INTERNATIONAL JOURNAL OF PLANT PROTECTION
VOLUME 11 | ISSUE 1 | APRIL, 2018 | 10-17

● e ISSN-0976-6855 | Visit us : *www.researchjournal.co.in*

IJPP

RESEARCH PAPER

DOI : 10.15740/HAS/IJPP/11.1/10-17

Assessing fungicides for seedling protection of cucumber to collar rot disease caused by *Sclerotium rolfsii*

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ARITCLE INFO

Received : 31.01.2018
Revised : 02.03.2018
Accepted : 10.03.2018

KEY WORDS :
Collar rot, Cucumber, Fungicide, *Sclerotium rolfsii*, Seedling protection

ABSTRACT

Sclerotium rolfsii Sacc. is known to be a serious pathogen on many crops of economic importance including cucurbits. Due to proliferic growth and ability to forming sclerotia, this pathogen is the major constraint in successful cultivation of cucumber. The present investigation was thus carried out to evaluate the potential of nine different fungicides *i.e.* carbendazim, thiophenate methyl, vinclozoline, captan, copper oxychloride, mancozeb, hexaconazole, mycobutanil and propiconazole at different concentrations of 10, 50, 100 and 150 ppm, on the growth inhibition of *S. rolfsii*. The primary assessment was made on *in vitro* screening of fungicides and its concentration whereby hexaconazole at lowest concentration (10 ppm) rendered the most vital effect ($P \leq 0.0001$) on growth reduction ability followed by propiconazole and mycobutanil. No growth of *S. rolfsii* observed on plates amended with hexaconazole when the concentration was further increased. Similar effect was traced in an experiment conducted on root trainer. The percent infected plant also provided the same impact of fungicides received in toxic-assay experiment. Maximum seedlings protection of cucumber was achieved through seed application of hexaconazole even at lowest concentration ($P \leq 0.01$); similarly, no mortality was detected on higher concentration of this fungicide. Although our result directly claiming the best effect of hexaconazole but we propose to use a combination of fungicides from different groups in order to avoid resistance development in *S. rolfsii* against a particular fungicide. A combination of carbendazim and hexaconazole is hereby proposed for seedling protection of cucumber to *S. rolfsii*.

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How to view point the article : Kumar, Ritesh, Ghatak, Abhijeet and Bhagat, Arun P. (2018). Assessing fungicides for seedling protection of cucumber to collar rot disease caused by *Sclerotium rolfsii*. *Internat. J. Plant Protec.*, **11**(1) : 10-17, **DOI : 10.15740/HAS/IJPP/11.1/10-17**.

INTRODUCTION

Sclerotium rolfsii is the fungal pathogen known for causing collar rot disease on a wide host species and causes severe economic loss worldwide. It causes disease over 270 host genera in the United States alone, and at least 100 families with 500 species of plants are

susceptible to this pathogen (Dandnaik *et al.*, 2006). *S. rolfsii* can infect seeds, seedlings, mature plants in the field, cause diseases to fresh vegetables and rhizomes, while in storage and transit (Dasgupta and Mandal, 1989). The pathogen is widely spread in tropical, subtropical and warm temperate regions of the globe, especially the southern United States, Central and South America, the West Indies, southern European countries bordering the Mediterranean, Africa, India, Japan, the Philippines, and Hawaii. It is rarely seen in the temperate area where the temperature falls below 0°C (Punja, 1985). This pathogen is grown well in a temperature range between 25 and 30°C; however, a recent report supported its adaptability to higher temperature regime *i.e.* 35°C making this pathogen of a great threat to the crops cultivated in tropical or sub-tropical areas (Kumar *et al.*, 2017).

S. rolfsii has a more diverse host range which mainly covers legumes, crucifers, and cucurbits (Kator *et al.*, 2015). Cucumber (*Cucumis sativus*) is a well-known host of this pathogen. Collar rot disease of cucumber is the major problem and has been a challenging task in the way of economical cultivation of cucumber crop. The first symptom of the disease is a mid-day wilting of the plant. Leaves turn yellow and within a few days, the plant collapses leading to plant death. The fungus develops a white mycelium around the collar portion, which may be fan-shaped, over the surface. Light brown bodies (sclerotia) may be observed embedded within the white mycelium. These sclerotia turn dark brown with age. The fungus also infects fruit in contact with infested soil, developing sunken, yellow spots which decay and collapse. Large amounts of white mycelium and sclerotia can form on the fruit as it decays. Sclerotia, which are the overwintering structure, allow the pathogen to survive for many years in the soil. Sclerotia are spread by movement of soil or by surface water. At an early stage, seedling decaying by collar rot infection is a major hindrance to cucumber cultivation. The disease is favoured by high temperatures (27–32ºC) and high soil moisture.

