendazim (0.1%)	13 (20.3)	13 (20.3)	13 (20.3)
Control	92 (73.6)	92 (73.6)	92 (73.6)
Figures in parentheses are arc sin transformed values			
*Observation on 25 sclerotia in each of three replications			
CD at 5%	3.20	3.02	2.67
SEm	1.09	1.03	0.91

Table 5 : Effect of plant extracts on ascospore germination of cauliflower stalk rot pathogen *Sclerotinia sclerotiorum in vitro*

Plants Extracts	% Germination of ascospores*			
	5 per cent concentration	10 per cent concentration	15 per cent concentration	Mean
Azadirachta indica	32 (34.4)	24 (29.3)	19 (25.8)	29.8
Allium sativum	25 (29.9)	18 (25.1)	10 (18.4)	24.5
Curcuma longa	38 (38.1)	28 (3.19)	22 (27.9)	32.7
Ocimum sanctum	35 (36.2)	27 (31.3)	20 (26.5)	31.4
Vinca rosea	30 (33.2)	24 (29.3)	20 (26.5)	29.7
Withamia somnifera	31 (33.8)	20 (26.5)	15 (22.8)	27.7
Ranunculus asiaticus	35 (36.3)	30 (33.2)	25 (29.9)	33.2
Moringa oleifera	40 (39.2)	36 (36.9)	32 (33.8)	36.6
Tagetes erecta	30 (33.2)	21 (27.2)	14 (21.9)	27.5
Polyalthia longifolia	40 (39.2)	34 (35.6)	30 (33.2)	36.0
Carbendazim (0.1%)	4 (11.5)	4 (11.5)	4 (11.5)	11.5
Control	43 (40.9)	43 (40.9)	43 (40.9)	40.9
Mean	33.8	29.9	26.6	

Figures in parentheses are arc sin transformed values

*Observationon 500 ascospores in each of three replications

	CD at 5%	SEm
Plant extracts	: 0.78	0.26
Concentration	: 0.49	0.17
Plant extracts x concentration	: 1.68	0.59

tion of sclerotia of *S. sclerotiorum* which recorded 89% germination as compared to 92% in control.

It is evident from the data (Table 5) that aqueous extracts of all plant extracts were significantly effective in reducing ascospore germination of *S. sclerotiorum* relative to control in all the three concentrations (5, 10 and 15%) of which 15% concentration was found to be superior to two other concentrations in respect of all the plant extracts. The bulb extract of *A. sativum* was found to be most effective, significantly reducing the germination of ascospore of *S. sclerotiorum* from 40.9% in control to 24.5% followed by *T. erecta* (27.5%) and *W. somnifera* (27.7%) which were statistically at par with each other. Similarly, the effect of *V. rosea* (29.7%) and *A. indica* (29.8%); *C longa* (32.7%) and *R. asiaticus* (33.2%) and *P. longifolia* (36.0%) and *M. oleifera* (36.6%) were found to be statistically non-significant.

This result is in agreement with the findings of Singh *et al.*, (2003) who reported that garlic leaf extract inhibited radial growth of *S. sclerotiorum*, the casual agent of steam rot of ajowan to the extent of 82.2%. When *A. sativum* and *Azadirachta indica* were compared in the present study, the *A. indica* was not much effective in reducing the radial growth of the fungus which was in corroboration with the reports of Sharma and Basandrai (1997) who found that leaf extracts of *A. indica* did not perform best in inhibiting the radial growth of *S. sclerotiorum* while *A. sativum* bulb extract performed the best. Neem extract was only as effective as fungicide when applied at higher concentration (5000 ppm) (Qais *et al.*, 2004). Inhibitory effect of garlic preparation (trade name Bioczos BR) was reported by Sapieha *et al.* (2000). The garlic preparation at 10% concentration stunted the growth of *S. sclerotiorum*. At 2% concentration 63-78% control of the colony diameter was achieved after 4 days of incubation.

Garlic bulb extract was found more effective than leaf extract and bark extract of *A. arabica* against *M. phaseolina*. Presence of antibiotic constituents such as phenolic substances and other unknown substances are suggested to have inhibiroty activity of plant extracts (Dubey and Dwivedi, 1991).

