Investigations on black rot of cauliflower (*Brassica oleracea* var. *botrytis* L.) caused by *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson

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Thesis

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Maharana Pratap University of Agriculture & Technology,

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by

Hanuman Prasad Bairwa

MAHARANA PRATAP UNIVERSITY OF AGRICULTURE AND TECHNOLOGY, UDAIPUR

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Dated : - -2005

This is to certify that **Mr. Hanuman Prasad Bairwa** has successfully completed the Comprehensive Examination held on **18th May 2004** as required under the regulations for **Post Graduate Studies.**

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Dated: - -2005

This is to certify that this thesis entitled **"Investigations on black rot of cauliflower (*Brassica oleracea* var. *botrytis* L.) caused by *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson"** submitted for the degree of **Master of Science** in the subject of **Plant Pathology**, embodies bonafide research work carried out by **Mr. Hanuman Prasad Bairwa** under my guidance and supervision and that no part of this thesis has been submitted for any other degree. The assistance and help received during the course of investigation have been fully acknowledged. The draft of the thesis was also approved by the advisory committee on …….., **2005.**

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This is to certify that this thesis entitled **"Investigations on black rot of cauliflower (*Brassica oleracea* var. *botrytis* L.) caused by *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson"** submitted by **Mr. Hanuman Prasad Bairwa** to the Maharana Pratap University of Agriculture and Technology, Udaipur in partial fulfillment of the requirements for the degree of **Master of Science** in the subject of **Plant Pathology** after recommendation by the external examiner was defended by the candidate before the following members of the examination committee. The performance of the candidate in the oral examination on his thesis has been found satisfactory; we therefore, recommend that the thesis be approved.

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This is to certify that **Mr. Hanuman Prasad Bairwa** student of Department of **Plant Pathology**, Rajasthan College of Agriculture, Udaipur has made all corrections/modifications in the thesis entitled **"Investigations on black rot of cauliflower (*Brassica oleracea* var. *botrytis* L.) caused by *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson"** which were suggested by the external examiner and the advisory committee in the oral examination held on - -2005. The final copies of the thesis duly bound and corrected were submitted on - -2005 and are enclosed herewith for approval.

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Enclose: One original and two copies of bound thesis forwarded to the Director, Resident Instructions, Maharana Pratap University of Agriculture and Technology, Udaipur through the Dean, Rajasthan College of Agriculture, Udaipur.

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INTRODUCTION

Cauliflower (*Brassica oleracea* var. *botrytis* L.) is one of the important vegetable crops of India. Cauliflower is known in different parts of country by various local names *viz.*, phulghobhi, fulwar, phulkhobi. It is probably a native of South Europe in the Mediterranean region (Thompson and Kelly, 1957). It was introduced into India during Moghul period. The name cauliflower goes by the Latin name caulis, which means cabbage, and floris, which means flower.

Cauliflower is the most important member of the genus *Brassica*. Many species of this genus are grown in North India as vegetables. It is cultivated for its shortened flower parts, which is used in many preparations like curries, soups and pickles. The edible portion of plant is the "*curd*" made up of numerous divided hypertrophic branches, which terminate the main stem at the plant and is highly suppressed with number of parts of flower apparent there. As vegetable it is cooked alone or mixed with potatoes, tomatoes, mushroom etc. Cooked cauliflower contains a good amount of vitamin like riboflavin, thiamine, nicotinic acid and fair amount of high quality protein in comparison to leafy vegetable. It is rich in minerals, such as potassium, sodium, iron, phosphorus, calcium, magnesium and carbohydrate.

Cauliflower is cultivated mostly in the Northern India, as it requires cool climate to grow. Proper climatic conditions are essential to the variety used because the early varieties sown late or late varieties sown early will not bear proper curd.

At higher temperature the curd becomes ricy, leafy, loose and yellow, resulting in poor quality. At relatively lower temperature the curd remains under sized, small, unmarketable also showing some signs of "buttoning". In a dry, hot weather the curd becomes small and hard. Thus, it is very essential that the early, main and late varieties are planted at proper time; otherwise it would result in improper growth. The total area under cauliflower cultivation was about 0.27 m ha and production 4.70 m tones during 2001-02 (Economic Survey 2001-02). At present the crop is grown in various parts of the country *viz.*, Bihar, Orissa, West Bangal, U.P. (Plains), Haryana, M.P., Assam, Gujrat and Rajasthan.

It is extensively grown in the state of Rajasthan including Udaipur Zone, because of prevailing suitable agroclimatic conditions. The farmer raise the nursery during August and transplant the seedling in the month of September in the field for gaining benefit by this early maturing crops.

The crop is prone to attack by pathogens and suffers from several diseases caused by *fungi*, *viruses* and *bacteria*. The black rot disease of this crop caused by *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson, is most common and wide spread. The black rot of cabbage was first observed in Kentucky, USA, in 1891 but the causal organism was identified only during 1895 by Pammel. Feyter and Gabriel (1991) later on identified the causal organism as *Xanthomanas campestris* pv. *campestris* which produced leaf spot symptoms and hydathode necrosis on crucifers. Patel *et al.* (1949) reported that the disease is known to be prevalent in most of the areas where crucifers are grown. Severe losses due to the disease in heavily manured soil near big towns has been reported by Patel *et al.* (1949) and Chakravarti *et al.* (1969). William (1980) described this disease as a serious disease of crucifer, occurring world wide and charactrized by appearance of "V" shaped lesions on leaf margins and blackened veins associated with systemic movement of bacterium. In India, black rot of cauliflower occurr in moderate to severe form in all the states inflicting substantial losses to grovers. It was first reported by Patwardhan from Pune (Maharstra) in 1928. Chakravarti *et al.* (1969) reported that disease occurred in severe form in Rajasthan at Udaipur, which caused extensive damage to cauliflower. The black rot is one of the most important disease problems of cauliflower in Rajasthan and it occurs almost every year in all cauliflower cultivating areas of the state. This disease occurred extensively in *rabi* season in nearby area of Udaipur, especially in surrounding area of city because this crop is grown by the farmers in city area only.

Since very little information is available on black rot disease on commercially important cauliflower crop. Therefore, proposed study is considered important. The objectives of present study are:

i. Isolation, purification, characterization and proving pathogenicity of bacterium.
ii. Effect of certain abiotic factors on disease development.
iii. Biochemical analysis for protein, chlorophyll, and phenols in diseased and healthy leaves.
iv. Testing various genotypes for the source of resistance.
v. *In-vitro* and *in-vivo* testing of various chemicals and herbal extracts against bacterium causing black rot disease.

REVIEW OF LITERATURE

Characterization and Identification of Bacterium:

Cauliflower (*Brassica oleracea* var. *botrytis* L.) suffers from bacterial black rot incited by *Xanthomonas campestris* pv. *campestris* is extremely common and wide spread. It has assumed considerable importance as it becomes destructive and devastative particularly under favourable environmental conditions.

Speciation in *Xanthomonas* had been controversial. Dye (1962), on the basis of 30 different tests conducted for a comparative study of 209 phytopathogenic *Xanthomonas* comprising of 57 recognised species, suggested that many species within the genus *Xanthomonas* could be regarded as single species comprising of special forms adopted to particular hosts. He stressed the necessity of further investigations to determine the most susceptible host, host range and the mechanism of phytogenicity of *Xanthomonas*. Young *et al.* (1978) suggested, the use of term "pathovar" at the infra sub specific level on the basis of their host range. Recognising the pathovar system, the committee on taxonomy of phytopathogenic bacteria, was constituted by the International society for plant pathology. Dye *et al.* (1980) named cauliflower black rot bacterium as *Xanthomonas campestris* pv. *campestris.*

Chakravarti *et al*. (1969) gave a brief review of the disease in India. Among the fungicides and antibiotics tested *in vitro* against *X. campestris*, streptocycline was found the best followed in order by streptomycin, terramycin, hexazir, bisdithane and achromycin. A black rot disease was first observed in the USA on cabbage in 1891 but causal bacterium could only be identified during 1895. In India, disease was first described in 1928 on cabbage from Pune, Maharastra (Patwardhan, 1928). Patel *et al.* (1949) reported that the disease is known to be prevalent in most of the crucifers growing areas of country. Severe losses due to bacterial black rot disease in heavily manured soils near the big towns have also been reported.

Detection and Isolation:

Srinivasan *et al.* (1973) developed a technique for detection of *X. c.* pv. *campestris* from crucifer seeds. Pretreated the seeds with aureofungin (200 ppm) for 3-4 h were sown on water agar. The plates were incubated at 20^0C in darkness and assessments were made after 8 and 12 days. Schaad *et al.* (1980) studied the relationship of incidence of seed borne *X. campestris* to black rot of crucifers, in lab assaying and field sowing. The pathogen was detected from samples in laboratory. Whereas, black rot developed in the field in 3 of 4 such samples. Bae Dang Won *et al.* (1997) isolated the *X. c.* pv. *campestris*, the causal agent of black rot of crucifers and identified as cruciferous hosts. Obradovic *et al*. (1999) isolated the pathogen (*X. c.* pv. *campestris*) from diseased leaf tissues of cauliflower and cole plants collected in Yugoslavia from 1995 to 1998. Muhiar and Khlaif (2000) obtained 45 different bacterial isolates from different diseased crucifer hosts suspected to be infected with black rot disease collected from different cole growing area in Jordan. They also isolated the pathogen by seed washing of all the tested cabbage and cauliflower cultivars.

Media:

Shekhawat *et al.* (1982) found that detection of the black rot organism from seeds by the water agar seedling method and seed borne infection was confirmed. Ali (1989) used antiserum for detection and identification of *Colletotrichum gloeosporioides* and *X. c.* pv. *campestris*. Chang *et al.* (1991) used five media: CS20A, CS20ABN, NSCA, NSCAA and F.S. for isolation of black rot bacterium. *X. c.* pv. *campestris* grew on all media except CS20ABN where 59 – 100 per cent colonies of *X. c.* pv. *campestris* were recoreded and these media were used for rapid detection of black rot pathogen from crucifers.

Symptoms:

Mozzucchi (1968) described the symptoms produced on cabbage and cauliflower and classified according to their susceptibility in Bologna, Forli, Italy. Symptoms of the disease have been described by Onsando *et al.* (1992). Caponero and Iacobellis (1994) found typical symptoms of black rot on cauliflower plants (*Brassica oleracea* var. *botrytis*). Leite *et al.* (1994) described the symptoms of black rot disease in rape (*Brassica napus*), and observed V-shaped chlorotic lesions on the leaf margins. Cabbage heads were also invaded and discoloured. Chun *et al.* (1997) isolated and purified the *X. c.* pv. *campestris* from liquid culture by ethyl acetate extraction.

Pathogenicity:

Chakravarti and Rangarajan (1968) isolated the pathogen from 9 of 60 varieties collected from different states in India. Inoculated seedlings with the Rajasthan strain was found immune. The bacterium could survive for prolonged period in seed. Nemeth and Laszlo (1983) confirmed the pathogenicity of *X. c.* pv. *campestris* by inoculation on cabbage and kohlrabi. Somjai Lalidvongsa (1987) studied pathogenicity of nine isolate of *X. c.* pv. *campestris* and these were found to induce the V-shaped blight symptoms on the plant leaves. Scortichini *et al.* (1994) identified the causal agent of black rot disease in cruciferae as *X. c.* pv. *campestris* by pathogenicity and biochemical tests. Lenka *et al.* (1997) reported that black rot caused by *X. c.* pv. *campestris* in cauliflower was most prevalent in Varanasi and neighbouring areas in U.P., in India and they have used different methods of inoculation to prove pathogenicity of bacterium. He observed that 10 days incubation period is required for development of black rot symptoms and also reported that infection had occurred through hydathodes of leaves. Jovanovic *et al.* (1997) prorved that the pathogenicity of 4 isolates on cabbage and other crucifers. Muhiar and Khlaif (2000) conducted various pathogenicity tests for identification of the causal organism of black rot of cauliflower.

Characteristic of Bacterium:

Morphological, biochemical and cultural characteristics of the causal bacterium have been described by Patel *et al.* (1949) Obradovic *et al.* (1999) confirmed the characteristics of bacterium (*X. c.* pv. *campestris*) and proved Koch's postulates.

Inoculation Methods:

A suitable, convenient and ensured method of inoculation is very important for various field and green house studies including testing of varieties for resistance. Bacterium enters leaves and stem mainly through stomata, lenticels and wounds. Among artificial methods of inoculation, spraying bacterial suspension under pressure on leaf surface causes visible water soaking lesions on the leave in field conditions. Klement (1963) reported that injection infiltration was the best method for rapid test of pathogenicity with phytopathogenic pseudomonads. However, this method was found unsatisfactory with leaves of plants, which overcome the difficulties encountered by Klement. Starr and Dye (1965) considered that the syringe method was excellent but found it to be tedious and preferred pricking with multineedle for inoculation of various phytopathogenic bacterial species into bean pods.

Gupta and Chakravarti (1983) found that multiplication of the pathogen (*X. c.* pv. *campestris*) in cabbage cultivar, pride of India was slightly higher when the injection in filtration method was used. Bandyopadhyay and Chattopadhyay (1985) suggested suitable method for inoculation to induce the black rot disease on varieties i.e. Eclipse, Drumhead of cabbage and snowball of cauliflower caused by *X. c.* pv. *campestris*. They claimed that infection was higher when inoculation was through injured veins than through hydathodes. Pin pricking in veins caused more infection than spraying. Spraying with bacterial suspension was more effective on guttating than non guttating plants.

Foder and Pesti (1986) inoculated cabbage plants by (i) wounding at the cotyledon stage, using suspension of 10^8 cfu / ml (ii) spraying plants at the 2 leaf stage with the same suspension and inoculating plants in the field before and after heading. Robeson *et al.* (1989) established that a hydathode inoculation technique for the stimulation of natural black rot infection of cabbage by *X. c.* pv. *campestris*, where the bacteria were taken into the plant via the hydathodes. This technique, while being highly efficient, possesses several advantages over commonly used methods of inoculation.

