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Population Density of *Sclerotinia* Species in Various Changes of Climate

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Abstract: Fungal pathogens are liable for a wide part of damages in agriculture economy. In the present research a survey was made to study occurrence of *Sclerotinia* species the causal agent of stem rot in Cauliflower, through agar disc method and dual culture bioassays. The number of apothecia reduced with decrease in temperature and relative humidity. Infection is dependent on long periods of leaf wetness provided by high humidity and high soil moisture and the optimal temperatures are between 15 and 18°C. Thus, control of the disease was targeted by studying the optimal conditions of infection taking *Streptomyces* with antagonistic properties as biocontrol agent.

Key words: Biocontrol · *Sclerotinia* sp. · *Streptomyces*

INTRODUCTION

High level of biosafety and non adverse effects on the environment of biocontrol strategies of pest management, are priorities of tomorrow's world agriculture. Actinomycetes are active biocontrol agents due to their antagonistic properties against wide range of plant pathogens particularly fungi. The soil-borne fungi *Sclerotinia* sp (Lib.) de Bary and *Sclerotinia minor* (Sm) Jagger have worldwide distribution in temperate and subtropical climates and cause significant losses to horticultural and ornamental crops. There are 408 plant species (75 families and 278 genera) that host *Sclerotinia sclerotioum*. In contrast, *Sclerotinia minor* may infect at least 94 plant species belonging to 21 families and 66 genera Kora, [1]. The genus *Sclerotinia* produces sclerotia that remain in the soil under adverse climatic conditions for several years. *Sclerotinia* sp. can also be known as cottony rot, watery soft rot, stem rot, drop, crown rot and blossom blight. A key characteristic of this pathogen is its ability to produce black resting structures known as sclerotia and white fuzzy growths of mycelium on the infected plant. These sclerotia give rise to a fruiting body in the spring that produces spores in a sac which is why fungi in this class are called sac fungi (Ascomycetes). When *Sclerotinia* sp. is onset in the field by favorable environmental conditions, losses can be great and control measures should be considered.

Sclerotinia (*sclerotioum* sp (Lib.) de Bary) rot of cauliflower is a destructive field and storage disease of cauliflower in worldwide. Losses in storage can exceed 30% [2].

Severe Sclerotinia rot in the field can weaken cauliflower foliage, causing it to break off during mechanical harvest, leaving cauliflower roots in the ground unharvested. The development of Sclerotinia rot in storage is dependent on infection occurring in the field during the growing season. However, once roots are infected, the severity of storage losses often is determined by storage conditions.Infection of cauliflower by *Sclerotinia* sp. in the field occurs primarily through ascospore germination and direct penetration of senescing leaves [3].

Infection is dependent on long periods of leaf wetness provided by high humidity and high soil moisture and the optimal temperatures are between 15 and 18°C [4]. Once the pathogen infects the leaves, mycelium can progress down the petioles of senescing leaves and into the crown, where storage rots are initiated. Optimal conditions for *Sclerotinia* sp. infection occur after full canopy closure later in the growing season. Thus, control of the disease is targeted to this stage of growth. There are no fungicides currently registered for controlling Sclerotinia rot on cauliflower; however, fungicides are applied regularly to control cauliflower leaf blight (CLB) and might have some effect in protecting foliage from *Sclerotinia* sp. infection.

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The effect of climate on the cauliflower foliage once or twice, with and without biocontrol agent (*Streptomyces*) application, was examined on cauliflower plants grown on organic soil for 3 years at the farmers'field in Farrukhabad. The number of *Sclerotinia* sp. apothecia, microclimate and total and marketable yield were assessed. The number of apothecia of *Sclerotinia* sp. and relative humidity in the canopy were reduced by *Streptomyces* at either the first observation of apothecia or at 100 days after seeding (DAS). In both cases, the effects of microclimate lasted between 2 and 4 weeks. This was attributed mainly to the lower requirement of the CLB pathogens for prolonged periods of high relative humidity and leaf wetness compared with *Sclerotinia* sp.

The pathogenic fungus *Sclerotinia sclerotioum* proliferates in moist environments. Under moist field conditions, *Sclerotinia* sp. is capable of completely invading a plant host, colonizing nearly all of the plant's tissues with mycelium. An optimal temperature for growth range was from 15 to 25 degrees Celsius. Under wet conditions, *Sclerotinia* sp. produced an abundance of mycelium and sclerotia. The fungus can survive in the soil mainly on the previous year's plant debris. Like most fungi, *Sclerotinia* sp.

