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PREVALENCE AND MANAGEMENT OF BLACK ROT OF CABBAGE

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Cabbage (*Brassica oleracea* var. *capitata* L.) is one of the most widely cultivated and popular vegetable crops in India, particularly during the cooler seasons. It is an important source of essential nutrients, including proteins, carbohydrates, minerals, ascorbic acid, and glycoalkaloids. Additionally, cabbage is rich in antioxidants. However, cabbage cultivation is vulnerable to a range of diseases, notably black rot, which inflicts significant economic losses globally, especially in temperate and subtropical regions. The causal agent of black rot, *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson, is a rod-shaped, aerobic, gram-negative, non-spore-forming bacterium belonging to the Proteobacteria class. Seven races of *X. campestris* pv. *campestris* have been documented worldwide (Taylor *et al.*, 2002). Black rot disease is complex, manifesting through local, systemic, head, and dormant infections. The disease is particularly aggressive in low-lying areas where plants remain wet for extended periods and during episodes of high temperature and high relative humidity. Under cool temperatures in late winter and early spring, the disease is less problematic; however, warm conditions can trigger severe outbreaks (Gupta *et al.*, 2017). While nine pathogenic races of *X. campestris* pv. *campestris* have been identified (Tonu *et al.*, 2013), races 1 and 4 are the most prevalent globally. Recent studies have identified races 1, 4, and 6 in India, with race 1, followed by race 4, being dominant in most states (Singh *et al*., 2016).

Black rot leads to substantial yield losses by causing premature defoliation and degrading head quality, rendering them unmarketable. The rapid bacterial spread under favourable conditions, particularly during seedling production, has been linked to significant crop losses (Berg *et al*., 2005). In cauliflower, losses due to black rot have exceeded 50% (Sharma *et al.*, 2017), with potential losses reaching up to 100% under high disease incidence (Roberts *et al.*, 2007). Initial symptoms include chlorotic lesions along leaf margins that progress towards the midrib, forming characteristic "V" shaped lesions (Gupta *et al.*, 2017). The pathogen's infection may be followed by secondary infections from the soft rot bacterium *Erwinia carotovora* or the fungus *Sclerotinia sclerotiorum*. Advanced systemic infections can cause darkened leaf veins and stem vascular tissues, extensive leaf yellowing, wilting, and necrosis (Popovic *et al*., 2013).

Control measures for black rot include the use of a seed treatment combining 0.01% streptocycline with 0.25% mancozeb for 3 hours and foliar sprays of the same combination, which have shown effectiveness (Verma and Shyam, 2003). Additionally, soaking seeds in 3% hydrogen peroxide for 30 minutes has been reported to eliminate 100% of bacteria from cabbage seeds (Sanna *et al.*, 2022). Pre-treatment with CAC-717, a disinfectant created using an electric field and water flow in distilled water containing calcium hydrogen carbonate, significantly reduces bacterial populations (Sakudo *et al.*, 2020). Despite these methods, many antibiotics and pesticides, including streptocycline, oxytetracycline, and chloramphenicol, pose environmental and health risks and can lead to bacterial resistance (Liu *et al.*, 2022). Thus, alternative disease management strategies are needed. This investigation aims to evaluate the efficacy of various chemicals and plant extracts against black rot both *in vitro* and *in vivo*, with the goal of identifying viable alternative management methods for this pervasive cabbage disease.

The prevalence of black rot disease in cabbage was assessed during the 2017 crop season across three major cabbage-growing districts in Himachal Pradesh—Solan, Shimla, and Kullu. Disease monitoring was conducted at various growth stages. In each district, 25 plants were sampled from ten fields to determine disease prevalence. Disease incidence was calculated using the formula: Disease Incidence (%) = (Number of Diseased Plants / Total Number of Plants) × 100. Disease severity was evaluated using a scale adapted from Verma and Shyam (2003), with slight modifications:

Disease grade	Infected leaf area (%)	Disease reaction
0	No visible infection	Immune (I)
1	1-5	Resistance (R)
2	5.1-15	Moderately resistant (MR)
3	15.1-30	Moderately susceptible (MS)
4	30.1-50	Susceptible (S)
5	>50	Highly susceptible (HS)

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The percent disease index was calculated by following formula:

$$\text{Percent disease index (PDI)} = \frac{\text{Total sum of numerical rating}}{\text{Total number of ratings} \times \text{Maximum disease grade}} \times 100$$

The bacterial pathogen was isolated from infected cabbage leaves by first swabbing the diseased tissues with rectified spirit and then washing them repeatedly in autoclaved, sterile distilled water. Small bits of the cleaned tissues were placed in sterile water droplets on a sterilized glass slide under aseptic conditions. Each tissue fragment was then incised with a sterile blade to release bacterial ooze. A loopful of the resulting bacterial suspension was streaked onto sterilized Nutrient Sodium Chloride Agar (NSA) plates under aseptic conditions. The Petri dishes were incubated at 30°C for 48 hours and subsequently examined for colony formation. Colonies exhibiting a circular, yellow, and mucoid morphology were selected. These colonies were further purified using the streak plate method and maintained on NSA slants at 4 ± 2°C for subsequent studies. The pathogen was identified based on its morphological, cultural, and biochemical characteristics, following the guidelines established by Verma and Shyam (2003).