Chaudhary and Chakravarti (1989) found that among various artificial methods of inoculation tried for *X. a.* pv. *ricini*, heavy infection developed when the leaves were inoculated after making injury with carborundum powder. Shah *et al.* (1995) observed that the bacterial blight of cowpea incited by *X.a.* pv. *vignicola* was more pronounced when the plants were inoculated through sprays twice at an interval of 24 h as compared to carborundum abrasion method. Spray inoculation method proved to be most convenient and suitable for field inoculation but required longer incubation period.

Shukla *et al.* (1995) observed surface injury to be essential for canker development in citrus. They found that symptoms developed only when inoculation were made by employing techniques like pricking with multineedle or rubbing cell suspension.

According to Kocks and Zadoks (1996) the disease development was most internsive with fresh than old refuse piles (RP). Refuse piles had 1 per cent disease plants per plot and on an av. of 0.02 diseased leaves / plant. Fresh refuse piles resulted in 30 – 70 per cent diseased plants and 1.3 – 3.5 diseased leaves per plant.

Epidemiology:

Environment conditions i.e. temperature, humidity and rain in particular decisively affect the intensity and spread of plant diseases. Warm and rainy season is known to influence the development of several bacterial diseases. Shekhawat (1975) found high humidity and temperature between 22 – 34^{0}C to be the most favourable for bacterial leaf spot of chilli (*X. a.* pv. *vesicatoria*). Similarly, Shekhawat and Patel (1977) reported that green gram leaf spot (*X. a.* pv. *phaseoli*) became severe during summers. The incidence of disease was only 0, 3 and 32 per cent from a inoculum load of 1, 10 and 100 per cent initial seed infection. They recorded 82 per cent incidence of cowpea blight during summers, which was about the same during monsoon. However, maximum increase in the incidence of cowpea blight occurred following periods with RH 75 per cent and temperature 25^{0}C. Better spread of blight to leaf, pod and seedling infection resulted during wet weather. According to them higher incidence of bacterial blight of cowpea even in summer may be because the pathogen is probably systemic in nature and can reach xylem vessels even during dry period. Jain (1982) and Singh (1987) also found warm rainy weather to be most congenial for the development of bacterial blight of guar (*X. a.* pv. *cyamopsidis*) and bacterial pastule of soybean (*X. a.* pv. *glycines*).

In Rajasthan, highest intensity of soybean bacterial pustules was recorded during September when warm weather (27^{0}C) coupled with mean RH of 86.4 per cent (Srivastava and Bais, 1985). Santiranjan *et al.* (1986) reported that cabbage planted in September, October and January and cauliflower planted in October showed higher incidence of black rot disease caused by *X. c.* pv. *campestris* than cabbage planted in October or February and cauliflower in February. Shah *et al.* (1989) reported that temperature between 28 – 30^{0}C and humidity above 80 per cent coupled with rain favoured rapid progress of cowpea blight.

Chaudhary and Chakravarti (1989) found that high relative humidity (above 70 – 75 per cent) and maximum temperature fluctuation around 30 – 35^{0}C were congenial for spot and blight development of castor (*X. a.* pv. *ricini*). Gupta (1991) reported that black rot disease of cabbage in Manipur appeared near the end of February. Ruissen *et al.* (1993) studied the growth of bacterium *X. c.* pv. *campestris* the causal agent of black rot of cabbage in static broth cultures at different constant temp. (10 – 35^{0}C).

LiMing Yuan and Wu DongFan (1995) conducted pot tests with Chinese cabbage cv. *Beijing* 106, the infection cycle of bacterial black rot caused by *X. c.* pv. *campestris* was observed after inoculation and incubation at 15, 20, 25 and 30^0C in a growth chamber for 48 h followed by transfer to room temperature conditions 11 days later. The disease developed quicker at higher temperature. Dzhalilow and Tiwari (1995) found the survival time 20 days at 20^0C and 47 days at 5^0C, whereas in stem debris survival time was 493 and 551 days on soil surface and at depth of 20 cm respectively.

According to Oliveira (1996) the incidence of black rot on cole crops was evaluated at two weeks intervals from 29th December 1992 to 14th December 1993 in Brazil. Major development of black rot had registered during the warm and humid period, whereas *Alternaria brassicae* had a higher incidence at low temperature. Ignatov *et al.* (1999) reported that *X. c.* pv. *campestris* a vascular pathogen of crucifers. His experimental results showed a potential effect of the environment on type of symptoms produced by *X. c.* pv. *campestris*. Singh (2001) reported that the incidence of cabbege black rot bacterium is higher in the month of November to January. He conducted experiments during October 2000 to February 2001 at RCA, Udaipur.

Biochemical Analysis:

Padmanabhan *et al.* (1973) reported that decrease in chlorophyll content, photo synthesis rate, Xanthophyll, carotene, starch content, total sugar content, glucose and sucrose per cent in *X. c.* pv. *citri* infected citrus leaves but increase in fructose per cent on dry weight basis. Similarly, Gupta *et al.* (1983) studied the loss of chlorophyll and electrolysis from cowpea leaves infected with *X. a.* pv. *vignicola.* Washnikar *et al.* (1998) showed reduction in moisture, chlorophyll, protein, phenole and total carotenoid contents in betel vine infected with *X. a.* pv. *betlicola.* Gour *et al.* (2001) reported that the blight affected leaves of cowpea with *X. a.* pv. *vignicola* exhibited the significant decrease in relative water contents, transpiration rate and chlorophyll contents as compared to healthy one.

Varietal Screening:

Henz *et al*. (1994) screened twenty-five *Brassica oleracea* var. *capitata* cultivars (including 13 Japanese 11 Dutch and the *Brazilian* cv. Master AG 325) for resistance to *X. c.* pv. *campestris* under greenhouse conditions. Pandey *et al*. (1995) worked on breeding for resistant at Bangalore. Cauliflower lines resistant to *X. c.* pv. *campestris* were identified as: IIHR 103, broccoli IIHR 104, IIHR 55, IIHR 82 and IIHR 106. Resistance to the bacterial pathogen in cauliflower was governed by additive and non-additive genes with the latter preponderant. According to Monakhos *et al*. (1995) screened the varieties Zimovka 1474, Decema, Extra and "V" yuga and F_1 hybrids kryumon, Ekstra and Kolobok were tested under field conditions in the Moscow province of Russia after artificial infection using two methods of inoculation. Kocks and Ruissen (1996) reported disease progress and gradient curves of black rot (*X. c.* pv. *campestris*) on cabbage were evaluated in field plotes of the cultivars Bartola, Erdeno, Perfect Ball, and Roxy in the Netherlands during 1991 and 1992. Plots were inoculated by single source in the centre of each plot. Ignatov *et al.* (1997) screened 106 entries from sub species of *Brassica rapa* (*B. campestris*) crop and 10 weekly populations were also evaluated for resistance to *X. c.* pv. *campestris* race 1, 4 and 5 in different geographical regions of the world. They reported first 1998 regarding resistance gene identified based on gene for gene interaction and different races of the pathogen.

Galli *et al.* (2002) evaluated commercial seed lots of cabbage and cauliflower in relation to *X. c.* pv. *campestris* infection. The degree of resistance was studied on the basis of genotype and the best inoculation method to detect the resistance to black rot were also determined. Taylor *et al.* (2002) worked on 276 accessions of *Brassica* spp. for resistance against *X. c.* pv. *campestris.*

Disease Management:

Many chemicals have been found effective *in vitro* in inhibiting the growth of phytopathogenic bacteria but few are effective in controlling the bacterial disease in field conditions. Many workers have reported the use of different antibiotics and bactericides, as seed treatment and foliar sprays, to control many bacterial disease in plant. (Morgan Goodman, 1995; Chakravarti and Rangarajan, 1968; Chakravarti *et al.,* 1969; Chakravarti *et al.,* 1969; Shekhawat, 1975; Kishun, 1983 and Singh, 1987).

Chakravarty and Rangarajan (1968) conducted an experiment and results showed terramycin, streptomycin, synermycin and Streptocycline to be effective against *Pseudomonas lapsa* and *Erwinia carotovora* causing maize stalk rot. Fungicides were much less effective.

Singh (1987) reported that both agrimycin-17 (1 : 2000) and streptocycline (1 : 27000) in water, lowered *X. oryzae* incidence on rice and increased yields as compared with the control.

Namasivayam and Hegde (1971) found streptocycline was best, followed by agrimycin or streptomycin, in an inhibiting the growth of *X. c.* pv. *campestris*. Shukla *et al.* (1995) reported that bavistin (500 ppm) at 3 weekly sprays gave the best control of *X. c.* pv. *campestris* among 15 fungicides and 3 antibiotics tested in the field. Areton (Methoxy ethyl mercury chloride) at 200 ppm was the most effective in laboratory bioassay test. According to Schaad (1980) treating seeds in cupric acetate at 40^0C should prove useful for eradicating *X. c.* pv. *campestris* from seeds. Sharma (1981) suggested that best control of *X. c.* pv. *campestris* in cauliflower was obtained by treating the seeds in water at 52^0C for 0.5 h followed by a dip in streptocycline at 100 ppm, plus spraying the crop with streptocycline (50 ppm) at transplanting and again at curd and seed-pot formation. Shekhawat *et al.* (1982) found that streptocycline (0.01 per cent) + captan (0.02 per cent) is most effective than hot water (50^0C for 30 min.). Agrimycin 100 + captan found most effective against infected cabbage seed with black rot pathogen (*X. c.* pv. *campestris*).

Kishun (1983) found that in trials against *X. c.* pv. *campestris*, soaking seeds in plantomycine (streptomycin sulphate + tetracycline + vitamin B_{12}) at 100 ppm for 30 min. gave best result in reducing incidence and increasing yield. Kim (1986) observed that seed soaking in 3 per cent hydrogen peroxide for 30 min. is most effective for eradication of bacterium from a naturally infected seed lot. Onsando *et al.* (1992) reported that the use of grass mulch to reduce soil splash and hence secondary spread of this disease through the hydathodes was as effective as application of cobox (50 per cent cu) in managing the disease and increasing yield. According to Fukaya *et al.* (1998) copper oxychloride-kasligamycin combination is the most effective against black rot disease. Huang and Lee (1988) suggested that soaking seed infested with *X. c.* pv. *campestris* in 0.1 m acidified zinc sulphate (pH 2.8) for 20 min. at 38 – 40^0C was the best treatment tested for eradicating the pathogen from crucifers and better than hot acidified copper-acetate, calcium hypochlorite or hot water treatments. Gupta (1991) reported good control of black rot by agrimycin 100 (1000 ppm), Streptocycline (250 ppm), carbendazim (0.05 per cent) and benomyl (0.05 per cent).

Shiomi (1992) reported that the seeds were effectively disinfected by hot air treatment at 75^0C for 6 days or 75^0C for 2 days for naturally infected seeds. No adverse effect on seed germination was observed with hot air treatment at 70^0C for 7 days, while the treatment at 75^0C even for 1 day prevented seed germination. It was suggested that pre drying seeds at 70^0C for 24 h followed by hot air treatment at 75^0C for 5 – 7 days is an effective method of disinfections, cabbage seeds infected by *X. c.* pv. *campestris*, without causing seed damage.

Minchinton (1994) found that treatment with hot water and sodium hypochlorite apparently completely killed the bacteria in naturally and artificially infected seeds. According to Dzhalilov *et al.* (1994) rhizoplan (*Pseudomonas* sp. Ap 33) applied as a seed treatment and 2 sprays in the field gave good control of black rot and other bacterial disease of cabbage and increased yield in trial conducted in Russia during 1990-93. Mochizuki and Alvarez (1996) mentioned that a single prophylatic treatment of fosetyl AI at 4800 mg / 1 kg a. i. as a spray and soil drench reduced the invasiveness of effect of fosetyl-AI treatment was more pronounced at 20^0C and decreased as temperature increased. Korobko (1997) mentioned that calcium hydrochloride gave the best control of black rot (*X. c.* pv. *campestris*) comparison to other treatment of vita gram and formalin on cabbage. Kumar and Sharma (1997) reported that application of boron alone or in combination with nitrogen significantly reduced black rot disease severity from 48.5 to 30.9. Singh (2001) reported that the maximum inhibition (25.06 mm) of *X. c.* pv. *campestris* growth recorded by Streptocycline (500 ppm) followed by plantomycine, and fytolan whereas bavistin failed to inhibit the bacterial growth. Bora and Bhattacharya (2002) reported good control of black rot of cabbage by aqueous extract of *Terminalia chebala* and *Sesbania aculata.*

Plant Extracts:

A large number of plants are known to have antiphyto-bacterial activities (Skinner, 1955; Allen and Rawson, 1960; Singh and Sharma, 1978; Kumar and Tripathi, 1991; Tripathi *et al.*, 1982). Garg and Kasera (1984) studied the strong antibacterial activity of the essential oil of *Anocardium occidentale* linn against *X. c.* pv. *campestris* causing black rot of crucifers and *Pseudomonas mangiferae* causing leaf spot of mango. Miles *et al.* (1990) obtained a new agrochemical, 3 Formyl -2, 4, 6 –trihydrozy-5 methyl-dihdrochalcone, from CH_2Cl_2 extract of leaves of *Psidium acuntangulum* plants, which showed activity against *X. c.* pv. *campestris.*

Csizinszky *et al*. (1993) reported that three strains of *X. c.* pv. *citri* causing citrus bacterial canker disease and bacterial spot in Florida, USA (F_1) were incubated with 3 concentration (0.5, 0.75 and 1 ml / litre) of extracts from *Matricaria recutita* (*Chamomilla recutita*) *Chamaemelum nobile* and *C. recutita* extract inhibited the growth of all strain except the Brazilian at all concentration. *C. nobile* extract at 0.75 and 1 ml / litre inhibited all strains; Gracia *et al.* (1994) reported that black rot of crucifers caused by *X. c. campestris* in cabbage and cauliflower can be controlled by using various plant extracts.

Lirio *et al.* (1998) Screened aqueous extract of 36 plant species for antibacterial activity against *Ervinia carotovora* pv. *carotovora, X. c.* pv. *campestris* and *Pseudomonas solanecearum.* And 21 plant species showed antibacterial activity. Garcia *et al*. (1994) reported that black rot of crucifers caused by *X. c.* pv. *campestris* in cabbage and cauliflower can be controlled by using various plant extracts.