Prefers darker, shadier conditions as opposed to direct exposure to sunlight.

Actinomyctes have been known as efficient biocontrol agents that naturally exist in soil and have the ability to inhibit growth of plant pathogens among which *Streptomyces* spp. had been shown to have characteristics which make them useful as antagonistic agents against soil-borne fungal plant pathogens. These characteristics include the production of different kinds of secondary metabolites and biologically active substances of high commercial value such as enzymes (which degrade the fungal cell wall directly) and antibiotics. Soil *Streptomyces* are of the major contributors to the biological buffering of soils and have roles in decomposition of organic matter conductive to crop production. Besides, they have been much studied as potential producers of antibiotics and exert antagonistic activity against wide range of bacteria and fungi [1, 5-7]. Biocontrol of soil-borne fungal or bacterial pathogens will be of increasing importance for a more sustainable agriculture. In the present research a survey was made to study occurrence of *Sclerotinia* species the causal agent of stem rot in Cauliflower under various climatic changes.

MATERIALS AND METHODS

Experiments were conducted on organic soil, (pH 6.4, organic matter 60%), naturally infested with *S. sp.* at the Farukhabad. There were 68.8±24.3 sclerotia of *Sclerotinia* sp. per square meter in the top 0.5 cm of soil within 25 m of these research plots. Cauliflower was direct seeded with a precision seeder (80 to 90 seeds/m) on 3 June 2002, 21 May 2003 and 18 May 2004 on raised beds 20 cm high and 86 cm apart. A randomized complete block design with four replications per treatment was used.

Each experimental unit consisted of four 5-m-long rows of cauliflower plants in 2002 and 2003 and four 10-m-long rows in 2004. The trial was a factorial design with one factor. The second factor was *Streptomyces* application at two levels: (i) untreated and (ii) the *Streptomyces.*

Air temperature and relative humidity measurements were recorded throughout the growing season from 10 cm above soil level in the cauliflower canopy. The data loggers recorded temperature and relative humidity every 2 h. A single datalogger was placed in one replicate of each of the treatments receiving Streptomyces sprays. Weather conditions at the test site are summarized in Table 1. Irrigation was applied when rainfall was insufficient to maintain soil moisture above 35%.

Table 1: Monthly mean temperature and rainfall at the experimental site 2002 to 2004 compared with 10-year mean

	Mean Temperature (°C)				Rainfall (mm)			
Month	2002	2003	2004	10-year mean	2002	2003	2004	10-year mean
May	9.9	12.2	12.4	12.3	113	105	108	89
June	18.2	17.3	16.3	18.0	106	75	50	87
July	21.7	19.9	19.3	19.9	76	29	102	73
August	19.6	20.4	17.8	19.2	18	81	103	62
September	17.5	15.0	16.6	15.4	40	110	25	77
October	7.2	8.0	9.1	8.9	49	78	26	65

Culture Media: Casein Glycerol (or starch) Agar (CGA) was used for screening and isolating Actinomycetes and composed of: Glycerol or starch 10 g; casein, 0.3 g; KNO3, 2 g; NaCl, 2g; k2HPO4, 2 g; MgSO4.7H2O, 0.05 g; $CaCO_3$, 0.02 g; FeSO4.7H2O, 0.01 g and agar, 18 g in 1 L of distilled H2O (pH 7.2).Actinomycetes colonies with different morphologies were selected and transferred to CGA slants for further studies.

Isolation of Actinomycetes from Soil: For isolation of Actinomycetes, soil samples were collected from grasslands and vegetable fields.Several samples were randomly selected from mentioned localities using an open-end soil borer (20 cm in depth, 2.5 cm in diameter). Soil samples were taken from a depth of 8-12 cm below the soil surface. Samples were air-dried at room temperature for 7-10 days and then passed through a 0.8 mm mesh sieve and were preserved in polyethylene bags at room temperature before use. Samples (10 g) of air-dried were mixed with sterile distilled water (100 ml). Mixtures were shaken vigorously for 15 min and then allowed to settle for 15 min. Portions (1 ml) of soil suspensions (diluted 10^{-1}) were transferred to 9ml of sterile distilled water and subsequently diluted to 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}. Inocula consisted of adding aliquots of 10^{-3} -10^{-6} soil dilution to autoclaved CGA (1 ml in 25 ml CGA) at 50°C before pouring the plates and solidifications. Three replicates were considered for each dilution. Plates were incubated at 28°C for up to 7 days. From eighth day on, Actinomycete colonies were isolated on CGA, incubated at 28°C for one week and stored refrigerated as pure cultures before use. For screening studies, pure Actinomycete isolates were collected and maintained in stock.