Pathogenicity tests were performed on healthy cabbage plants using a bacterial suspension with a concentration of 3×10^8 cfu/ml. Three inoculation methods were employed: hydathode inoculation, vein inoculation, and clip inoculation, as described by Nazir *et al.* (2010). Following inoculation, the seedlings were watered and covered with polyethylene bags for 48 hours to create a high humidity environment, which was maintained using periodic water sprays inside the bags. Control plants were inoculated with sterile distilled water under identical conditions. Leaves were monitored regularly for symptom development. Observations on the incubation period and disease severity were recorded systematically from the onset of symptoms.

The efficacy of various chemicals and plant extracts against *Xanthomonas campestris* pv. *campestris* was evaluated under both *in vitro* and pot conditions. Six chemicals were tested: streptomycin sulfate, Bacterinashak (2-bromo-2-nitropropane-1,3-diol), mancozeb, copper oxychloride, and cuprous oxide. Additionally, five plant extracts were assessed: *Ocimum sanctum* (tulsi), *Melia azedarach* (darek), *Vitex negundo* (bana), *Aloe vera* (aloe), and *Cymbopogon schoenanthus* (lemongrass). *In vitro* efficacy was determined using the agar well diffusion method, while pot experiments involved foliar spraying. The plant extracts were prepared according to the method described by Adikshita *et al.* (2018) and tested at three concentrations: 10%, 25%, and 50% (Balouiri *et al.*, 2015).

A 1 ml aliquot of the standard bacterial suspension (3×10^8 cfu/ml) was evenly spread across the surface of agar plates (7.5 cm in diameter). Subsequently, a well with a diameter of 6 to 8 mm was punched in the center of each Petri dish. A 0.5 ml volume of each test chemical and plant extract, prepared at various concentrations, was pipetted into the well. Sterilized distilled water was used as a control. Each treatment

Table 1. Prevalence of black rot disease of cabbage in Shimla, Solan and Kullu districts during 2017

District	Location	Disease incidence (%)	Percent Disease Index (PDI)
Shimla	Gajeri	55.15	44.30
	Theog	88.64	51.38
	Kaljair	57.25	22.07
	Bharmali	77.70	21.03
	Mean	69.68	34.69
Solan	Kandaghat	39.01	26.15
	Nauni	14.01	12.98
	Kotla	21.05	17.34
	Jaunaji	65.25	31.54
	Mean	34.83	22.00
Kullu	Bajaura	69.00	42.01
	Baragraon	22.00	21.78
	Katrain	51.55	35.06
	Mean	47.51	32.95
Overall Mean		50.96	29.60

Fig. 1. Symptoms of black rot on cabbage

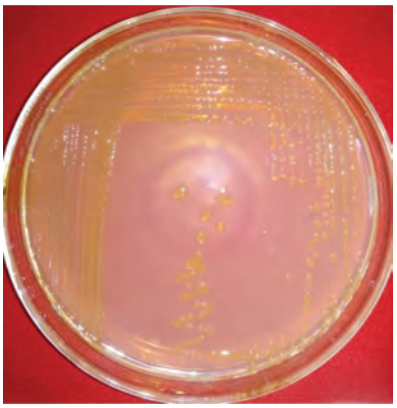

Fig. 2. Pure culture of *Xanthomonas campestris* pv. *campestris* on nutrient agar medium

was replicated three times in a completely randomized design (CRD). The plates were incubated at 30°C, and the diameters of the inhibition zones (measured in cm) were recorded after 48 hours.

The chemicals and plant extracts were further tested as foliar sprays against black rot of cabbage in a completely randomized design under controlled glasshouse conditions. One-month-old cabbage seedlings were transplanted into pots (10 cm diameter) filled with sterilized soil. After seven days, the first foliar application of the chemicals and plant extracts was conducted. Three days later, the seedlings were inoculated with a standard bacterial suspension (3×10^8 cfu/ml) using the vein inoculation method and covered with polyethylene bags for 48 hours to maintain high humidity through periodic water sprays inside the bags. Four days after inoculation, a second foliar spray of the treatments was administered. Disease severity and plant height were monitored and recorded regularly.