MATERIALS AND METHODS

Glasswares Used:

In all experiments, corning a Borosil made glwaswares were used. Before conducting the experiments, the glasswares were cleaned with 6 per cent chromic acid ($K_2Cr_2O_7$-60g, concentrate H_2SO_4-60 ml and distilled water 1000 ml) followed by several washing in running tap water and finally cleaned with distilled water and air dried.

Survey of Disease:

Periodical surveys were carried out from November to February for bacterial black rot of cauliflower during the year 2003-04 in the vicinity of Udaipur district for collection of diseased samples during rabi season in the four villages *viz.*, Hawala, Bhujeda, Choti Nokha and Manwa Khera. In the surveyed fields, incidence of black rot was also recorded. To assess the incidence of disease three fields were observed in each village and five plots from each field was randomly selected for sampling and than per cent disease incidence was calculated. In each field diseased and healthy plants were counted from each randomly selected five plots and per cent incidence of disease was calculated by using following formula given below:

$$\text{Pre cent disease incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants observed}} \times 100$$

The disease sample were brought to the laboratory and thoroughly washed with sterile distilled water. For the confirmation of the presence of bacterium ooze test was performed in laboratory under the light compound microscope at low power objective.

Isolation, Purification and Pathogenicity:

Cauliflower leaves showing symptoms of bacterial black rot were collected from Nokha village in Udaipur District. The presence of the bacterium was confirmed by ooze test. For isolation of the causal bacterium, diseased portions of leaves were cut, surface sterilized for 2-3 min. with 2 per cent sodium hypochlorite solution and washed throughly thrice with sterile distilled water. The bits were then transferred individually into a few drops of sterile water on a sterilized glass slide in aseptic condition. The diseased bits were given a cut with sharp sterilized blade. The cuts bit were left for two minutes to allow bacterial ooze to come out in water. A loopful of ooze, so obtained was streaked on nutrient agar Petri plates. Three Petri plates were streaked at a time, without recharging the needle loop.

This procedure was repeated many times using fresh sets of plates each time. The inoculated plates were incubated at 28±1^{0}C for 48 h in a inverted position. The yellow, single bacterial colonies developing on the medium were picked up, under a stereoscopic microscope, with inoculating needle and streaked on same medium in same way as was done earlier for the purification of bacterial culture. The process was repeated until all colonies developing on the medium had similar cultural appearance. Such culturally identical single colony was then restricted on yeast chalk agar slant and maintained by preserving in a refrigerator and making periodic transfers after every fortnight.

Pathogenicity tests were conducted by inoculating two months old cauliflower (cv. Pusi) plants raised in earthen pots, by carborundum abrasion technique (Leben *et al.*, 1968). Inoculated plants were kept in cage house under high humid condition for 48 h and then under natural cage house conditions. The plants were watered at frequent interval and regularly observed for disease development. The pathogen was isolated from inoculated plants and compared with original culture.

Sterilization:

The medium used was sterilized at 1.045 kg / cm^2 for 20 minutes in an autoclave while all the glasswares were sterilized in the hot air oven at 180^{0}C for two h. The cork borer, inoculation needle, forceps ets. were initially dipped in rectified spirit and finally starilized on flame of spirit lamp and allowed to cool down before use.

Characterization and Identification of Bacterium:

For understanding and identifying the bacterium, its morphological, cultural and biochemical characteristics were studied according to the procedures described by Pelczar *et al*. (1957), Dowson (1957), Dye (1962) and Ryu (1980).

Microscopic Studies by Standard Preparation:

Following standing methods were used to study the morphological characteristics of bacterial cells.

(i) **Gram's Differential Staining (Dowson, 1957):**

A thin bacterial smear was prepared from fresh culture (24 h) on grease free glass slide. It was air dried, heat fixed and covered with ammonium oxyalate crystal violet[1] solution at least for one minute and washed in tap water for not more than 2 seconds. Gram's iodine solution[2] was then applied for one min. and washed in distilled water. Ethyl alcohol (95 per cent) discolouring agent was added drop by drop to decolourize the stain and then counter stained with Safranin[3] for 30 seconds, washed with distilled water, air dried and examined under oil immersion objective lens of a compound microscope.

(ii) **Flagella Staining (Ryu, 1980):**

Solution 1: 5 per cent Carbolic acid 10 ml, powdered Tannic acid 2g, saturated solution of Potassium alum. 10 ml.

Solution 2: Saturated alcoholic solution of crystel violet 12 per cent.

Ten parts of solution 1 (Mordant) and one part of solution 2 were throughly mixed right before staining.

Smear prepared from 24 h old culture, was air dried on clean grease free slide. Poured the mixed solution on the smear and allowed it to act at room temperature for 3-5 min., washed with distilled water and examined.

(iii) **Capsule Staining (Pelczar *et al.*, 1957):**

Solution 1: Crystal violet (85 per cent dye content) 0.1 g, Glacial acetic acid 0.25 ml, distilled water 100 ml.

Solution 2: 20 per cent aqueous copper sulphate ($CuSo_4 .5H_2o$)

Smear from 48 h old culture was prepared on clean glass slide and air dried, poured the acetic crystal violet solution 1 on the smear for 4-7 min. and then washed with 20 per cent copper sulphate solution 2. The slide was air dried and examined under oil immersion objective of light compound microscope.

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1. Ammonium Crystal Violet: Solution A (Crystal violet 0.2 g, 95 per cent ethyl alcohol 20 ml), Solution B (Ammonium oxyalate 0.8 g, distilled water 80 ml) mix solution A and B.
2. Gram's iodine solution: Iodine 1 g, KI 2 g, distilled water 300 ml.
3. Safranin Solution: Safranin (2.5 per cent solution in 95 per cent ethyl alcohol) 10 ml distilled water 100 ml.

Cultural Characteristics:

The bacterium was grown on nutrient agar[4], potato dextrose agar[5] and yeast extract glucose chalk agar[6] in Petri plates under aseptic condition. Inoculated Petri plates were inocubated at 28±1^{0}C. After 3 days of incubation, characters of bacterial colonies were studied under microscope. The colonies were observed up to 10 days.

Biochemical Characteristics:

The biochemical characteristics of black rot bacterium were determined by following thirteen tests. Fresh 48 h old bacterial culture was used for all the tests.

(i) **Oxygen Requirement (Dye, 1962):** The aerobic and anaerobic nature of bacterium was determined by inoculating culture by stabbing in nutrient agar medium[7] containing 0.005 per cent bromocresol purple. Half of inoculated tubs were filled with sterilized paraffin liquid to cover the agar surface, whereas the others were left as such. All the tubs were incubated at 28±1^{0}C for 7 days and observed daily for change in colour from blue to yellow.

(ii) **Sodium Chloride Tolerance (Dye, 1962):** Tubes containing 1 to 5 per cent sodium chloride in yeast extract salt broth[8] were inoculated, incubated at 28±1^{0}C and observed for 7 days. The broth without Sodium chloride was used as check.

(iii) **Mode of Glucose Utilization (Hayward, 1964):** 90 ml of the agar medium[9] was autoclaved at 1.045 kg/cm^2 for 15 min. 10 per cent glucose solution was separately sterilized at 0.703 kg/cm^2 for 10 min, mixed at the rate of 10 ml per 90 ml of the melted medium, dispensed in sterile tubes to a depth of 4 cm, allowed to solidify and then inoculated by stabbing method. Half of the inoculated tubes were sealed by covering the surface of the medium with sterilized paraffin liquid, incubated at 28±1^{0}C and observes at regular interval up to 7 days.

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4. Nutrient Agar: beef extract 3 g; Peptone 5 g; agar 20 g; dist. water 1 lit., pH 7.
5. Potato dextrose agar: Potato 250 gm, dextrose 20g; agar 20g; dist. water l lit., pH 7.
6. Yeast extract glucose chalk agar: Yeast extract 10 g; dextrose 20 g; Calcium carbonate 20 g; agar 20 g; dist. water 1 lit., pH 7.
7. Nutrient dextrose agar medium: beef extract 3 g; peptone 5 g; dextrose 10 g; agar 20 g; dist. water 1 lit., pH 7.
8. Yeast extract salt broth: $NH_4H_2PO_4$ 0.5g; K_2HPO_4 0.5 g; $MgSO_4$ $7H_2O$ 0.2 g; Nacl 5 g; Yeast extract 5 g; dextrose 5 g; dist. water 1 lit. pH 7.
9. Medium for mode of glucose utilization (Hayward, 1964): Peptone 1 g; $NH_4H_2PO_4$ 1 g; KCl 0.2 g; $MgSO_4$ $7H_2O$ 0.2 g; bromothymole blue 0.03 g, agar 10 g; dist. water 1 lit. pH 7.

(iv) **Starch Hydrolysis (Pelczar *et al.*, 1957):** Inoculations were made at three places on starch agar medium[10] in Petri plates. Incubated Petri plates were incubated at 28 ± 1^0C for 7 days and then flooded with Lugol's iodine solution (iodine 1 g, IK 2g, dist. water 300 ml). The formation of clear zone around the growth of bacterium indicated the hydrolysis of starch.

(v) **Gelation Liquefaction (Pelczar *et al.*, 1957):** Inoculations were made at different spots on gelation medium[11] in Petri plates which were then, incubated at 28 ± 1^0C for 2 days. Then Petri plates were flooded with 8-10 ml mercuric chloride solution (H_gCl_2 15 g, conc. HC1 20ml, dist. water 100 ml). Liquefaction of gelatin was indicated by clear zone around bacterial growth.

(vi) **Urease Production (Dye, 1962):** Modified yeast extract salt broth[12] (0.1 per cent) was inoculated after adding 2 per cent urea aseptically to the medium. Thymol blue was used as an indicator. The tubes were incubated at 28 ± 1^0C for 7 days. A blue colour developed in case of urease positive reaction.

(vii) **Catalase Activity (Pelczar *et al.*, 1957):** A loopful of bacterial culture was mixed with a drop of 10 per cent hydrogen peroxide on slide and examined for any gas bubbles, which indicated positive reaction.

(viii) **Production of Ammonia:** Peptone water tubes[13] were inoculated and incubated at 28 ± 1^0C for 7 days. Culture tubes were tested by adding Nessler's reagent[14]. A yellow orange precipitate indicated the presence of ammonia.

(ix) **Hydrogen Sulphide Production:** Dry sterilized filter paper strips soaked in saturated lead acetate solution, were suspended in inoculated peptone water tubes along with cotton plugs and incubated at 28 ± 1^0C for 14 days. The blackening of strips indicated liberation of H_2S.

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10. Medium for starch hydrolysis: Peptone 10 g; beef 5 g; soluble starch 2 g; agar 20 g; dist. water 1 lit., pH 7.
11. Medium for gelatin liquefaction: Peptone 10 g; beef extract 5 g; gelatin 4 g; agar 20 g; dist. water 1 lit., pH 7.
12. Yeast extract salf broth: $NH_4H_2PO_4$ 0.5 g; K_2HPO_4 0.5 $MgSO_4$ $7H_2O$ 0.2 g; NaCl 5 g; Yeast extract 5 g; dextrose 5 g; dist. water 1 lit., pH 7.
13. Peptone water: Peptone 10 g; NaCl 4 g; dist. water 1 lit., pH 7.
14. Nessler's reagent: 62.5 kg KI was dissolved in 250 ml dist. water. About 10 ml of this solution was reserved. A cold saturated solution of $HgCl_2$, stirred continuously and increasing the volume of $HgCl_2$ until a permanent precipitate was formed was added in remaining KI solution. The reserve KI solution and additional $HgCl_2$ solution were added until a distinct precipitate remained. Then 150 g of KOH was dissolved in 150 ml dist. water, cooled and added to the above solution. It was then diluted to 1 lit. with distilled ester, allowed to stand for one week and decanted for use.

(x) **Methyl Red Test (Pelczar *et al.*, 1957):** Bacterium was grown in methyl red test medium[15] and was tested after 7 days of incubation at 28±1^{0}C by adding few drops of methyl red (methyl red 0.01 g, 95 per cent ethyl alcohol 300 ml, dist. water 500 ml). A distinct red colour indicated a positive reaction.

(xi) **Voges-Proskauer Test (Pelczar *et al.*, 1957):** Bacterium was grown in methyl red test medium, incubated at 28±1^{0}C for 7 days and then tested by adding 0.6 ml of 5 per cent L-naphthol in absolute alcohol and 0.2 ml of 40 per cent potassium hydroxide in 1 ml of culture broth. These tubes were left for 2-4 h for colour development. A crimson colour indicated positive reaction.

(xii) **Reduction of Nitrates:** Bacterial inoculation was made in nitrate broth[16] and incubated at 28±1^{0}C for 15 days. The test was done by adding few drops of sulphonilic acid (0.8 per cent in 5 M acetic acid) and dimethyl L-naphthyl-amine (0.5 per cent in 5 M acetic acid) to the broth culture. A distinct pink or red colour in broth indicated the presence of nitrate.

(xiii) Utilization of Asparagine as a Sole Source of Carbon and Nitrogen: Inoculations were made in ml of asparagine medium[17] incubated at 28±1^{0}C for 7 days and observed for the growth of bacterium.

Inoculation Methods:

The details of all the five inoculation methods used are as under:

(i) **Spraying The Inoculum With Hand Atomizer (Klement, 1968):** The bacterial suspension (1 x 10^8 cfu/ml) was sprayed on cauliflower plants with hand atomizer. The inoculated plants were kept under high humid condition for 48 h by covering them with polyethylene bags lined with wet cotton swabs and then left as such under natural condition.

