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Research Article

BIOLOGICAL CONTROL OF POWDERY MILDEW DISEASE *SPHAEROTHECA FLUIGINEA* OF *CUCURBITA MAXIMA* (PUMPKIN) SURFACE OF LEAF ANTAGONISTS

***Vasantrao P. Pawar**
Arts and Science College Bhalod, Tal. Yawal
**Author for Correspondence*

ABSTRACT
Cucurbita maxima (Pumpkin) are cultivated crop members of family cucurbitaceae. The Bottle gourd in the field is affects by several pathogens like bacteria, virus, mycoplasma, nematodes and fungi. Out of 20 surface of leaf isolates of fungi or bacteria. Seven viz. *Trichoderma viride Ampelomyces quisqualis, Cladosporium cladosporioides, Fusarium pallidoroseum, Aspergillus niger, Alternaria alternata, and Pestalotiopsis dissminata.* However identified and found antagonistic to *Sphaerotheca fuliginea* (Schlecht ex. Fr.) Pollacci in dual culture on detached leaves of Bottle gourd. *Ampelomyces quisqualis* showed the mycoparasitic activity in dual culture. On the study of antagonism test in vitro. *Fusarium pallidoroseum, Cladosporium cladosporioides, Pestalotiopsis dissiminata,* and *Ampelomyces quisqualis* were adjudged as potential antagonists of *Sphaerotheca fuliginea.* All the antagonists significantly controlled powdery mildew over check as pre-and post. Inoculation sprays of spore suspension of antagonists on potted plants in glass house. Disease control was better in pre-inoculation spray application of biological control agent as compared to post inoculation application.

Keywords: *Cucurbita Maxima (Pumpkin), Biocontrol, Fungi*

INTRODUCTION
Cucurbita maxima (Pumpkin) is Rabi and Kharif season crop grown all over India in the tropical and sub tropical region. The crop field is attached by a number of bacterial, fungal viral diseases. Kapoor (1967); Shooty (1971); Goswami and Dasgupta (1981) and Mital *et al.,* (1985) observed powdery mildews on it mainly infected due to *Sphaerotheca fuliginea*. The disease appears in epiphytotic proportions every year and causes considerable loses. None of the management methods provide adequate level of disease control because of sulphur, Bavaistine and Calixin injury to cucurbits, and chemical hazards and non availability of powdery mildew resistant variety (Moursi and Sirry, 1956).
In the literature cited very little information was available regarding the host range of the pathogen; bio chemical changes occurs due to the pathogen and the eco-friendly management of the disease. The study was undertaken with an aim to isolate surface of leaf mycroflora, test their antagonistic activity and control of powdery mildew by potential antagonists in glass house.

MATERIALS AND METHODS
Isolation of surface of leaf bacteria, yeast and fungi were taken from powdery mildew infected and healthy sponge gourd plants by leaf wash method on potato dextrose, malt extract and nutrient broth agar in petriplats. Fungal cultures were purified by hyphal-tip method and single spore isolations. Cultures of bacteria were maintained on PDA and nutrient broth agar slants respectively, under refrigerated conditions. Culture of *Sphaerotheca fuliginea* was maintained on *Cucurbita maxima* plants. Antagonism in dual spore suspension was studied by putting 0.2 ml spore suspension (20 to 25 spores per microscopic field 40x). Each of an antagonists and sphaerotheca fuliginea in cavity slides and incubated in humid chamber at 28^0C for 24 h. Conidial germination and germ tube length were recorded. Similarly, antagonism on detached leaf was studied by dual culture technique. Inoculated Bottle gourd leaves place in sterilized petriplates containing 200 ppm benzimidazole solution. The distance between was kept at 5 mm. Each treatment was replicated thrice. Colony diameter of borth antagonists and *S. fuliginea* was measured 120 h after incubation at 25 ± 1^0C percent inhibition in growth of test pathogen over control was

calculated (Vincent, 1947). Effect of pre-and-post-inoculations with antagonists on powdery mildew development was studied by spraying spore suspension (5.44 x 106 ml $^{-1}$) with the help of atomizer on detached leaves of bottle gourd 12 h before and after inoculation and *S. fuliginea*. The inoculated leaves without antigonist served as check. Inoculated leaves in petriplates containing benzimidazole solution were inoculated at 25±1^{0}C for one week and data on powdery mildew development was recorded. Culture filtrates were obtained by growing antagonist in 250 ml Erlenmayer flask, each containing 50 ml of potato dextrose broth. The flask was inoculated at 25±1^{0}C for 10 days. Spore suspension of *S. fuliginea* (20 to 25 spores) was prepared in culture filtrates of each antagonists and dispensed (0.2 ml) on clean cavity slides. The slides were incubated in humid chamber at 28±1^{0}C. Data on germination was recorded 72 h after inoculation. In another experiment, culture filtrates were sprayed separately with the help of atomizer on detached sponge gourd leaves in petriplates.

