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Antibiotic substance produced by *Streptomyces carcinomycicus* strain from *Brassica campestris* linn. Antagonistic to *Alternaria brassicae* (BERK.) and *Alternaria brassicicola* (SCHW.) leaf spot disease

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Abstract
The antibiotic substances are produced by strain of *Streptomyces carcinomycicus* (S-9) was isolated from soil of *Brassica campestris* Linn. crop field of Agra district. It was found to be strongly antagonistic to *Alternaria brassicae* (Berk.) and *Alternaria brassicicola* (Schw.) which causing the leaf spot disease of crucifers. Its growth characters on various media have been determined and organism placed in section and classification which identified as a strain of *Streptomyces carcinomycicus* on the basis of growth and biological characteristics. *Streptomyces carcinomycicus* strain is strongly antagonistic on agar medium produced active principle in liquid culture media. The culture filtrate inhibits the spore germination of *Alternaria brassicae* (Berk.) and *Alternaria brassicicola* (Schw.) on leaf. It is clear that the antifungal activity of culture filtrate decrease with increase in the storage time at all the temperatures. The antibiotic substance can be stored without any appreciable loss in its activity for 30 days at pH 6-8 and temperature 6^0C. It is soluble *n*-butanol in, ethanol, isopropyl alcohol, benzene and toluene.

Keywords: antibiotic strain, *Streptomyces carcinomycicus*, Leaf spot on *Brassica campestris*, *Alternaria sps*

Introduction
Brassica campestris Linn. plant [1] belongs to the Family-Cruciferae and commonly called as *Sarson* is an important Rabi crop and seeds yielded seed oil [2] and used in medicinal purposes. Actinomycetes have received increasing attention because they produce a large number of antibiotic substances capable of inhibiting the growth of other micro-organism [3]. They vary considerably in their utilization of available nutrient. The latter have profound role in the production of antibiotic substances. Quantitative yield of antibiotic is greatly influenced by the nature and concentration of nutrients. It is therefore, worthwhile to develop the specific nutrient medium in order to obtain the maximum antibiotic production.
The present investigation mainly deals with the antibiotic substances are isolated by *Streptomyces carcinomycicus* (S-9) antagonistic to *Alternaria brassicae* (Berk.) and *Alternaria brassicicola* (Schw.) from *Brassica campestris* Linn plant. The effect of different nutrient broths on growth of organism and the production of antibiotic substances and also some of properties of the antibiotic substance produced by it.

Materials and methods
A large number of soil samples were collected from the cultivated fields of different places at Dayal bagh, Bodla, Sewla and Patholi of Agra district (U.P.) and were platted for the isolation of Actinomycetes on Thornton's medium [4] adopting the Dilution Plate Technique [5]. The plates were incubated for about 10 days at 28^0C ($\pm 2^0$C). The colonies developed were picked up on the basis of their pigment difference and other morphological characters and were further purified. Their screening for antagonistic activity was done by placing the plugs cut from 10 days old culture of various Actinomycetes isolated in petri plates seeded with the test organisms *Alternaria brassicae* (Berk.) and *Alternaria brassicicola* (Schw.) wiltshire separately. After 4 days of incubation at 28^0C ($\pm 2^0$C), the inhibition zone have a clear distance between the growth of the test organism and Actinomycetes plugs. It was measured by a centimetre scale from 3 different angles and average was taken. The growth characters, colour of aerial and substrate Mycelium of a selected isolate (S-9) on different media and some biochemical characteristics were studied [6]. The colour terminology [7] refers to that sporophore morphology was studied [8]. In order to select a basal medium supporting the maximum antibiotic production by selected strain. The organism was cultured on 6 different broths including both natural and synthetic.
Each broth (30 ml) was taken in Erlenmeyer flasks (250 ml) in triplicate and autoclaved at 10 lbs pressure for half an hour. The flasks were then inoculated with a fresh spore suspension prepared from 10 days old culture of the isolate (S-9) and incubated at 28^0C ($\pm 2^0$C). After 15 days of incubation, the Mycelia mat from each flask was removed by filtration through previously weighed on Whatman No. 1 filter paper. They were thoroughly washed and dried to constant weight in an electric oven at 70^0C for about 40 hours and the mean dry weight in gm was determined. The antibiotic activity of culture filtrate was assayed in terms of percentage inhibition of spores germination of *Alternaria brassicae* (Berk.) and *Alternaria brassicicola* (Schw.) after 5, 10 and 15 days incubation adopting hanging drop method [9].