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15. Medium for methyl red test and Voges Prokauer test: Peptone 5 g; dextrose 5 g; K_2HPO_4 5 g; dist. water 1 lit., pH 7.
16. Nitrate broth: Peptone 10 g; beef extract 5 g; KNO_3 (nitrate free) 1 g; dist. water 1 lit., pH 7.
17. Medium for utilization of asparagines as a sole source of carbon and nitrogen (Dye, 1962)
    Solution 1: K_2HPO_4 8 g; KH_2PO_4 2 g; dist. water 100 ml.
    Solution 2: $MgSO_4$ 0.02 g; H_2SO_4 (Nitrogen free) 0.1 ml; dist. water 100 ml.
    Solution 3: Na_2MoO_4 0.02 g; dist. water 100 ml.
    Solution 4: Copper sulphate saturated solution in dist. water 10 ml of each solution in order 3, 4, 2 and 1 was filtered and added to 960 ml water in which 2 g L-asparagine was dissolved; pH 7.

(ii) **Carborundum Abrasion Method (Leben *et al.*, 1968):** The plants were inoculated with help of cotton Swab on both the surfaces of leaves. The cotton swab was soaked in inoculum and carried some carborundum powder (300 mesh) for making gentle injury and applied inoculum simultaneously.

(iii) **Multineedle Pricking Method (Andrus, 1948; Starr and Dye, 1965):** The two month old plant leaves were gently injured with the help of multineedle prepared by fixing 8-10 fine entomological pins (No.20) in a cork in such a way that ends remain projected by 1 mm. The opposite surface of the cork dipped in melted sealing wax to fix needles. The injury to the leaves was gentle so that it did not tear or perforate leaf surface. The bacterial suspension (1×10^8 cfu/ml) was then automized on the leaf surface.

(iv) **Injection Infiltration Method (Klement, 1963):** Inoculations were made by injecting 0.1 ml of bacterial suspension (3.5×10^6 cfu/ml) in the two month old leaf mesophyl by hypodermic syringe (needle No. 24).

(v) **Hydathode Inoculation Method (Robeson *et al.*, 1989):** In this method, the bacterial suspension (1×10^8 cfu/ml) was introduced into guttation droplets on the leaf margin. Bacteria are taken into the plant via the hydathodes, thereby avoiding the mechanical injury to the plant and findout the possibility of disease symptoms development.

Suitable controls were maintained in each case using only distilled water in place of inoculum suspension. The inoculated plants were kept in cage house under high humid condition for 48 h. The plants were then, observed regularly for disease development. For all inoculations, 72 h old culture was used.

For close observation of the disease, disease rating was made on twenty plants for each inoculation method. To measure the quantum of disease, following disease rating key was devised based on disease development in which infected plants were categorised in six arbitrary classes (Scale 0-5).

0 = No infection
1 = Very slight bacterial infection covering 1-10 per cent leaf area.
2 = Slight bacterial infection and covering 11-25 per cent leaf area.
3 = Moderate bacterial infection which covering 26-50 per cent leaf area.
4 = Severe bacterial infection, covering 51-75 per cent leaf area.
5 = Very serve bacterial infection and covering more than 75 per cent leaf area.

Per cent infection index was calculated by using following formula (Mc Kinny, 1923; Chester 1959 and Wheeler, 1969):

$$\textit{Per cent Infection Index} = \frac{\textit{Sum of individual rating}}{\textit{No. of plants or leaves assessed X maximum disease rating}} \times 100$$

Environmental Factors on Disease Development:

Effect of weather parameters on black rot development on cauliflower was studied during crop season of rabi, 2003-04.

Cauliflower variety Pusi* seeds were sown in seed bed at weekly intervals. After 35-40 days, the cauliflower seedlings were transplanted in 30 cm earthen pots at every weekly interval so as to provide the plants of same age for inoculation. In each pot two plants were accommodated and five pots were used for each inoculation. Plants were inoculated after 10-12 days of transplanting in pots from seed bed by carborundum abrasion technique with bacterial suspension (concentration 10^8cfu/ml) at 7 days interval. The infection index was recorded 21 days after inoculation on each plant. The weather data viz. weekly temperature, relative humidity and rainfall were obtained from farm observatory, Rajasthan College of Agriculture, Udaipur.

Observation in respect of disease development under cage house conditions was made at Plant Pathology cage house at Rajasthan College of Agriculture (RCA), Udaipur In rabi, 2003-04 and were correlated with the meteorological data obtained from the farm weather observatory of RCA, Udaipur. To record the observation, ten plants on which initial symptoms of the disease observed on 20th October 2003 were tagged and thereafter observation on disease development were recorded at weakly interval.

Biochemical Changes:

Estimation of Chlorophyll:

The quantitative estimation of chlorophyll was done by the method of Arnon (1956), Leaf samples (1 g each) from diseased and healthy plants were cut into small pieces and homogenized with excess chilled acetone (80 per cent). The supernatant was decanted into flask filtering through a Buchner funnel using Whatman No.42 filter paper. The extraction was repeated 3-4 times and the total volume raised to 100 ml in volumetric flask. Five ml of the extract was transferred into a 50 ml volumetric flask and diluted by making up the volume with 80 per cent acetone. The absorbance (optical density) was measured at 663, 645 nm with systronics spectrophotometer.

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* J.K. Seeds, Lakhnow

The chlorophyll a, b and total chlorophyll contents were calculated on a fresh weight basis employing the following formulae:

$$\textit{Total chlorophyll}\ (mg/g) = \frac{20.2\,A_{645} + 8.02\,A_{663}}{a \text{ X } 1000 \text{ X } W} \text{ X } V$$

$$\textit{Chlorophyll a}\ (mg/g) = \frac{12.7\,A_{663} - 2.69\,A_{645}}{a \text{ X } 1000 \text{ X } W} \text{ X } V$$

$$\textit{Chlorophyll b}\ (mg/g) = \frac{22.7\,A_{645} + 4.68\,A_{663}}{a \text{ X } 1000 \text{ X } W} \text{ X } V$$

Where,

A	=	Absorbance at 663, 645, 450 nm in a 1.0 cm cell
V	=	Volume of original extract in ml
W	=	Fresh weight of the sample (mg)
a	=	Length of light path in the cell (1 cm, usually)

Estimation of Protein:

The micro-Kjeldhal method was used to estimate the nitrogen of protein content in the leaf samples. For this 100 mg of leaf sample ground and put in a dried Kjeldhal's flask. To this 3 ml of concentrated H_2SO_4 was added and digested on a heater for about one hour till the colour of the content became dark brown to black. Flasks were cooled and 1 ml of H_2O was added, again digested till the content became colourless and H_2O_2 fumes escaped. The volume was made to 50 ml out of this 1.5 ml aliquot was taken in another 50 ml flask and to this, 1 ml each 10 per cent NaOH, 10 per cent sodium silicate and Nessler's reagent were added. The volume was made to 50 ml with distilled water. The colour thus developed was read at 625 nm with maintaining a suitable control of distilled water by same procedure.

A standard curve was prepared by using 0.1185 g ammonium sulphate $[(NH_4)_2\ SO_4]$ in distilled water and volume was made to 1 litre which gave the concentration of nitrogen 0.2513g/litre i.e. 25 ppm. In 50 ml volumetric flask 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 ml solution of ammonium sulphate was taken out by pipette, colour was developed by procedure and a graph of ppm versus optical density was plotted. The protein content was calculated by multiplying the nitrogen content with a constant factor 6.25.

Estimation of Total Phenol:

Estimation of phenols with Folin-ciocalteu reagent was based on the reaction between phenols and an oxidizing agent phosphomolybdate which results in the formation of a blue complex (Bray and Thorpe, 1954). To prepare reagent; 100 g sodium tungstate (Na_2 Mo_4. $2H_2O$) and 25 g sodium molydate ($NaMoO_4$. $2H_2O$) were dissolved in 700 ml water in 1 litre flask. To this 50 ml 0f 80 per cent or the phosphoric acid (H_2PO_4) and 100 ml of concentration HCl was added and the mixture was boiled reflux for 10 h. Then it was cooled and 150 g lithium sulphate was dissolved in 50 ml water and 4.5 drops of liquid bromide were added. This mixture was boiled about 15 minutes to remove excess of bromine; cooled volume raised to one litre and filtered through Whatman No. 42 filter paper. This golden yellow coloured reagent was stored in brown bottle at 4^0C when not in use. The reagent was used after diluting it with 2 volumes of water.

Ethanol extract prepared earlier for estimation of sugars was used. One fifth ml (0.5 ml) extract was taken in a graduated test tube, and evaporated to dryness on a water bath 1 ml distilled water was added .To this 1 ml of Folinciocalteu reagent followed by 2 ml of Na_2CO_3 (20 per cent aq.) was added. The mixture was shaken gently and placed in boiling water bath for exactly 1 min. Thereafter, the contents were cooled, diluted to 25 ml and the absorbance of reaction mixture was measured at 650 nm. Blank containing all the reagents except plant extract was used to adjust the absorbance at zero. The amount of total phenol in sample was calculated from a standard curve prepared from catechol.

For standard curve, stock solution of catechol was prepared by dissolving 10 mg catechol in small volume of ethanol and volume raised to 100 ml. From this 0.1, 0.2, 0.4, 0.6, 0.8 and 1 ml aliquots were taken in tubes and the volume was raised to 1 ml in each case by adding distilled water. These were processed in the same way and the percent transmission was plotted against concentration of catechol.

Screening of Different Varieties Against Black Rot of Cauliflower:

Six cauliflower varieties *viz.*, Pusi, Kartiki specia, Snowball, Vigro Ageni, Pusa Deepali and Vigro Kartiki were evaluated for resistance to black rot of cauliflower caused by *X. c.* pv. *campestrics* under artificial inoculation condition during rabi 2003-04. These varieties were consisted of different maturity group's *viz.*, early, medium and late maturity. Seeds were received from local market of Udaipur city. Two seedlings were transplanted in each pot. Between two varieties infector replication were also maintained.

The 15 days after transplanting each plant individually inoculated with the bacterial suspension. Humidity was maintained by frequent irrigations. Observations were recorded 21 days after inoculation by following standard 0-4 disease rating scale.

Grade	Percent leaf area affected	Disease Reaction
0	0	Highly resistant
0.1 – 1.0	0.1 – 5	Resistant
1.1 – 2.0	5.1 – 15	Moderately resistant
2.1 – 3.0	15.1 – 50	Susceptible
3.1 – 4.0	Above 50	Highly susceptible

Management of Disease:

Different chemicals and plant extracts were tested for their efficacy against black rot bacterium in (1) Laboratory by paper disc method (Waksman and Reilly, 1945), (2) Effect of seed treatment on seed germination.

The chemicals used during this study were: Streptocycline (90 per cent) streptomycine sulphate + 10 per cent Tetra cycline hydrochloride), Plantomycine (75 per cent streptomycine sulphate + 25 per cent Chlorotetracycline hydrochloride).

The extracts of four plant species i.e. Garlic, Neem, Tulsi and Zinger were tested for their efficacy.

S.No.	Name of plant	Botanical name	Plant part use for extraction
1.	Garlic	*Allium sativum* L.	Bulbs
2.	Neem	*Azadirachta indica A. Juss*	Leaf
3.	Tulsi	*Ocimum sanctum* L.	Leaf
4.	Ginger	*Zingiber officinale Rosc.*	Rhizomes

Laboratory Assaying:

(i) Assaying of Chemicals on The Growth of The Bacterium: One ml of bacterial suspension in sterile distilled water prepared by suspending 48 h old bacterial culture from yeast extract glucose chalk agar slants in 5 ml sterile water, was incorporated in 250 ml of bioassay medium[18] mixed thoroughly 20 ml of medium by equal volume was poured in each sterilized Petri plates. Sterilized Whatman No. 1 filter paper discs of 10 mm diameter, soaked in different concentrations (50, 100, 200, 250, 300, 400 and 500 ppm) of test chemicals, were placed on the medium (3 disc/plate). The Petri plates were then incubated at 10^0C for 2 h for allowing the chemical from disc to diffuse in the medium and then incubated at 28 ± 1^0C for 72 h and then inhibition zone recorded.

(ii) Assaying of Plant Extracts on The Growth of The Bacterium: The extracts of four plant species were used to test the efficacy against black rot causing bacterium. After washing, the plant materials were surface sterilized (2 per cent sodium hypochlorite solution) and kept in sterilized covered beaker.

All plant materials were crushed with sterilized water (1 : 1 w/v) and the material was homogenized for five minutes and the mixture was filtered through sterilized filter paper (Whatman No. 1). The resultant extracts were tested for efficacy by paper disc method at 5, 10, 20, 25 and 50 per cent concentrations.

Effect of Antibiotics on Seed Germination:

Two above mentioned chemicals i.e. Streptocycline, Plantomycine and Extracts of different plant species were tested under laboratory conditions on seed germination. Inoculated seeds with *X. c.* pv. *campestris* were treated with these chemicals and plant extracts at different concentrations by seed soaking methods for 2 h.

Treated seeds were plated on three layers of moistened blotter paper kept in sterilized Petri plates @ 10 seeds per Petri plate and incubated at temperature (28-30^0C). Sterilized distilled water was used to maintain humidity. Untreated seeds served as control. Observations were recorded on percent seeds germination after 10 days of incubation. The experiment was repeated thrice.

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18. Bioassay medium (Waksman and Reilly, 1954): Peptone 10 g; beef extract 3 g; yeast extract 5 g; distilled water 1 lit., pH 7.

EXPERIMENTAL RESULTS

Survey of Disease:

A preliminary survey was carried out in Hawala, Bhujeda, Choti Nokha, and Manwa Khera villages of Udaipur district of Rajasthan during Rabi 2003-2004. During the survey discussions were held with the concerned farmers. As a result of this discussion, it was revealed that disease appeared in most of the fields wherever, the cauliflower (*cv. Snowball*) crop was grown. It was observed that the PDI was more in the month of January and February. Observations recorded on the incidence of disease are presented in Table-1.

Table 1 Per cent incidence of black rot of cauliflower caused by *X. c.* pv. *campestris*

Time / Place	PDI (per cent disease incidence)*				
	Nov.	Dec.	Jan.	Feb.	Mean
Choti Nokha	Trace	20.1	24.5	30.8	18.85
Bhujeda	1.3	25.5	30.3	38.6	23.92
Hawala	1.6	27.3	31.6	40.3	25.2
Manwa Khera	Trace	22.6	26.8	37.4	21.92

*Average of five replications.