During the middle of the 20th century, *S. rolfsii* was controlled by fumigation. But this method of seedling protection is impractical in certain areas. Application of chemicals to the soil is cost-expensive and affects the environment severely. Now-a-days, control of this pathogen is still the subject of many research projects which involves chemicals, biological agents, soil amendments, cultural modifications, disease physiology, nutrition studies and resistant varieties (Kator *et al.*, 2015). Despite these efforts, *S. rolfsii*, like several other soil-borne fungal disease-causing agents, continues to be proved a difficult pathogen to control. The wide host range, prolific growth and ability to produce persistent sclerotia contribute to the large economic losses associated with the pathogen. In the line of management, strategic attempts have been done by various workers for *S. rolfsii* which also include, deep ploughing (at least 20 cm), organic amendments, soil solarization, biological or chemical control; however, it has not yet received a satisfactory result (Gour and Sharma, 2010). But in the recent past, considerable work has been done in the field of disease control through chemicals. The chemicals used for control of fungal diseases are mainly fungicides and antibiotics. At current scenario, fungicides are known to be the most effective method of the fungal pathogen and disease control. The prevailing condition of this host-pathogen interaction leads to economic loss of cucumber cultivation. Although, the information of suitable fungicide for collar rot disease management of cucumber, particularly, under the seedling condition is yet to appear through researches. We conducted this research for identifying a suitable fungicide effective to protect the cucumber seedlings from collar rot disease. The objective of this research was to screen out a number of fungicides commonly available in the market for *S. rolfsii* management in cucumber that can be easily used by the farmers and protect their fields from the infection by this dreaded pathogen.

MATERIAL AND METHODS

Isolation of the pathogen :

The pathogen, *Sclerotium rolfsii*, was isolated from infected collar region of infected cucumber (*Cucumis sativus*) plant on potato dextrose agar (PDA) medium (Chaurasia *et al.*, 2014a). The infected sample was collected from the vegetable farm of Bihar Agricultural University, Sabour. The diseased samples were processed within a day after appearing the laboratory. The infected samples were cut into 0.5–1.0 cm pieces and were surface sterilized with 1 per cent sodium hypochlorite for 1 minute and thereafter rinsed thrice in sterilized distilled water for 20 seconds at each time. The infected cut pieces were placed on PDA medium in Petriplate and incubated at 25±1°C for 3-4 days.

Identification of *Sclerotium rolfsii* :

On PDA medium, the growth of white mycelial threads along with small, uniformly sized, globoid sclerotia in larger number were observed. Sclerotia measured 1–3 mm in diameter and were initially white then turned to dark brown at maturation. Based on cultural and morphological features, the fungal pathogen was identified as *Sclerotium rolfsii* (Punja *et al.*, 1982). The further confirmation was made with satisfactory infection on cucumber seedlings using the pathogenicity tests, and by re-isolating the pathogen producing similar pattern of sclerotia distribution on PDA medium.

Purification and storage of the pathogen :

The actively growing mycelium was picked, and inoculated onto fresh Petri dishes containing PDA medium. These plates were incubated at 25±1°C for 3-4 days. The mycelium was looped and transferred into a PDA slant and incubated as described above. The fungal mycelium in the slants allowed to grow until the surface of PDA was covered with a dense layer of the fungal colony and thereafter the resting structure *i.e*. sclerotia. Three sets of slants were developed and stored in refrigerator at 4°C. The stock culture was maintained by periodic transfer for further studies.

Fungicidal assay :

Nine different fungicides (Table 1) from three groups of mode of action (three fungicides under a category) were evaluated for their efficacy on mycelial growth of *S. rolfsii* by poisoned-food technique (Chaurasia *et al.*,2014b) by using different concentrations of 0, 10, 50, 100 and 150 ppm. The fungus was grown on 90 mm Petriplate on PDA mixed with different concentrations of various fungicides. Mycelial growth was measured after 7 days of incubation at 25°C. The growth of the fungus was measured by averaging the bisecting assessments of the radial growth from back of the Petriplate (Das *et al*., 2014 and Fig. 1). Duncan's multiple range test ($P \leq 0.05$) was performed to delineate treatment mean for suppression of radial growth by different fungicides using statistical software SAS, NY version 9.4. This experiment was repeated twice.