The properties of the antibiotic substances and culture (S-9) was grown on basal medium (glucose asparagines) in many Erlenmeyer flasks (250 ml). After 15 days of termination, the flasks were harvested. Mycelium was filtered out and the filtrate from all the flasks were pooled. Biological activity of antibiotic substances, the culture filtrate (0.1 ml) was pipetted out in the circular activities of about 8 mm diameter, made on agar medium by sterilized cork borer in the centre of each petri plates seeded separately with test micro- organism. After 4 days incubation at 28^0C ($\pm 2^0$C), the inhibition zone if formed was measured in each case.

Light absorption of antibiotic substance produced by *Streptomyces carcinomycicus* was dried and redissolved in methanol for absorption analysis. Absorption spectrum was determined the optical density at different wave length of UV- light. IR- Spectrum of the antibiotic substances was recorded on 577-Perkin Elmer Double Beam Spectrophotometer at 4000-200 cm^{-1} in methanol medium and calibrated by standard method [10].

Results and discussion

The various Actinomycetes isolated from *Streptomyces carcinomycicus* (S-9) was found to be strongly antagonistic to *Alternaria brassicae* (Berk.) and *Alternaria brassicicola* (Schw.) causing leaf spot disease in *Brassica campestris* Linn. plant and others micro-organism including some of the important plant pathogens. Thus there is a scope of its use in the control of the important plant disease. The growth of the organism is tough textured in agar medium. The vegetative Mycelium does not fragment in bacillary forms is white non-septet, monopodally branched. Aerial Mycelium is pale yellowish in colour. Sporophre developed on aerial Mycelium are straight to flexuous. Spores are oval 1.2-1.4 × 1.8-2.0µ and smooth walled. The isolate (S-9) was placed in section Rectus flexibly and yellow series [7].

Carbon utilization showed positive for lactose, fructose, glucose, mannitol, raffinose, galactose and starch while negative for xylose and cellulose. In its morphology and culture characteristic and some biochemical feature have in its antagonistic activity. This organism are best agreed with *Streptomyces carcinomycicus* [11], however there are a few marked differences between the two. The isolate (S-9) unlike *Streptomyces carcinomycicus* does not hydrolysed the starch and liquefy gelatin slowly. It grows luxuriantly on sucrose nitrate as well as starch whereas in case of *Streptomyces carcinomycicus* is thin transparent. *Streptomyces carcinomycicus* strain [12] is mainly antibacterial whereas isolate (S-9) is antifungal. Therefore, considering sporophore morphology and spore surface configuration as stable taxonomic criteria and isolate (S-9) has been regarded as a new strain of *Streptomyces carcinomycicus*.

Strains of *Streptomyces carcinomycicus* is strongly antagonistic on agar medium also produces active principle in liquid media. The culture filtrate was inhibits the spore germination of *Alternaria brassicae* (Berk.) and *Alternaria brassicicola* (Schw.). The production of active substances was found to increase significantly in most of the treatments as incubation period advance from 5-10 days. The maximum activity was observed after 15 days of incubation in all the media. Actinomycetes produced antibiotic substance in all the media tested but the amount synthesized various considerably, according to the nature and composition of the medium. It is clear that the antifungal activity of culture filtrate decreases with the increase in the storage time at all the temperatures. The loss of activity was relatively loss at lower than at higher temperatures. The filtrate retained its original potency up to 30 days when stored at pH 6.8. The higher pH does not favour the stability of active principle. When it stored at n30-35^{0}C, the most suitable pH and temperature for storage of active substance is at pH 6-8 and temperature at 7^0C.

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