Besides inhibition of radial growth of the fungus, plant extracts also inhibited the myceliogenic, carpogenic and ascospore germination of *S. sclerotiorum*. Based on the perusal of earlier literature, it is understood that there is no report of inhibition of myceliogenic and carpogenic germination of

sclerotia and ascospore germination of *S. sclerotiorum* by GBE. Therefore, this may be regarded as the first of inhibition of myceliogenic, carpogenic and ascospore germination by garlic bulb extract.

However, volatic compounds from crude aqueous extracts of garlic bulbs are known to inhibit germination of microconidia and hyphal extension in *F. oxysporum* f. sp. *lycopersici* in axenic culture. At higher concentration the volatiles of garlic also inhibited production of microconidia and chlamydospores (Tariq and Magee, 1990). Garlic bulb extract also showed considerable amount of inhibition on growth of *A. solani*. A high reduction of enzymatic activities was observed for the fungi treated with garlic extract compared with untreated fungal cultures which may be a mechanism of inhibition by garlic bulb extract (Muhsin *et al.*, 2001). Antifungal property of garlic was attributable to vapours of the essential oil of garlic by Horberg (1998). Mycelial growth was completely inhibited by the vapours from garlic oil at 10-80 ppm concentration.

On chromatographic analysis of GBE (Fig. 6) four major spots were noticed with Rf value of 0.81, 0.42, 0.21 and 0.12. Compounds from all the spots were further purified by preparatory TLC and tested against *S. Sclerotiorum* by paper disc method. Compound D with Rf value 0.81 was found to inhibit completely the growth of *S. sclerotiorum* (Fig. 7) while in rest of the compounds complete growth was observed 5 days after inoculation.

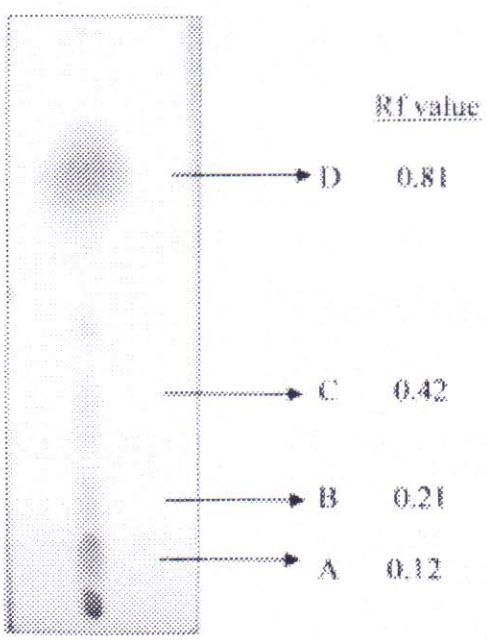

Fig 6. Thin Layer Chromatograph (TLC) of hexane extract of garlic bulb

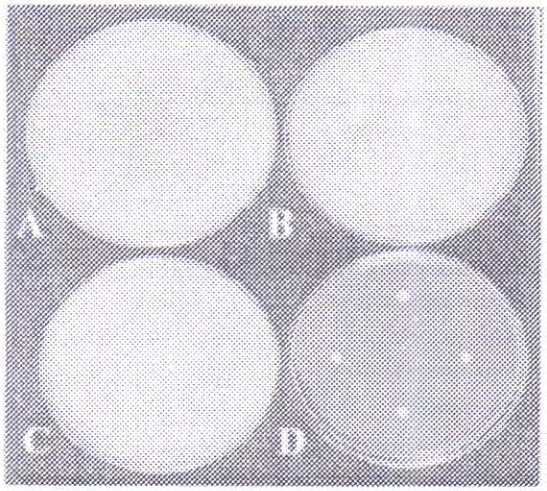

Fig 7. Effect of TLC separated compounds (A-0. 12, B-0-21, C-0.42 and D-0.81) of GBE on Growth of *S. sclerotiorum*

It is understood from the available literature that compound with Rf value 0.81 is being reported for the first time to be antifungal in the present study. However, compounds of different Rf values from garlic bulb extract were reported to possess different properties like promoting male or female flower production (Abou Hussen *et al.*, 1975b) and marked enhancement of flowering of squash plants at Rf 0.8 (Abou Hussein *et al.*, 1975 a).

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