Seedling evaluation to fungicides :

The sclerotia of *S. rolfsii* produced on PDA medium were thoroughly mixed in the double-autoclaved pot-mix (sand and soil, 1:1). The sclerotia were added with a rate of 100 sclerotia/ 100 g pot-mix (Yaqub and Shahzad, 2005). The experiment was laid out in root trainers. The cucumber seeds were surface sterilized with 1 per cent sodium hypochloride followed by water rinsing. Upon shade drying of the seed, fungicide coating of the seeds were performed for individual fungicide. The cucumber seeds (20-22) were then sown for evaluation of a fungicide concentration; and 15 seedlings were finally maintained on 7-10 days after sowing. Seedlings were raised with application of sterilized water and chemical fertilizer. The incidence of disease was recorded three times recorded weekly after 14 days of sowing. The experiment was conducted two times.

Statistical design and data analysis :

The Petriplates were subjected to factorial arrangement according to Completely Randomized Design (Gomez and Gomez, 1984). Analysis of variance (ANOVA) was performed to identify the effect of response of radial growth to the concentration of various fungicides as well as their concentrations (Table 2). The experiment was performed with three replications for each treatment. Radial growth of the fungus for each isolate was measured using a transparent ruler bisecting the colony (Fig. 1). Average value was, therefore, calculated from the two bisecting assessments and used for analysis (Ghatak *et al.*, 2017). The analysis was performed with a statistical software SAS, NY version 9.4.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

Fungicide efficacy assessments :

This experiment was conducted to determine the effect of nine fungicides of three different groups (mode of action) comprising of three fungicides in each group (Table 1) at different concentrations *viz.*, 0 (control), 10, 50, 100 and 150 ppm by poisoned food technique (Fig. 1). The radial growth of the pathogen recorded after 48, 72, 96, 120 and 144 h after inoculation (hai). However, the analysis was done with the final assessment (144 hai) when the control plates attained full colony growth. We found the effect of experiment significant