The bacterial black rot disease was observed in all four villages during Rabi 2003-2004, maximum black rot (25.2 per cent) was recorded at village Hawala, followed by Bhujeda (23.92 per cent), Manwa Khera (21.92 per cent) and Choti Nokha (18.85 per cent).

The incidence of the disease was successively increased during this period but comparatively high during January and February. The disease incidence varied from trace to 40.30 per cent. The disease made its appearance after 15 days of transplanting.

Symptoms:

The bacterium, *X. c.* pv. *campestris* initially produced minute irregular water soaked lesions 3-4 days after inoculation, mostly on the leaf. Infection at the periphery of leaf was quite common in nature. These lesions were brown yellow in colour produced near the leaf tip become angular with age and increased in size. These lesions which initially appeared at leaf tips indicated that the pathogen probably entered through the hydathodes and moved through the veins of the leaf and then whole leaf become blighted. The affected area later turn brown and dry. The pathogen produced V-shaped chlorotic lesions on the leaf margins (Plate-3), chlorosis and necrosis of the vascular system were observed. Severe infection resulted in defoliation of diseased leaves. The symptoms of black rot of cauliflower are restricted to the stem leaves and curds and no other parts of the plant get infection. The disease first appear on cotylendery leaves and then in advance stage to the younger leaves.

Isolation, Purification and Pathogenicity:

Black rot pathogen, *X. c.* pv. *campestris* was isolated from diseased leaves. Isolation of black rot pathogen was made by streaking plate method on nutrient agar medium. Infected leaf bits were surface sterilized in 2 per cent sodium hypochlorite solution, placed on sterilized slides in sterile water and then given a sharp cut with sterilized blade. The emerging bacterial ooze was taken in a loop of inoculation needle, streaked on the nutrient medium and incubated at 28 ± 1^{0}C. After 72 h of incubation, small yellow circular entire smooth and shining colonies appeared on the medium (Plate-1). Single colonies were picked up under a stereoscopic microscope and restricted on the same medium for further purification. The procedure was repeated to obtain single, identical individual colonies. Such purified cultures were used for pathogenicity test.

Pathogenicity test was conducted by inoculating one month old cauliflower plants after transplanting in pots from nursery with 72 h old culture of the bacterium using carborundum abrasion technique (Plate-5). The inoculated plants were covered with polythene bags for maintaining high humid condition. Minute water soaked lesion developed after 4-5 days of inoculation (Plate-4). The lesions increased in size and turned brown yellow to brown in colour. The pathogen produced chlorotic lesion on leaf. The pathogen was reisolated from inoculated plants. The culture was found identical with the original one.

Identification and Characterization of Bacterium:

Morphological characteristics:

The morphology of bacterial cell was studied with different staining techniques i.e. Gram staining, Flegella staining and Capsule staining. The method used is described in detail under materials and methods.

The study clearly indicated that *X. c.* pv. *campestris,* black rot pathogen of cauliflower is Gram, negative, short rod with single polar flagellum and capsulated, mostly single, rarely in chains and had no involuted forms (Table-2 & Plate-2).

Table 2 Morphological characters of black rot causing bacterium *X. c.* pv. *campestris*

S.No.	Staining	Result
1.	Grams staining	Gram "–" ve
2.	Flegella staining	Single polar flagellum
3.	Capsule staining	"+"ve

Cultural Characteristics:

After 48 h incubation at 28±1^{0}C bacterial growth appeared, on all the three media, as minute and transparent colonies. The colonies became light yellow on nutrient agar and yeast extract glucose chalk agar and light creamish yellow on 'potato dextrose agar' media after 72 h of incubation. The size and colour of bacterial colonies on different solid media after 5 days of incubation at 28±1^{0}C are presented in Table-3.

Table 3 Cultural characteristics of *X. c.* pv. *campestris* on different solid media after 5 days of incubation at 28±1^{0}C

Colony characters	NA*	PDA**	YGCA***
Colour	Pale lemon yellow	Empire yellow	Straw yellow
Form	Circular	Circular	Circular
Margin	Entire smooth	Entire smooth	Entire smooth
Elevation	Convex	Flate	Convex
Size	10 mm	8 mm	10-12 mm
Pigmentation	Light yellow	Light creamish yellow	Yellow

* Nutrient Agar ** Potato Dextrose Agar *** Yeast Extract Glucose Chalk Agar

The bacterium formed biggest colonies, measuring 10-12 mm in diameter on yeast extract glucose chalk agar medium. The colonies on all the media were circular with entire smooth margin. They were raised and glistening on nutrient agar and yeast extract glucose chalk agar media (Table-3).

Biochemical Characteristics:

Biochemical characteristics of the black rot of cauliflower pathogen were also studied as mentioned in materials and methods and observations on various tests are given in Table-4.

The bacterium *X.c.* pv. *campestris* was strictly aerobic and tolerated sodium chloride upto 3 per cent. Bacterium utilized glucose oxydatively, liquefied gelatin and hydrolysed starch. Hydrogen sulphide and ammonia were produced by one per cent proteose peptone and peptone water, respectively (Table-4).

Methyl red and voge-proskaur test were negative. Bacterium did not reduce nitrate into nitrite. It produced catelase but not urease. Asparagine was inadequate as sole source of carbon and nitrogen.

Table 4 Biochemical Characteristics of black rot causing bacterium *X. c.* pv. *campestris*

S.No.	Biochemical Characteristics	Result
1.	Starch hydrolysis	+
2.	Gelatin liquefaction	+
3.	Ammonia production	+
4.	Urease production	-
5.	Utilization of asparagines as a source of carbon & nitrogen	-
6.	Sodium chloride tolerance	Upto 3%
7.	Oxygen requirement	Strictly aerobic
8.	Hydrogen sulphide production	+
9.	Catalase test	+
10.	Reduction of nitrates	-
11.	Methyl red and voge-proskaur test	-
12.	Mode of glucose utilization	Utilized oxydatively

Method of Inoculation:

Cauliflower plants were inoculated by five different method *viz.*, (1) Atomizing the inoculum with hand atomizer on uninjured surface (2) Carborundum abrasion, (3) Pin prick method, (4) Syringe method and (5) Hydathode inoculation method. The details of the techniques are given in materials and methods. Inoculations were made on two months old potted plants during rabi seasion. The inoculated plants were then observed daily for the appearance and development of the disease upto 20 days. The data so obtained are presented in Table-5.

Table 5 Development of black rot in cauliflower plants by different inoculation methods

Inoculation methods	Number of inoculated plants	Number of plants infected	Infection (%)	Incubation period (days)
Spray inoculation on uninjured surface	80	21	26.25	8-10
Carborundum abrasion	80	45	56.25	4-5
Pin prick	80	26	32.50	4-5
Syringe	80	17	21.25	6-7
Hydathode	80	42	52.50	5-6

It is clear from Table-5 that cauliflower black rot pathogen could infect the plants irrespective of methods used. The earliest expression of disease was noted 4-5 days after inoculation in carborundum abrasion, multineedle Pin-prick method and hydathode inoculation techniques (5-6 days). Symptom expression took longer time i.e. 8-10 days, when spray inoculation were made on uninjured surface and 6-7 days in case of syringe method.

It was interesting to note that maximum disease (56.25 per cent) developed in carborundum abrasion method followed by hydathode inoculation method (52.50 per cent), Pin pricking (32.50 per cent) and spray inoculation on uninjured surface (26.25 per cent). The disease development was slow (21.25 per cent) in the syringe method. The carborundum abrasion method was found most convenient and suitable method of inoculation for producing disease on potted plants in Cage house.

Effect of Environmental Factors on Disease Development:

The disease development in relation to environmental factors was studied in cage house (RCA) by inoculating (conc. 10^8 cfu/ml) one month old cauliflower plants at 7 days interval. The observations were recorded and data were correlated with meteorological data obtained from weather observatory located in the RCA farm Udaipur.

The results presented in Table-6 and Fig-1 revealed that the disease could be produced artificially during the crop season although, its infection index was varied significantly and affected by the environmental conditions.

Table 6 Black rot development on cauliflower plants on weekly inoculation made during Oct. 2003 to Feb. 2004 (In pots)

Date of inoculation	Per cent infection index
20 Oct. 2003	Trace*
27 Oct. 2003	Trace*
03 Nov. 2003	Trace*
10 Nov. 2003	1.8
17 Nov. 2003	3.6
24 Nov. 2003	4.2
01 Dec. 2003	6.8
08 Dec. 2003	14.3
15 Dec. 2003	23.1
22 Dec. 2003	30.4
29 Dec. 2003	34.6
05 Jan. 2004	39.3
12 Jan. 2004	46.7
19 Jan. 2004	53.1
26 Jan. 2004	50.5
02 Feb. 2004	46.4
09 Feb. 2004	42.8
16 Feb. 2004	37.1
23 Feb. 2004	32.1

* Trace = Less than one per cent.

Statistical analysis was done to clearly understand the effect of various environmental factors on the progress and decline of the disease and regression and correlation coefficient obtained are presented in Table-7.

Table 7 Effect of different environmental factors on black rot development in cauliflower plant on weekly inoculation made during Oct. 2003 to Feb. 2004 (In pots)

Environmental factors	Regression equation (Y = a + bx)	Correlation coefficient (r)
Maximum temperature	Y = 161.07** - 4.78 x**	-0.807**
Minimum temperature	Y = 76.25** - 5.20 x**	-0.885**
Maximum humidity	Y = 107.94** + 1.69x	0.585*
Minimum humidity	Y = 42.82** - 0.060	-0.180

Y = Estimated disease infection, a = Intercept, b = Regression coefficient,

* = Significant at 5%, ** = Significant at 1%

In the crop season (October 2003 to February 2004) the disease was in trace to low on plants inoculated in months of October and November 2003. During this period a poor correlation existed between temperature and humidity conditions. The temperature was high (maximum temperature 30–33^{0}C). The humidity was comparatively low, minimum humidity ranged between 31–34 and maximum between 84–86 per cent. The precipitation was negligible. Disease development was better from second week of December onwards and maximum disease was recorded on plants inoculated on 19th Jan. 2004, followed by 26th Jan., 12th Jan and 2nd Feb; high humidity and temperature were favourable for disease development. The most favourable period of disease development was from last week of Dec. 2003 to second week of Feb. 2004, which decline thereafter. During favourable period the maximum humidity ranged between 82–89 per cent and maximum temperature between 23–30^{0}C.

Biochemical changes:

Three component *viz.*, chlorophyll (a, b and total), protein and total phenols were analysed for quantitative estimation in diseased and healthy plant leaves.

Effect of *X. c.* pv. *campestris* on Chlorophyll, Protein and Phenols:

The quantitative estimation of chlorophyll, protein and phenol was done as per detailes given in materials and methods. Results presented in Table-8 and Fig.-2 revealed that chlorophyll "a" decreased 69.02 per cent and chlorophyll "b" decreased 9.53 per cent whereas, total chlorophyll decreased 55.48 per cent over healthy leaves. Date presented in Table-8 and Fig.-2 also revealed that protein decreased 44.56 per cent in diseased plants. Similarly total phenols decreased 21.4 per cent in diseased plants as compared to healthy leaves.

Table 8 Effect of *X. c.* pv. *campestris* on protein, chlorophyll and phenol contents of cauliflower (mg/gm leaf tissue)

S.No.	Constituent	Amount (mg/gm)		Per cent decrease over healthy
		Healthy	Disease	
1.	Chlorophyll "a"	1.317	0.408	69.02
2.	Chlorophyll "b"	0.388	0.351	9.53
3.	Total Chlorophyll	1.705	0.759	55.48
4.	Protein	71.11	39.42	44.56
5.	Total phenol	0.673	0.529	21.4

Therefore, we concluded that *X. c.* pv. *Campestris* affects the above mentioned three components chlorophyll (chlorophyll a, chlorophyll b), protein and total phenols.

Host Resistant:

In order to search the stable source of host resistance to black rot of cauliflower (*X. c.* pv. *campestris*), six (6) cauliflower entries were evaluated. The entries belonging different maturity groups were tested against *X. c.* pv. *campestris* through artificial inoculation, during the Rabi 2003-04.

Table 9 Reaction of cauliflower germplasms against *X. c.* pv. *campestris* under artificial inoculation conditions

S.No.	Variety	Average disease rating (0-4 scale)	Reaction
1.	Vigro kartiki	2.4	S
2.	Snowball	2.8	S
3.	Pusa deepali	1.2	MT
4.	Kartiki specia	1.7	MT
5.	Vigor ageni	3.6	HS
6.	Pusi*	2.7	S

*J.K.Seeds Lakhnow

S = Susceptible, MT = Moderate Tolerant, HS = Highly susceptible

Out of 6 cauliflower entries, two entries *viz.,* pusa deepali and kartiki specia exhibited moderate resistant reaction (1.1-2.0) against black rot under artificial inoculation. Three entries *viz.,* vigro kartiki, snowball and pusi showed susceptible reaction (2.1-3.0). One entry *viz.,* vigro ageni was categorised as highly susceptible (3.1-4.0). In all of 6 entries no one variety found highly tolerant and tolerant in Udaipur (Raj.) condition (Table-9).

Management of Disease:

Evaluation of Chemicals and Herbal Extracts in Laboratory:

Experiments were conducted as to observe the efficacy of chemicals and herbal extracts against black rot pathogen *in vitro*:

1. Two antibacterial chemicals *viz.,* streptocycline and plantomycine were tested for their efficacy against black rot bacterium of cauliflower in laboratory by diffusion paper disc method at 50, 100, 200, 250, 300, 400 and 500 ppm concentration. The zone of inhibition was measured 72 h after inoculation and the average data are presented in Table-10. The perusal of data presented in Table-10, Fig.-3 and Plate-6 revealed that maximum inhibition (23.40 mm) of *X. c.* pv. *campestris* growth was induced by streptocycline (500 ppm) followed by streptocycline 400 ppm and 300 ppm which have inhibition zone 19.20 mm and 15.80 mm respectively. The similar trend was also recorded in case of plantomycine. The plantomycine was less effective at all concentration used as compared to streptocycline.