Preparation of Fungal Isolate: Pure culture of *Sclerotinia* sp, the causal agent of stem and head rot disease in cauliflower was obtained from IARI, New Delhi. The fungus was grown at 25°C and maintained on potato dextrose agar (PDA, Difco, 39 g PDA L^{-1} of distilled H_2O, pH = 7.2). All cultures stored at 4°C and sub-cultured as needed.

Screening Procedure and *in vitro* Antifungal Bioassays: To evaluate the antifungal activity of isolated *Streptomyces* against the pathogen, bioassays were performed in agar disk method. Antifungal activity around the *Streptomyces* agar disks was evaluated.s

Agar-Disk Method: From the refrigerated stocks, each Actinomycete isolate was smeared on CGA medium as a single streak and after incubation at 28°C for 4-6 days from well-grown streaks, 6 mm agar disks of Actinomycetes colony mass was prepared by using sterile cork borers. Disks were then aseptically transferred to PDA plates having fresh lawn culture of *Sclerotinia* sp.. Controls included using plain disks from CGA medium. Plates were incubated at 28°C for 6 days and bioactivity was evaluated by measuring the Diameter of Inhibition Zones.

***In vivo* greenhouse studies:** Greenhouse tests were accomplished in a conventional greenhouse using 7-10 day old cauliflower seedlings with approximately 10 cm uniform stem sizes grown in sterilized soil mix in Styrofoam containers. Tests performed on a complete randomized plan with the repetition of ten pots for each treatment. Four treatments included as follows: (A) *Sclerotinia* sp., (B) Streptomyces isolate No. 125, (C) *Sclerotinia* sp. plus *Streptomyces* isolate No. 226 and (D) Control. To prepare inocula, *Streptomyces* isolate No. 125 was cultured on CGA and *Sclerotinia* sp. was grown on PDA media. All seedling stems were wounded as surface slashes using a sterile razor blade. Wounds in control received sterile wet cotton pads and covered with Parafilm® to avoid dryness. Similarly other treatments received a 2 cm^2 of the media mat of the well grown pathogen and/or the antagonist and covered similarly. All treated plants covered with transparent plastic bags for 48 h to prevent accidental dryness and provide high relative humidity for onset of pathogen/antagonist activities. Symptoms recorded 10 days after inoculation at 25°C. To compare the relative growth of the plants in the four treatments, seedlings were carefully desoiled through detailed rinsing in tap water, stems and roots cut apart and dried at 60°C for 48 h and weighed with accuracy. The experiment was repeated twice and means were recorded.

RESULTS

Screening and Bioassays: In screening for Actinomycetes with antifungal activity, 50 isolates were screened from which 10 isolates showed activities against *S. sp.* (Fig. 1). Based on the screening results, in comparision to others, *Streptomyces* isolate No. 125 had maximum inhibition zone against *S. sp.* and selected for further evaluations. Clear inhibition zones around the *Streptomyces* agar disks are representatives of lack of growth and bioactivity against the pathogen.

Table 2: Total month and season-long numbers of *Sclerotinia sp.* apothecia per 5 m of row following treatments with or without *Streptomyces* application

	Number of apothecia				Yield (t ha^{-1})	
Treatment	First to 100 DAS	100 DAS to harvest	Total	Foliar weight (kg m^{-1})	Total	Marketable
No treatment	1.2bc	2.3b	3.5c	12.7c	89.9bc	81.2a
Streptomyces	0.5c	1.1b	1.6c	15.5b	92.6a–c	83.2a

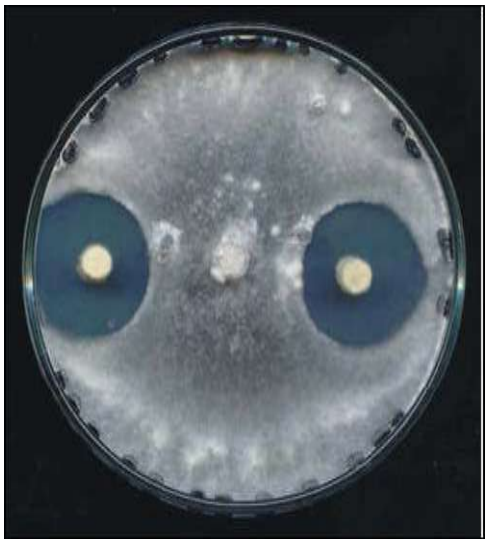

Fig 1: Bioassay result in Agar Disk-Method of Streptomyces isolate No. 125 against Sclerotinia sp.. *Center disk is Sclerotinia* sp. *agar inoculum disk*