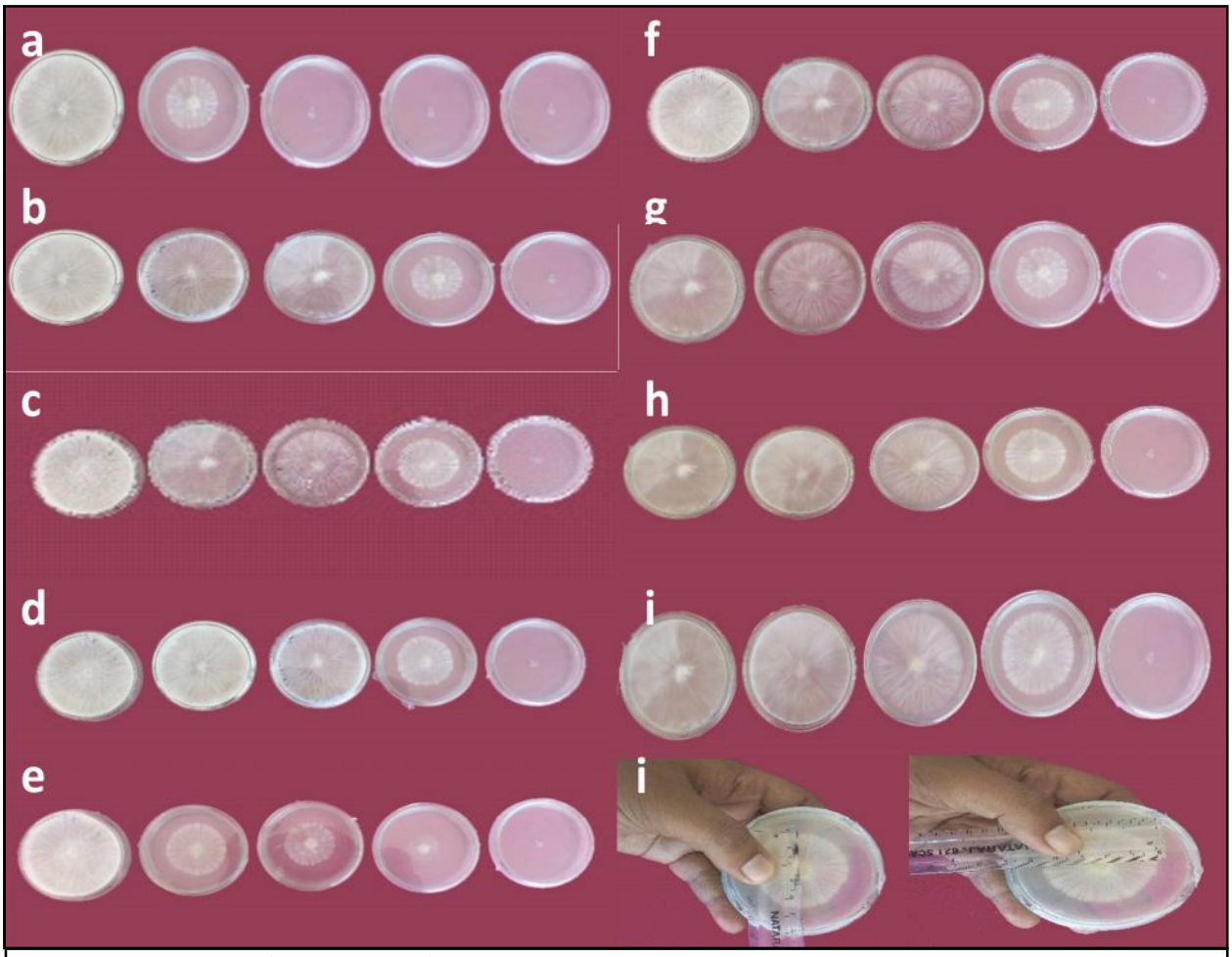

Fig. 1: Radial growth of *Sclerotium rolfsii* supplemented with a) Hexaconazole, b) Carbendazim, c) Mancozeb, d) Copper oxychloride, e) Propiconazole, f) Mycobutanil, g) Captan, h) Thiophenate methyl, i) Vinclozoline at 5 concentrations *viz.*, 0, 10, 50, 100, 150 ppm, and j) measurement of radial growth. In a series of plates, under a category: control, 10 ppm, 50 ppm, 100 ppm, and 150 ppm (from L-R). No growth found on 150 ppm amongst the fungicides used

Table 1 : Fungicides used in the study

Common name	Trade name	Active ingredient	Manufacturer
Cell division inhibitor			
Carbendazim	Bavistin	Carbendazim 50% WP	BASF Chem. Co.
Thiophenate methyl	Kirin	Thiophenate methyl 70% WP	Parijat Ind. pvt. ltd.
Vinclozolin	Apparent	Vinclozolin50% WP	APDANT pvt. ltd.
Multisite action			
Captan	Captan	Captan 50% WP	Arya laboratories
Copperoxychloride	Blitox	Copper oxychloride 50% WP	RaLIS India ltd.
Mancozeb	Shree	Mancozeb75% WP	JaishreeRasayanUdyog ltd.
Sterol inhibitor			
Hexaconazole	Hexa	Hexaconazole 5% EC	MahabirBajrangAgrochempvt. ltd.
Mycobutanil	Index	Mycobutanil 10% WP	NagarajunaAgrichem ltd.
Propiconazole	Tilt	Propiconazole 25% EC	Syngenta

($P \leq 0.0001$) on the radial growth of *S. rolfsii*. Therefore, separate analyses were performed to identify the effect of fungicide and concentration, and their interaction on radial growth of the fungus (Table 2). The various fungicides and concentration affected the fungal radial growth significantly ($P \leq 0.0001$) in both the experiments. This could be interpreted that the suppression of radial growth is largely affected by the type of fungicide used and their different concentrations. The result showed that all the fungicides significantly inhibited radial growth of the pathogen (Fig. 1). Different combinations of fungicides were found effective to inhibit soybean pathogens appearing in late season (Carmona *et al.*, 2017).