Table 10 **Efficacy of different concentrations of antibiotics against *X. c.* pv. *campestris* after 72 h incubation at 28±1^{0}C**

Chemicals	Inhibition zone (mm) at different concentration (ppm)							Mean
	50	100	200	250	300	400	500	
Streptocycline	4.8	6.7	11.3	13.6	15.8	19.2	23.4	13.54
Plantomycine	3.0	5.2	10.6	11.5	12.6	14.7	16.11	10.53
	Concentration					Chemicals		
SEM±	0.0772					0.0413		
C.D. 5 %	0.2235					0.1195		

2. Four herbal extracts and streptocycline (control) were used in different concentrations i.e. 5, 10, 20, 25 and 50 per cent / ppm for their efficacy against black rot of cauliflower bacterium in laboratory by paper disc method. The zone of inhibition was measured 72 h after inoculation (28±1^{0}C). The average data are presented in Table-11 and Fig.-4.

Table 11 **Efficacy of different concentrations of herbal extracts against *X. c.* pv. *campestris in vitro* after 72 h incubation at 28±1^{0}C**

Extract / chemical	Inhibition zone (mm) at different concentration (ppm / percentage)					Mean
	5	10	20	25	30	
Neem	1.20	2.50	3.80	4.00	4.80	3.26
Garlic	0.50	1.60	2.10	2.50	4.60	2.26
Tulsi	0.40	1.30	1.90	2.30	4.30	2.04
Ginger	0.40	1.00	1.80	2.00	3.20	1.68
Streptocycline	1.80	2.90	4.10	4.30	5.10	3.63
	Concentration			Plant extract / chemical		
SEM±	0.0209			0.0209		
C.D. 5 %	0.0593			0.0593		

Out of four herbal extracts and one chemical, streptocycline, Neem leaves, Garlic bulbs and Tulsi leaves effectively inhibited the growth of *X. c.* pv. *campestris* as presented in Table 11, Fig.-4 and Plate-7. Best inhibition was recorded in case of Streptocycline (5.1 mm) followed by Neem leaves extract (4.8 mm) and Garlic bulbs extract (4.6 mm). Moderate inhibition was recorded in case of Tulsi leaves and Ginger rhizomes extracts.

Evaluation of Antibiotics and Plant Extracts Against *X. c.* pv. *campestris* on Cauliflower Seeds:

1. Two antibiotics *viz.,* streptocycline and plantomycine were used as seed dressers *in vitro* as detailed given in materials and methods. Seeds were inoculated with *X. c.* pv. *campestris* by seed soaking method and then treated with different concentrations of antibiotics and plated on blotter papers and then kept at room temperature. The results revealed that both the antibiotics enhanced the seeds germination of "Pusi" variety inoculated with inoculum of *X. c.* pv. *campestris* upto 250 ppm of both the antibiotics and then at higher concentration (i.e. 300, 400 and 500 ppm) germination of seeds were gradually decreased as presented in Table-12, Fig.-5 and Plate-8.

Table 12 Effect of different antibiotics concentrations on germination of inoculated cauliflower seeds in Petri plates

Chemicals	**Per cent seed germination at different concentrations (ppm)**							**Mean**
	50	**100**	**200**	**250**	**300**	**400**	**500**	
Streptocycline	56.33	60.67	63.33	66.67	56.67	50.00	43.33	56.67
Plantomycine	50.00	56.67	60.00	63.33	60.00	53.33	40.00	54.76
Control								23.33
	Concentrations					Chemicals		
SEM±	1.7961					0.9600		
C.D. 5 %	5.1803					2.7690		

Maximum germination was recorded with Streptocycline 250 ppm (66.67 per cent) followed by Streptocycline 200 ppm (63.33 per cent) as compared to control (23.33 per cent). However, as concentrations of both chemical increased proposnatly germination of seeds gradually declined. So it can be concluded from data that streptocycline was found most effective against *X. c.* pv. *campestris* as compared to plantomycine.

2. The extracts of leaves of two plant species, extract of bulb, extract of rhizome and one chemical were prepared, as mentioned in materials and methods. These extracts were used to test their efficacy against seed germination of inoculated cauliflower seeds. Seeds were inoculated with fresh culture of *X. c.* pv. *campestris* and then treated with different concentration of herbal extracts and chemical and then seeded on blotter papers. Results (Table-13 and Fig.-6) represent that out of four plant extracts and chemical tested. Maximum germination was recorded with streptocycline (60.00 per cent) followed by Neem (56.67 per cent) and Garlic (53.33 per cent). Whereas, Tulsi and Ginger extracts has shown poor effectivity on seed germination.

Table 13 Effect of different concentrations of herbal extracts on germination of inoculated cauliflower seeds

Herbal extracts	**Per cent seed germination at different concentrations (ppm / percentage)**					**Mean**
	5	**10**	**20**	**25**	**50**	
Neem	33.33	40.00	46.67	53.33	56.67	46.00
Garlic	30.00	36.67	40.00	43.67	53.33	41.33
Tulsi	30.00	33.33	40.00	43.33	50.00	39.33
Ginger	26.67	33.33	36.67	40.00	46.67	36.67
Streptocycline	40.00	46.67	53.33	56.67	60.00	51.33
Control						20.00
	Concentrations			Herbal extract		
SEM±	1.1694			1.1694		
C.D. 5 %	3.3185			3.3185		

DISCUSSION

Cauliflower (*Brassica oleracea* var. *botrytis* L.) is an important vegetable crop of Rajasthan. The crop, however suffers with black rot (*Xanthomonas campestris* pv. *campestris*) disease in different growing areas of Rajasthan, causing severe loss in nursery as well as in the field.

The symptoms of black rot of cauliflower have been described by many workers i.e. Mazzuccni (1968) and Onsando *et al.* (1992). Leite *et al.* (1994) were also found similar in rapa (*Brassica napus*). In present case, the symptoms initially appeared as minute water soaked lesions on the leaf and later these lesions turned into V-shaped chlorotic lesions on the leaf margins, which caused chlorosis and necrosis of the vascular system. These observations suggest that perhaps a higher initial inoculum was responsible for wilting of the seedlings by black rot pathogen.

On the basis of cultural, morphological and biochemical characteristics, the bacterium was identified as *X. c. pv. campestris*. The morphology of black rot bacterium was studied with different methods of staining i.e. Gram staining, flagella staining and capsule staining. The bacterium was found to be rod-shaped with a single polar flagellum and capsulated. The other cultural and Physio-Biochemical characterstics of the bacterium were also similar to that described by Patel *et al.* (1949a) for cabbage black rot bacterium *X. c.* pv. *campestris.*

Production of disease by inoculation is essential to understand various aspects of its development. Most bacterial plant pathogens have been reported to develop heavy infection when inoculations were made on injured surface i.e. carborundum abrasion (Chaudhary and Chakravrti, 1989), injection infiltration (Klement, 1963), Multineedle pricking (Stars and Dye, 1966) and hydathode inoculation (Robeson *et al.*, 1989). In present case best disease was produced by carborundum abrasion method followed by hydathode inoculation method. The disease was also reproduced by spraying the inoculum on uninjured surface but it had taken longer time for expression of symptos. Spray inoculation on multineedle pricking and injection infiltration techniques were however, tedious and were not suitable for mass inoculation.

Besides methods of inoculation, initial bacterial population used for inoculation also influenced the symptoms expression and disease development. Several workers *viz.*, (Shekhawat and Chakravarti, 1977; Gupta and Chakravarti, 1983; Bandyopadhyay and Chattopadhyay, 1985 and Shah *et al.*, 1995) reported that a minimum inoculation level was necessary for pathogenesis in different host pathogen combinations.

In present study a minimum level of 10^4 cfu / ml was found necessary for infection and development of black rot of cauliflower similarly, Shukla *et al.* (1995) also proved that surface injury is essential for canker development in citrus plant.

It was found that bacterial population used for inoculation had influenced the symptoms expression and disease development. It was observed that at higher concentration of 10^8 cfu / ml the disease development was significantly high with a shorter incubation period. Similar observations were made for *X. c.* pv. *vignicola* (Shah, 1988).

Environmental factors essentially affect the intensity and spread of plant disease and thus, the knowledge concerning epidemiology can help in better management of the disease. During the present study, cauliflower plants inoculated at one week interval, throughout experimentation during the period from Oct. 2003 to Feb. 2004, developed severe disease only in the month of January when humidity between 82 – 89 per cent and temperature ranges between 23 – 30^0C prevailed. Disease developed only in traces during October and November and was low during cool December. Apart from this, the studies have also shown that the disease progressed faster during the crop growth period in January and February under cage house conditions.

Oliveira *et al.* (1996) found that major development of black rot (*X. c.* pv. *campestris*) on cole crop was registered during the warm and humid period. Present observations also indicated that the maximum disease development in warm and humid environmental conditions.

Regression and correlation coefficients were calculated for important environmental factors in relation to the progress of the disease. These observations led to an important conclusion that the disease progress curve had three distrinct phase (periods) *viz.*, initial inoculum build up or disease establishment, rapid disease progress and disease stabilization. The conditions prevailing during these specified periods were much different and therefore, correlation coefficients for environmental factor were calculated separately for disease establishment and rapid disease progress periods. This method had given a very clear positive picture and negative correlation was obtained for maximum and minimum temperature, which signified their role of the rapid progress of the disease. In the available range of relative humidity (RH), any change in humidity did not affect the disease progress. However, maximum infection was noticed, when RH ranged between 82 – 89 per cent. It was observed that disease development gained momentum when the maximum temperature started lowering down from 33^0C minimum temperature below 23^0C adversely affected the disease.

It is clear from these observations that the best conditions for rapid progress of the disease were temperature between 23 – 30^{0}C and humidity above 84 – 86 per cent. However, temperature upto 30 – 33^{0}C on higher side was found limits of disease development. Singh (2001) also concluded that temperature between 24 – 27^{0}C and humidity between 69 – 88 per cent was favourable for maximum disease development, which is almost similar to present investigations.

Padmanabhan *et al.* (1973), Gupta *et al.* (1983) and Washnikar *et al.* (1998) reported reduction of chlorophyll content, protein and total phenols in diseased leaves as compared to healthy one. Similarly, Sain (2002) also reported that chlorophyll content; protein and total phenol reduce in diseased plant leaves. In present investigation almost similar results were recorded which show reduction in chlorophyll content, protein and total phenols.

Therefore, we can conclude that *X. c.* pv. *campestris* reduced chlorophyll "a", "b" and total in order to 69.02, 9.53, 55.48 per cent respectively, protein 44.56 per cent and total phenols 21.4 per cent as compared to healthy one.

Henz *et al.* (1994), Mankhos *et al.* (1995), Kocks *et al.* (1996), Ignatov *et al.* (1997) and Galli *et al.* (2002) evaluated cabbage and cauliflower in relation to *X. c.* pv. *campestris* infection and degree of resistance on the basis of genotype. Similarly, Taylor *et al.* (2002) reported *Brassics spp.* for resistance against *X. c.* pv. *campestris.* In present investigation six cauliflower cultivars namely: Pusi, Pusa deepali, Snowball, Kartiki specia, Vigro ageni and Vigro kartiki were screened for resistance against black rot and not found any resistant variety against *X. c.* pv. *campestris.* Only Pusa deepali and Kartiki specia exhibited moderate resistant against black rot hence no variety was found resistant.

One of the best methods of plant disease control is by seed treatment and foliar spraying of chemicals. In present investigation it was found that antibiotics, streptocycline (250 ppm) as seed soaking and foliar sprays was found best and gave 66.67 per cent seed germination of infected seeds followed by streptocycline 200 ppm. The antibiotic streptocycline (500 ppm) was also found best in inhibiting the growth of *X. c.* pv. *campestris* in *in-vitro* conditions. Kishun (1983) found plantomycine was found best for seed soaking as well as foliar spray, while Sharma (1981) reported sprays of streptocycline is most effective for control of black rot of cauliflower. Plantomycine was not found as effective as streptocycline in present studies.

A large number of herbal extract have been reported to be effective against bacterial pathogen (Miles *et al.*, 1990; Dimitriev *et al.*, 1991 and Garcia *et al.*, 1994). The use of herbal extracts is beneficial since it involves no health hazards and eco-friendly and cause no environmental pollution problems. In present study the Neem and Garlic extract effectively inhibited the growth of *X. c.* pv. *campestris* in *in-vitro* condition. The extract of neem leaves was found to be highly effective under *in-vitro* condition.

Dimitriev *et al.* (1991) tested tesibuline as antibiotic substance from onion and found that Gram-positive bacteria were more sensitive than Gram-negative. Gracia *et al.* (1994) reported that black rot of crucifers caused by *X. c.* pv. *campestris* can be controlled by using various herbal extracts.

SUMMARY

Cauliflower (*Brassica oleracea* var. *botrytis* L.) is an important vegetable crop of Rajathan. In Southern Rajasthan black rot of cauliflowr, caused by *Xanthomonas campestris* pv. *campestris* is a common and has been observed in moderate to severe form around Udaipur and other cauliflower growing area of the state.

The disease appeared as small water soaked lesions on the leaf periphery, which later on increased in size. The yellowish lesions, which initially appeared at leaf tips indicated that the pathogen probably entered through the hydathodes. The pathogen produced V-shaped chlorotic lesions on the leaf margin caused chlorosis, and necrosis of the vascular system. Based on host specificity, morphological and biochemical characteristics, the causal bacterium was identified as *Xanthomonas campestris* pv. *campestris*. It produces straw yellow, circular, smooth, raised glistering colonies on yeast extract glucose chalk agar, potato dextrose agar and nutrient agar media.

The bacterium was Gram-negative and strictly aerobic. Under oil immersion objective of the compound microscope it appeared as straight and capsulated rod. It had a single polar flagellum. The bacterium utilized glucose oxidatively; produced hydrogen sulphide and ammonia; liquefied gelatin and hydrolysed starch; tolerated sodium chloride upto 3 per cent; produced catalase but not urease; not reduced nitrate into nitrite; methyl red and Voges-Proskauer tests were negative. Asparagine was inadequate source of carbon and nitrogen.