***In vivo* Greenhouse Studies:** Symptoms as stem rot appeared 4-6 days after inoculation in seedlings inoculated with *Sclerotinia* sp, while other treatments did not develop signs of the disease. The results of this experiment which indicates promising biological control of *Streptomyces* isolate No. 125 against *Sclerotinia* sp. the causal agent of head and stem rot in cauliflower in greenhouse.

Streptomyces application had significant effect on the number of apothecia in any period of the season. *Streptomyces* application resulted in higher foliar weights than in untreated cauliflower. *Streptomyces* application also increased total yield compared with untreated cauliflower, but temperature had no significant effect on total or marketable yield. There were no treatment–year interactions for yield and yield data were pooled among years.In all plots, Streptomyces application reduced CLB severity compared with no biocontrol sprays (Table 2).

Daily maximum air temperatures in the cauliflower canopy were higher in the Streptomyces treatments than in the control for the entire period from the first foliar Streptomyces treatment until harvest in 2003. In 2004, although the datalogger malfunctioned in the Streptomyces treatment, mean daily maximum air temperatures in the canopy were higher in the control.Mean daily minimum air temperatures were not affected by treatments in any period; however, in both 2003 and 2004, minimum temperatures tended to be lower in Streptomyces treatments during the final period before harvest.

No disease developed in storage in any of the 3 years and data are not reported.. High temperature did not improve the efficacy of *Streptomyces* applications for CLB control. Thus, microclimate has potential to improve the management of Sclerotinia rot of cauliflower at first observation of apothecia and at 100 DAS could reduce apothecia production and relative humidity within the climate created.

DISCUSSION

Overdose usage of chemicals as pesticides and fertilizers is common in farming in most parts of the world which threatens food safety of individuals. Some pesticides can be hardly cleaned from nature and have a potential capability to have adverse effect or destroy useful microorganisms which have positive effects in fertility of soil and growth of plants. To lower or avoid side effects, biological control is an alternative and proper choice in pest management. In ideal biological control measures, proper microorganisms are those having well adaptation in soil and rhizosphere exerting effective antagonistic activity against soil pathogens persistently. In the *in vitro* study to fight cauliflower stem rot, a natural agent (Actinomycete) was used that had effective antagonistic characteristics against *S. sp.*. In green house study, similar to *in vitro* tests, *Streptomyces* isolate 125 suppressed fungal diseases in inoculated plant without any contamination in the soil and plant. It also increased growth of plants. A commercial product containing *Streptomyces* isolate 125 or its effective metabolite is suggested to avoid head and stem rot in cauliflower.

Streptomyces treatment of the cauliflower canopy reduced the number of apothecia of *S. sp.* in all 3 years of the trial. Treating the canopy upon first observation of apothecia resulted in a lower number of apothecia compared with untreated.

Streptomyces treatment on the canopy of 100 DAS reduced the number of apothecia compared with the control. Because the canopy reclosed and the microclimatic effects of *Streptomyces* treatment declined within 3 or 4 weeks of treatment. *Streptomyces* treatment could be beneficial in reducing apothecia numbers. low apothecia development was late in the season as a result of dry conditions in September and October in 2 of the 3years. Based on apothecial number alone during the season appears to be beneficial for the management of *Sclerotinia* rot in the field.

The effects of foliar trimming on the canopy microclimate appear to last between 2 and 4 weeks after the treatment. Consequently, the benefit of foliar *Streptomyces* treatment twice during the season is a longer time period in which there is a less favorable environment for apothecial development.

Daily maximum air temperatures in the canopy increased with increasing number of foliar treatments. The data suggest that infection and development of Sclerotinia rot within the canopy, even in areas protected by leaf cover, could be reduced as a result of air temperatures increasing above the preferred range of *S. sp.* in biocontrol treatments. Optimal temperatures are between 10 and 20°C for apothecial expansion [3, 5] and between 15 and 18°C for infection [6, 7]. Although mean daily maximum air temperatures were above these ranges for all treatments during August and early September, the lower maximums in the control suggests that temperatures would have been in the optimum range for a longer period of time in this treatment.

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