Overall, hexaconazole, a potent sterol inhibitor at a very low concentration (10 ppm) showed 85.8 per cent suppression of the radial growth of *S. rolfsii* after 144 hai but no growth was observed at 50 ppm and all above concentrations (Table 3). Mycobutanil and propiconazole had poor inhibitory effect at all concentrations as compared to hexaconazole. Out of cell division inhibitor fungicides, carbendazim performed well for fungal growth inhibition followed by vinclozolin and thiophenate methyl. Among multi-site action inhibitors, copper oxychloride followed by captan and mancozeb proved well for suppression of fungal growth. It was found that analyses for both the experiments were resulted similar for a particular fungicide in terms of radial growth inhibition. Our result is in agreement with Yang *et al*. (2011). The tolerance of this pathogen towards some of the used fungicides (e.g. thiophenate methyl, vinclozoline, captan, copper oxychloride, mancozeb and mycobutanil) in which considerable radial growth has been reported even at high concentration, can be correlated with increasing ability of this fungus to synthesize extracellular melanin under fungicidal stress (Amany *et al.*, 2003).

Fungicide combination effect on *Sclerotium rolfsii* :

Significant effect of the interaction of fungicide and concentration indicated that a particular fungicide could be useful at a specific concentration (Table 2). In both of the experiments, the interaction of fungicide and concentration was found highly significant ($P \leq 0.0001$). Selection pressure on the pathogen, by applying

Table 2: Analysis of variances determining the effect of fungicide and concentration on radial growth of *Sclerotium rolfsii*[a]

		Experiment 1			Experiment 2		
Variable	*df*	MSS	F-value	Pr > F	MSS	F-value	Pr > F
Fungicide (F)	8	2668.26	4383.11	<0.0001	2533.62	6438.26	<0.0001
Concentration (C)	4	25522.49	4192.50	<0.0001	27427.47	6969.70	<0.0001
F × C	32	853.43	1401.93	<0.0001	550.94	1400.00	<0.0001
Error	96	0.61			0.39		
Total	179	842.60			824.95		

[a]Effect of experiment found significant on radial growth of *Sclerotium rolfsii*. Therefore, analyses for both experiments were conducted separately

Table 3: Radial growth of *Sclerotium rolfsii* against various fungicides[a]

Fungicide	Experiment 1[b]			Experiment 2		
	10 ppm	50 ppm	100 ppm	10 ppm	50 ppm	100 ppm
Carbendazim	90	40.5	9.3	90	40	10
Thiophenate methyl	90	87.3	65.5	90	87	65
Vinclozoline	90	90	62	90	90	62.5
Captan	90	85.8	45.3	90	85.3	45
Copperoxy chloride	90	90	41.5	90	90	41.8
Mancozeb	90	90	85.5	90	90	85.3
Hexaconazole	12.8	0	0	12.3	0	0
Mycobutanil	90	74.5	64.5	90	75	64
Propiconazole	50	21	11.8	50.3	20.3	11.5
No fungicide (control)	90	90	90	90	90	90

[a]Values are means over four replications, observation was taken at 144 hours after inoculation (hai)
[b]No growth observed at 150 ppm, therefore, removed from the table

inappropriate dose of fungicides should not be increased as it improves the fitness of resistant form of the pathogen (Solunke *et al.*, 2001). Therefore, for effective, judicious and economical use of fungicide, proper concentration of the fungicide should be applied in order to achieve greater impact of management of a pathogen affecting many hosts like *Macrophomina phaseolina* (Ghatak *et al.*, 2017). This research advocated for hexaconazole

Table 4: Percent mortality of seedlings germinated from fungicide treated seeds

	Concentration	Infected plants[a]			Disease incidence		
Fungicide	(ppm)	14 days	21 days	28 days	14 days	21 days	28 days
Carbendazim	10	0	3	6	0	20	40.2
	50	3	3	3	20	20	20
	100	0	0	0	0	0	0
	150	0	0	0	0	0	0
Thiophenate methyl	10	3	6	12	20.3	40.2	80
	50	3	6	9	20	40	60
	100	0	0	3	0	0	20
	150	0	0	0	0	0	0
Vinclozolin	10	3	6	9	20.5	40.4	60
	50	3	6	9	20	40	60
	100	0	3	6	0	20.6	40
	150	0	0	0	0	0	0
Captan	10	3	6	9	20.3	40.2	60
	50	3	6	9	20	40	60
	100	3	3	6	20	20.8	40
	150	0	0	0	0	0	0
Copper oxychloride	10	6	9	12	40.5	60.4	80
	50	3	6	9	20	40	60
	100	3	3	6	0	20	40
	150	0	0	0	0	0	0
Mancozeb	10	6	12	12	40.3	80.2	80
	50	3	6	12	20	40	80
	100	3	6	9	20	40.2	60
	150	0	0	0	0	0	0
Hexaconazole	10	0	0	3	0	0	20
	50	0	0	0	0	0	0
	100	0	0	0	0	0	0
	150	0	0	0	0	0	0
Mycobutanil	10	3	6	6	20.5	40.4	40
	50	0	3	6	0	20	40
	100	0	0	3	0	0	20
	150	0	0	0	0	0	0
Propiconazole	10	3	3	6	20	20.3	40
	50	0	3	6	0	20	40
	100	0	0	0	0	0	20
	150	0	0	0	0	0	0
Control	-	3	8	13	20	53.3	93.3