At the same time fresh conidia *S. fuliginea* were dusted on leaves with the help of camel hair brush incubated at 25±1^{0}C for one week and data on powdery mildew development was recorded. In the glass house experiment, spore suspension (5.44 x 10^6 spores ml^{-1}) of promising antagonists were sprayed separately with the help of atmizer 12 h before and after inoculation of *S. fuliginea* on one month old potted plants of bottle gourd (CV. Poinsettle). Four plants were kept in each pot and each treatment was replicated four times. Control plants were maintained without spray of any antagonist. Both the sets of plants were kept in a glass house (29±3^{0}C) for one month. Data on powdery mildew development was recorded by using 0-4 scale and percent disease index (PDI) was calculated (Mickinney, 1923). The experiment was repeated thrice.

RESULTS AND DISCUSSION

Table 1: Effect of antagonists and its cultural filtrates on conidial germination and germ tube length of *S. fuliginea*

Antagonist	Direct antagonism			Cultural titrate	
	conidial germination %	Inhibition %	Germtube length (µm)	Germination %	Inhibition
Alternaria alternata	14.18	75.00	28.10	5.06	78.10
Cladosporium cladosporioides	15.00	73.76	32.73	11.50	61.38
Fusarium pallidoroseum	08.18	75.16	17.19	008.87	70.34
Aspergillur niger	47.00	23.60	29.00	07.33	81.53
Trichoderma viride	53.13	13.80	29.10	14.80	61.35
Pestalotiopsis dissiminata	22.15	62.47	23.17	12.67	58.21
Ampelomyces quisqualis	08.45	87.18	18.33	04.23	78.87
Check	62.55	---	52.10	34.00	--
LSD (P-0.05)	02.38	04.88	11.50	04.17	9.45

Table 2: Inhibition of *Sphaerotheca fuliginea* by antagonists and their cultural filtrates on detached leaves of sponge gourd

Antagonist	Direct antagonism			Cultural titrate	
	Pathogen	Antagonist	Inhibition	PDI	Disease control (%)
Alternaria alternata	3.43	2.43	88.97	30.00	82.80
Cladosporium cladosporioides	3.77	2.00	85.70	26.43	87.87
Fusarium pallidoroseum	0.00	4.43	100.00	07.00	92.00
Aspergillur niger	9.43	4.40	35.35	54.00	28.95
Trichoderma viride	5.00	2.40	73.84	71.77	12.10
Pestalotiopsis dissiminata	0.00	5.00	99.80	12.00	91.10
Ampelomyces quisqualis	0.00	4.00	100.00	8.00	99.80
Check	12.00	--	---	83.77	--
LSD (P-0.05)	2.05	2.99	13.47	3.10	4.22

Figures were sine transformed prior to analysis PDF percent diseuse index.

Table 3: Inhibition of *Sphaerotheca fuliginea* by pre-and-post-inoculation sprays of spore suspension of different antagonists on detached leave

Antagonist	Pre-Inoculation Spray			Post-Inoculation Spray		
	LFA (%) Antagonist	LFA(%) Pathogen	Inhibition %	LFA (%) Antagonist	LFA(%) Pathogen	Inhibition %
Alternaria alternata	03.00	15.00	75.89	00.00	14.67	75.09
Cladosporium cladosporioides	00.00	11.00	83.03	12.33	13.33	79.19
Fusarium pallidoroseum	92.00	00.00	98.05	32.67	00.00	100
Aspergillur niger	05.00	52.00	32.23	02.67	54.00	25.97
Trichoderma viride	00.00	28.33	62.88	06.00	22.00	68.36
Pestalotiopsis dissiminata	30.00	00.00	100	06.00	8.77	100
Ampelomyces quisqualis	09.38	00.00	100	03.67	9.43	87.35
Check	---	72.67	---	---	78.33	--
LSD (P-0.05)	07.65	2.22	5.35	13.10	4.32	5.69

LFA = Leaf are covered; figures were are sine transformed prior to analysis.