Carborundum abrasion method was found as the best inoculation technique followed by hydathode inoculation method. The disease also developed by spraying inoculum on uninjured surface but it took longer time for expression of symptoms.

The disease could by produced artificially throughout the crop season but disease index varied significantly with change in weather. It was in trace to low during the month of October, November and maximum in January and February. The date of crop season have shown that temperature between 30 – 33^0C and relative humidity above 82 – 89 per cent favoured good development of black rot.

Bacterial black rot causing bacterium *X. c.* pv. *campestris* was reduced the quantity of chlorophyll (a, b and total chlorophyll), protein and total phenols in disease affected leaves. In case of cauliflower black rot, the *X. c.* pv. *campestris* reduce chlorophyll a, b and total chlorophyll in order to 69.02, 9.53 and 55.48 per cent respectively, as well as protein and total phenols were also reduced 44.56 and 21.4 per cent respectively as compared to healthy leaves.

Out of six cauliflower varieties namely Pusa deepali, Vigro kartiki, Snow ball, Kartiki specia, Vigro ageni and Pusi, no any variety was found resistant against black rot of cauliflower caused by *X. c.* pv. *campestris.* Only cultivar Pusa deepali and Kartiki exhibited moderate resistant against black rot, other wise not any variety was found resistant under Udaipur condition.

In laboratory screening of chemicals maximum inhibition of *X. c.* pv. *campestris* was observed with streptocycline (500 ppm) followed by other concentrations of streptocycline and plantomycine. The streptocycline (250 ppm) also proved effective against *X. c.* pv. *campestris*, when used seed protectant and gave significant increase in germination percentage as compared to plantomycine.

Amongest the various plant extracts, maximum inhibition of *X. c.* pv. *campestris* was induced by neem extract, followed by Tusli, Garlic and Ginger at higher concentration under *in vitro* studies.

The neem leaves extract was also found most effective against the pathogen, when used as seed protectent and gave significant increase in germination percentage as compared to other plant extracts.

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Investigations on black rot of cauliflower (*Brassica oleracea* var. *botrytis* L.) caused by *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson"

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ABSTRACT

Bacterial black rot is a major disease of cauliflower (*Brassica oleracea* var. *botrytis* L.). The disease has been observed in all cauliflower growing areas of the state including Udaipur.

The disease initially appeared as small water soaked lesions on the leaf periphery, which later on increased in size. The pathogen produced V-shaped chlorotic lesions on the leaf margins upon invasion through hydathodes.

Morphological, cultural and biochemical characteristics were studied. The bacterium appeared as straight, capsulated rods with single polar flagellum under the oil immersion objective of compound microscope. It was identified as *Xanthomonas campestris* pv. *campestris.*

Best infection was obtained by carborundum abrasion method. The disease was favoured by temperature between 30-33^{0}c and relative humidity above 82-89 per cent. High and low temperatures and dry weather were found adverse for its development.

The bacterium, *X. c.* pv. *campestris* causing black rot reduced the quantity of chlorophyll, protein and phenol in disease affected plant leaves.

Under climatic conditions of Udaipur none of the varieties were found tolerant against bacterial black rot of cauliflower. However, Pusa deepali and Kartiki exhibited moderatly tolerant.

Streptocycline (500 ppm) gave maximum inhibition of the bacterial growth *in vitro*. Out of four plant extracts, Neem and Garlic extract inhibited the growth of *X. c.* pv. *campestris,* effectively.

Streptocycline (250 ppm) was found most effective in seed germination (66.67 per cent) against *X. c.* pv. *campestris* and in case of plant extract Neem leaf and Garlic bulb extract was found most effective.

* Assistant Professor Department of Plant Pathology, R.C.A., Udaipur.
**P.G. Research Scholar, Department of Plant Pathology, R.C.A., Udaipur.

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bl vul/kku e jkx d y{k.k] 'kkdk.k d vkdkfjdh;] to jklk;fud ,o lof/kr x.kk dk v/;;u fd;k x;kA l;Dr l{en'kh d ry fueTtu vfHkn'; e thok.k n.Mkdkj] lifVd ,o ,d dk'kkHkh; fn[kk;h fn;kA bl 'kkdk.k dk tUFkkekuk/ dEifLVl ihoh- dEifLVl d #i e igpkuk x;kA

jkx ljki.k gr dkckj.Me fof/k lokf/kd mi;Dr ,o mùke ik;h xbA bl jkx d fodkl d fy,] rkide 30&33^0 l-] ,o vkif{kd vknrk 82&89 ifr'kr l vf/kd gkuk vko';d gA mPp ,o fuEu rkide ,o l[kk ekle bl jkx d fy, ifrdy gA

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t- d- ihoh- dEifLVl d foifjr LVlVkIkbfDyu cht vdj.k ¼66-67 ifr'kr½ e lcl T;knk iHkkoh ik;k x;k ,o ikni vd dh n'kk e uhe dh iùkh ,o yglu d dn dk vd iHkkoh ik;k x;kA

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* lgk;d&vkpk;] ikni 0;kf/k foKku foHkkx] jktLFkku Ñf"k egkfo|ky;] mn;ij
** Lukrdkrj 'kk/kNk=] ikni 0;kf/k foKku foHkkx] jktLFkku Ñf"k egkfo|ky;] mn;ij

Mean weekly weather data of Rabi 2003-2004

Date	Temperature (^{0}C)		Relative humidity (%)	
	Maximum	Minimum	Maximum	Minimum
20 Oct. 2003	33.2	14.9	73	33
27 Oct. 2003	32.8	14.7	73	34
03 Nov. 2003	33.4	15.2	71	32
10 Nov. 2003	32.2	13.9	70	31
17 Nov. 2003	30.4	12.5	76	29
24 Nov. 2003	29.1	12.7	72	32
01 Dec. 2003	28.9	10.4	78	31
08 Dec. 2003	30.8	10.3	84	25
15 Dec. 2003	28.1	10.8	83	33
22 Dec. 2003	26.8	6.4	82	34
29 Dec. 2003	25.0	5.9	84	42
05 Jan. 2004	24.1	7.3	86	35
12 Jan. 2004	26.2	7.0	84	27
19 Jan. 2004	27.8	8.5	85	30
26 Jan. 2004	23.0	6.5	89	36
02 Feb. 2004	23.3	5.8	86	26
09 Feb. 2004	26.7	5.8	65	17
16 Feb. 2004	29.3	9.1	77	27
23 Feb. 2004	31.3	10.9	74	19

APPENDIX – II

Analysis of variance for the efficacy of different concentrations of antibiotics against *X. c.* pv. *campestris in vitro*

Source of variance	d.f.	S.S.	M.S.S.	F.Cal.
Treatment	13	1299.5806	99.9677	2797.4181
Chemicals	1	95.3117	95.3117	2667.1283
Concentration	6	1158.5444	193.0907	5403.2986
Chemicals X Con.	6	45.7244	7.6207	213.2526
Error	28	1.0006	0.0357	

APPENDIX – III

Analysis of variance for the efficacy of extracts of neem and tulsi leaves, garlic bulbs, ginger rhizomes and streptocycline against *X. c.* pv. *campestris* *in vitro* at different concentrations

Source of variance	d.f.	S.S.	M.S.S.	F.Cal.
Treatment	24	151.7352	6.3223	967.6990
Extracts	4	41.6365	10.4091	1593.2347
Concentration	4	104.7605	26.1901	4008.6939
Extract X Con.	16	5.3381	0.3336	51.0663
Error	50	0.3267	0.0065	

APPENDIX – IV

Analysis of variance for the effect of different concentrations of antibiotics on germination of inoculated cauliflower seeds

Source of variance	d.f.	S.S.	M.S.S.	F.Cal.
Treatment	13	5231.1111	402.3932	20.7903
Chemicals	1	38.0952	38.0952	1.9683
Concentration	6	2128.5714	354.7619	18.3294
Chemicals X Con.	6	128.5714	21.4286	1.1071
Control X Rest	1	2935.8730	2935.8730	151.6868
Error	31	600.0000	19.3548	

APPENDIX – V

Analysis of variance for the effect of different concentrations of herbal extract on germination of inoculated cauliflower seeds

Source of variance	d.f.	S.S.	M.S.S.	F.Cal.
Treatment	24	7805.1282	325.2137	15.8542
Plant extract (T)	4	2021.3333	505.3333	24.6350
Concentration (C)	4	4168.0000	1042.0000	50.7975
T X C	16	98.6667	6.1667	0.3006
Control X Rest	1	1517.1282	1517.1282	73.9600
Error	52	1066.6667	20.5128	

Fig 1 Effect of different environmental factors on black rot development in cauliflower plant during Oct. 2003 to Feb. 2004

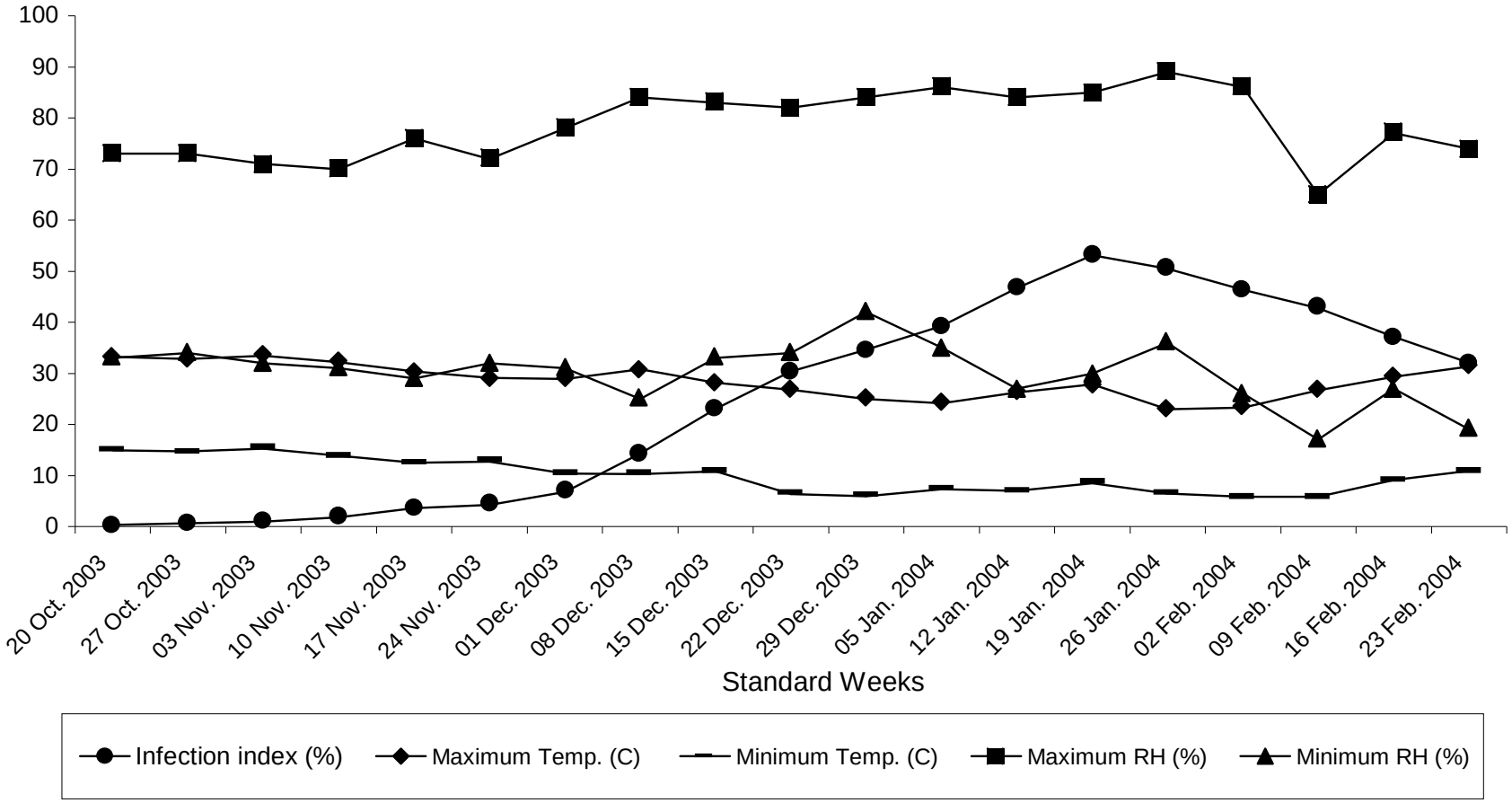

Fig 2 Effect of *X. c.* pv. *campestris* on protein, chlorophyll and phenol contents of cauliflower leaves (mg/gm leaf tissue)