[a]Total 15 seedlings were evaluated under a concentration of fungicide

followed by propiconazole for mycelium suppression of *S. rolfsii*. This result is in agreement with Das *et al.* (2014) who also found hexaconazole as a promising fungicide restricting *S. rolfsii* growth. Evaluation of fungicidal mixtures against the pathogen revealed that, hexaconazole should be a part of combination for effective control the *S. rolfsii* (Kumar *et al.,* 2014).

Seedling protection assessments :

All the nine fungicides were also tested for their potentiality to control collar rot disease of cucumber seedlings of variety Cucumber Supergreen (Maharaja). The fungicides were assayed on the basis of per cent disease incidence after 14, 21 and 28 days after sowing of seeds. Table 4 reveals that the results obtained from fungicide assay experiment are in agreement of the experiment conducted in root trainers. Disease incidence was found to be highest in thiophenate methyl at 10 ppm, in copperoxy chloride at 10 ppm and in mancozeb at 10 ppm. Our experiment rendered 60 per cent infected seedlings for thiophenate methyl at 50 ppm, vinclozolin at 10, 50 ppm, captan at 10, 50 ppm, copper oxychloride at 50 ppm and mancozeb at 100 ppm. A range between 40 and 45 per cent disease incidence was recorded for carbendazim at 10 ppm, vinclozoline at 100 ppm, captan at 100 ppm, copperoxy chloride at 100 ppm, mycobutanil at 10, 50 ppm and propiconazole at 10, 50 ppm. We assessed lowest infected seedlings (15-20%) for carbendazim at 50 ppm, thiophenate methyl at 100 ppm, hexaconazole at 10 ppm, mycobutanil at 100 ppm and propiconazole at 100 ppm. No infected seedlings were obtained at 150 ppm of any fungicide. The 50 ppm concentration of hexaconazole was found to be the best chemical for controlling of collar rot disease in cucumber followed by propiconazole at 100 ppm (Table 4). At 150 ppm, many of the fungicides used (e.g. carbendazim, thiophenate methyl, vinclozoline, captan, copperoxy chloride, mancozeb, mycobutanil) are able to control the disease completely but the toxic and residual effect should be encountered before advice. Our result is strongly supported by Das *et al.* (2014) who assessed many systemic and non-systemic fungicides and reported the great effect of hexaconazole and poor effect of carbendazim in reduction of *S. rolfsii* colony. Hexaconazole reported to be the best fungicide restricting *S. rolfsii* infection in brinjal (Chaurasia *et al.*, 2014b). No infection of *S. rolfsii* was observed for the finger millet plants treated with hexaconazole (Manu *et al.*, 2012).

Conclusion :

In this study, hexaconazole found to be the most effective fungicide among the all tested fungicides under sterol inhibition activity. Sterols and their derivatives promote and maintain growth and development of fungi as well as plants also by acting as membrane constituents and may be also as hormones, involved in control of metabolism. Inhibitors of sterol biosynthesis, operating at various stages in the pathway, are useful for investigating these functions. Inhibitors have been expected for their significant commercial importance as agricultural fungicides. There is also potential for using similar compounds to regulate plant growth (Burden *et al.*, 1989). Since, it is not feasible to manage the disease complex in our conditions through soil treatment or drenching of fungicides due to high cost involved. It is advised to give chemical treatment of fungicides against the diseases, so that it can be easily adopted by the farmers. Based on the result presented in this paper it can be suggested that the hexaconazole at 10 ppm along with carbendazim @ 50 ppm can be recommended to control *S. rolfsii* under field condition in the cucumber crop against collar rot disease for economical and effective control. This tactics may secure the possibilities of resistance development usually occur due single fungicide use.

Acknowledgment:

Technical assistance received by Mr Mukesh Kumar Ram is appreciated. This research article bears BAU Communication Number 370/2018.

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