Table 4: Effect of pre-and post-inoculation spray of potential antagonists on sponge gourd powdery mildew development under glass house conditions

Antagonist	Pre-inoculation spray		Post-inoculation spray	
	PDI	Disease control %	PDI	Disease control %
Fusarium pallidoroseum	28.05	68.00	42.85	48.47
Cladosporium cladosporioides	53.89	29.14	59.00	55.79
Pestalotiopsis dissiminata	74.90	09.46	74.18	10.32
Ampelomyces quisqualis	15.75	89.38	18.50	80.30
Check	82.77	---	82.68	---
LSD (P-0.05)	6.50	8.20	08.50	11.45

Figures were sine transformed prior to analysis.

To the 20 surface of leaf isolates of fungi and bacteria seven fungi antagonist namely; *Alternaria alternata, Aspergillus niger, Ampelomyces quisqualis, Cladasporium cladosporioides, Fusarium pallidoroseum, Trichoderma viride* and *Pestalotiopsis dissiminata* were identified and found to be antagonists of *Sphaerotheca fuliginea*. Most of these antagonists have been reported by earlier workers (El-Hammaldey *et al.,* 1977; Besada, 1978; Srivastava and Bisht, 1986; Sztejnberg *et al.,* 1989; Falk *et al.*, 1996; Abo-Foul *et al*., 1996; Gu and Ko, 1997).

It was observed (Table 1) the results that all the antagonists inhibited conidial germination and reduced germ tube length *Sphaerotheca fuliginea* in dual spore suspension. Maximum conidial inhibition was obtained with *Ampelomyces quisqualis* (87%) followed by *Fusarium pallidoroseum*, *Fusarium pallidoroseum* and *Ampelomyces quaisqualis* are resulted in maximum reductions in germ tube length. Barner (1971); Fokkema and Lorbeer (1974); Yadva and Tripathi (1991) and Howell (2003) reported that inhibition of germ tube by antagonists had direct germination of several pathogenic fungi by saprophytic mycroflora.

The culture filtrate of antagonists also inhibited conidial germination of *S. fuliginea* on cavity slides. Maximum inhibition was obtained with *Ampelomyces quisqualis Fusiarium Pallidoroseum* and *Pestalotiposis dissiminata*. Similarly, spray of culture filtrate of *Ampelomyces* on detached sponge gourd leaves of sponge gourd resulted 100 per cent control of powdery mildew. However, culture filtrate of *Fusarium pallidoreoseum* as 92 percent disease control respectively. In dual culture on detached bottle gourd leaves, *Fusarium pallidoroseum, Pestalotiopsis dissiminata* and *Ampelomyces quisqualis* resulted complete inhibition of *Sphaerotheca fuliginea* (Table 2).

Hashioka and Nakai (1980) reported that the mycoparasitism of *A. quisqualis* on *Erysiphe choracearum*, *Sphaerotheca fuliginea* and other powery mildew causing fungi. Complete inhibition of *S. fuliginea* in present study may be due to mycoparasitism competition for space, nutrient and antibiosis Pre-and Post-inculation spray of spore suspension of *F. pallidoroseum* and *Pestalotiopsis dissiminata* resulted cent percent control of powdery mildew on detached leaves of sponge gourd (Table 3).

Puzanova (1984) and Dhanbir (2004) reported significant reduction in powdery mildew of cucumber by post inoculation of *Ampelomyces arteminsiae A- gypsophitae A. gypsophilae* and *A. novoae Ampelomyces guisqualis, Trichoderma viride, Aspergillius fumigantus* in present study showed myloparasitism in dual culture.

It is clear from the (Table 4) that pre-and post-inoculations of all test antagonists gave significant control of sponge gourd powdery mildew on potted plants in glass house. However pre inoculation spray with *Ampelomyces quisaqualis* gave maximum disese control (80.30%). The next best was *Fusarium pallidoroseum* (68%). In general pre inoculation spray was comparatively more effective than post inoculation spray with antagonists. *Cladosporium cladosporioides* as post inoculation spray resulted 56% disease control. Stenijnberg (1979) was reported successful control of *Sphaerotheca fuliginea* on cucumber of *A quisaualis* post inoculation spray of *A. quisqualis* in present investigation resulted 80% disease control.

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