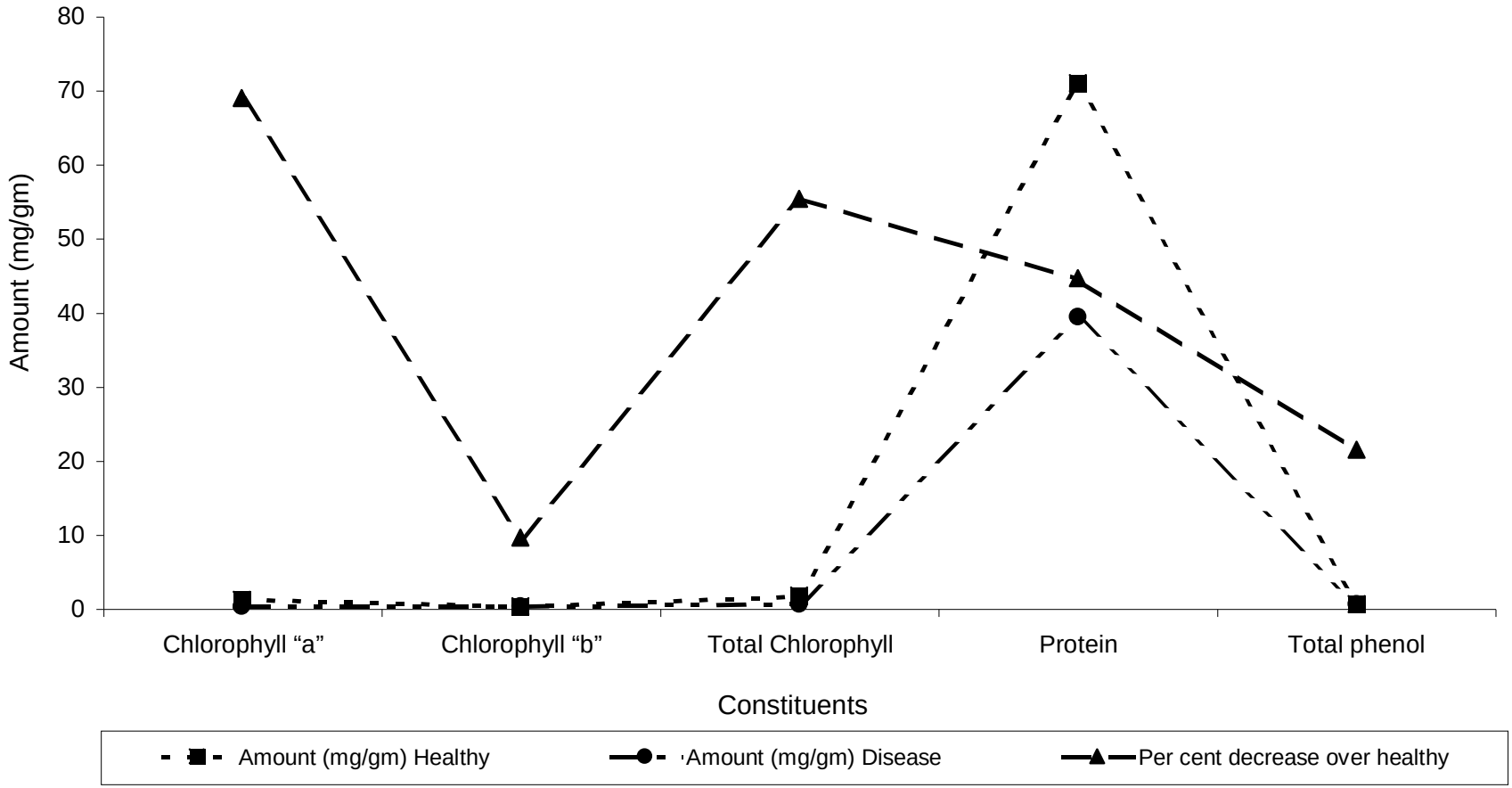

Fig 3 Efficacy of different concentrations of antibiotics against *X. c.* pv. *campestris* after 72 h incubation on bio-assay medium at 28±1^{0}C

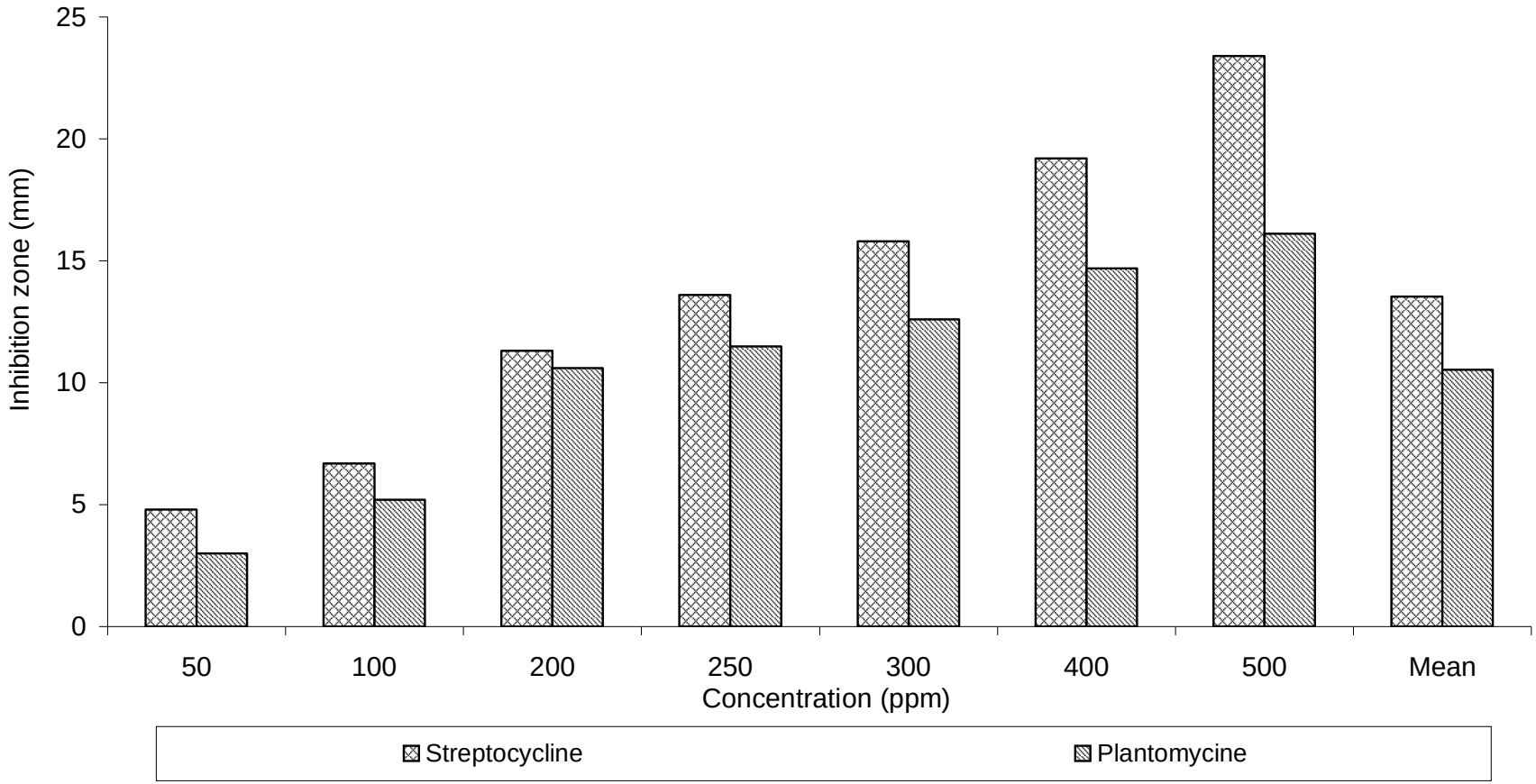

Fig 4 Efficacy of different concentrations of herbal extracts against *X. c.* pv. *campestris in vitro* after 72 h incubation at 28±1^{0}C

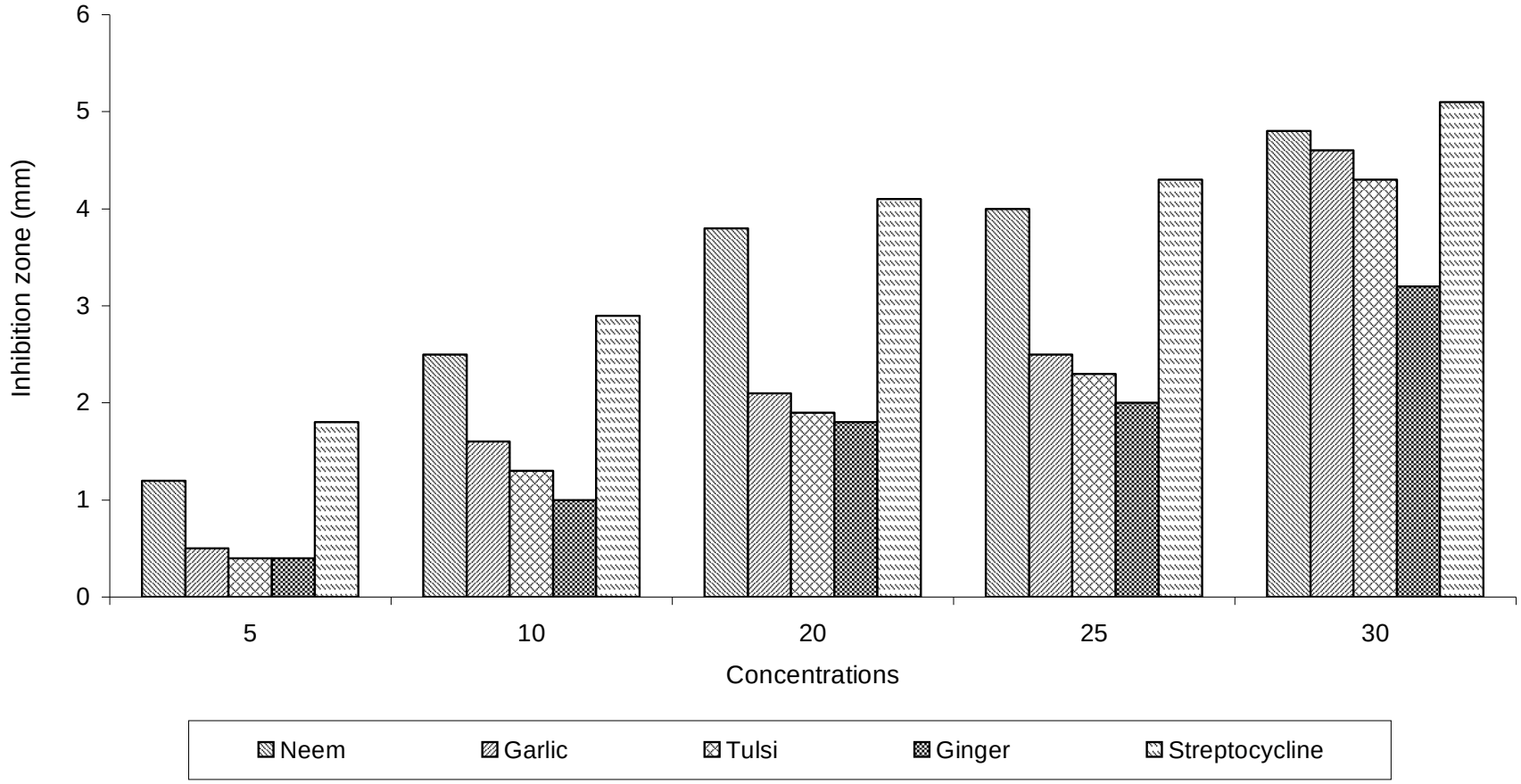

Fig 5 Effect of different concentrations of antibiotics on germination of inoculated cauliflower seeds in Petri plates

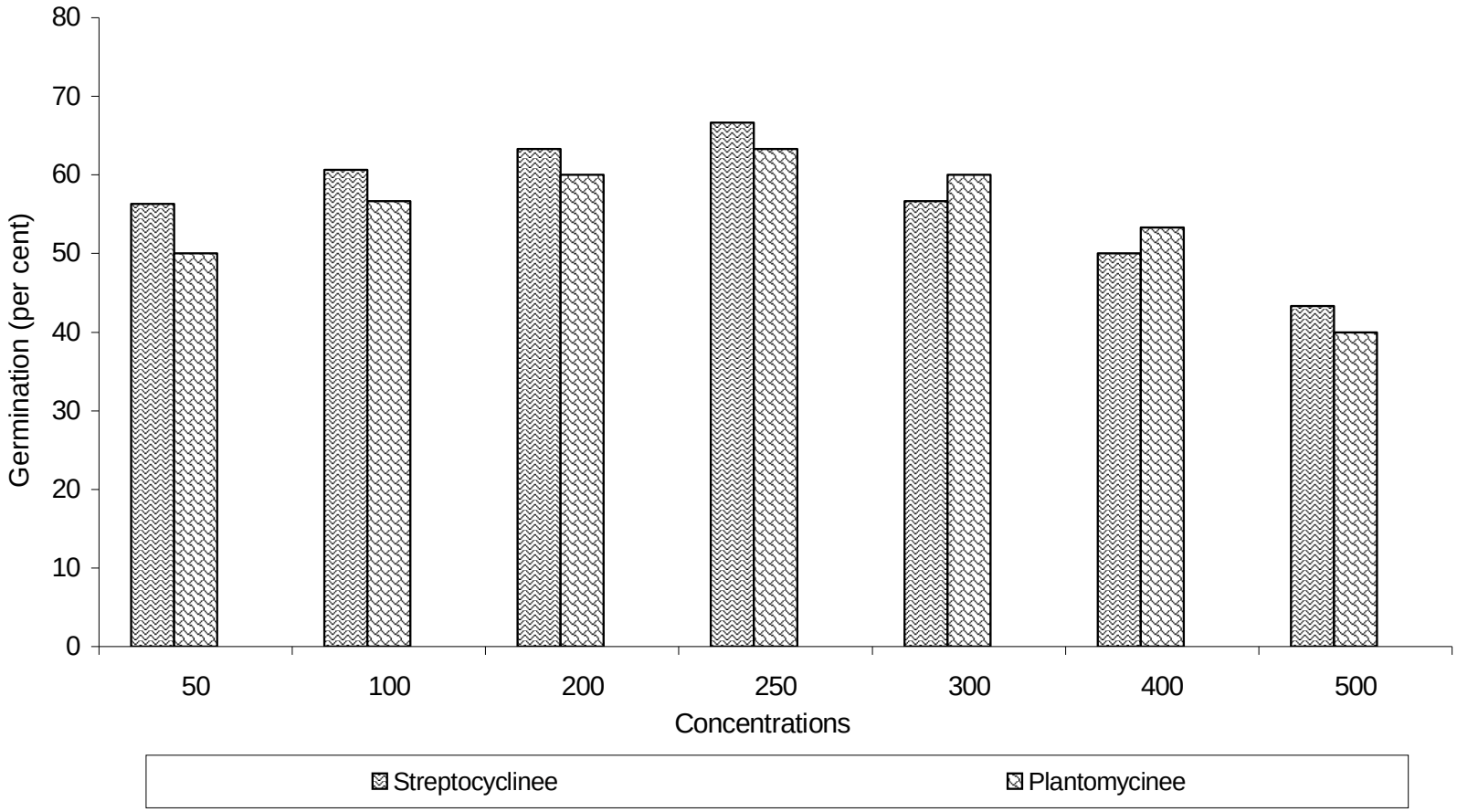

Fig 6 Effect of different concentrations of herbal extract on germination of inoculated cauliflower seeds