The analysis of black rot disease prevalence in cabbage across the Solan, Shimla, and Kullu districts of Himachal Pradesh revealed widespread occurrence (Table 1) and characteristic symptoms, including typical V-shaped lesions and blackened veins and veinlets (Fig. 1). The minimum disease incidence recorded was 14.01% at Nauni, Solan, while the maximum incidence was 88.64% at Theog, Shimla. Overall, Shimla district exhibited the highest disease incidence at 69.68%, followed by Kullu district at 50.96%, and Solan district at 34.83%. In terms of percentage disease index (PDI), the minimum value of 12.98% was observed at Nauni, Solan, whereas the maximum PDI of 51.38% was noted at Theog, Shimla. District-wise, Shimla had the highest average PDI at 34.69, followed by Kullu at 32.94, and Solan at 22.00.

The bacterium isolated from infected plant parts was identified as *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson based on its morphological, cultural, and biochemical characteristics. The bacterial cells were rod-shaped and gram-negative. On nutrient agar, the colonies appeared yellow, smooth, flat, glistening, convex, and round with fringed margins, measuring approximately 1 cm in diameter (Fig. 2). On Nutrient Sodium Chloride Agar (NSA), the colonies were mucoid, glistening, convex, and exhibited circular to entire margins. These distinctive features confirmed the identification of the bacterium as *Xanthomonas campestris* pv. *campestris*.

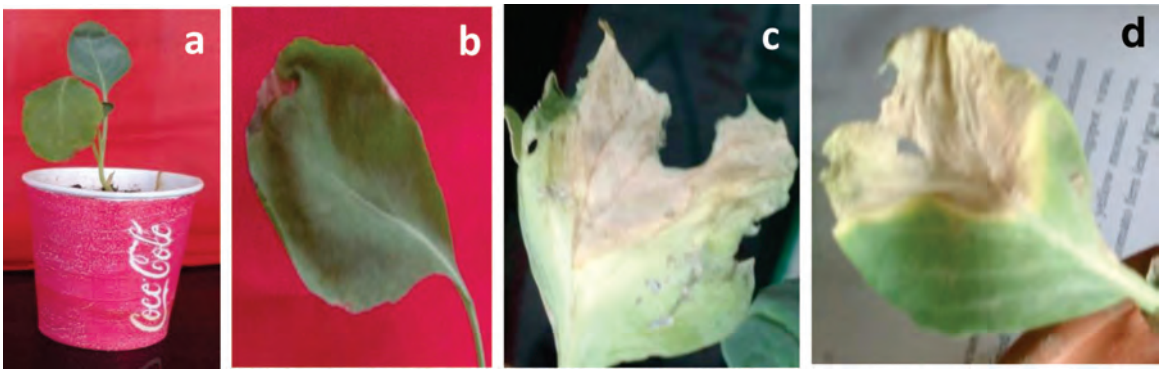

Fig. 3. Pathogenicity of *Xanthomonas campestris* pv. *campestris* a) Healthy green leaves, b) Hydathode inoculation method, c) Vein inoculation method, d) Clip inoculation method

Table 2. Pathogenicity of *Xanthomonas campestris* pv. *campestris*

Inoculation method	Incubation period (days)	Disease incidence (%)	Percent Disease Index (PDI)
Hydathode inoculation	5	85.03	46.76
Vein inoculation	2	100.00	74.22
Clip inoculation	3	80.21	38.01

The results of the pathogenicity tests demonstrated that all three inoculation methods produced typical V-shaped lesions along the leaf margins (Fig. 3). Among these methods, vein inoculation led to quicker symptom development compared to the other techniques (Table 2). Consequently, vein inoculation was selected for subsequent experiments. This finding is consistent with the observations of Nazir *et al.* (2010), who also reported that vein inoculation resulted in faster symptom expression compared to the hydathode inoculation method.

The *in vitro* evaluation of chemicals against bacterial growth revealed that streptomycin sulfate was the most effective, producing inhibition zones of 1.94 cm, 2.03 cm, and 2.20 cm at concentrations of 25 ppm, 50 ppm, and 100 ppm, respectively (Table 3; Fig. 4). This was followed by Bactrinashak at 1000 ppm, which resulted in an inhibition zone of 1.61 cm. Copper oxychloride at 1000 ppm and Bactrinashak at 500 ppm exhibited inhibition zones of 1.43 cm and 1.42 cm, respectively, placing them next in effectiveness. Cuprous oxide at 1000 ppm and mancozeb at 1000 ppm were the least effective, with inhibition zones of 0.73 cm and 0.68 cm, respectively. No inhibition zones were observed with mancozeb at 250 ppm and 500 ppm, copper oxychloride at 250 ppm, and cuprous oxide at 250 ppm and 500 ppm.

The evaluation of chemicals for managing black rot disease in cabbage demonstrated that all tested chemicals significantly reduced the percentage disease index (PDI) and increased plant height compared to the untreated control (Table 4). Streptomycin sulfate was found to be the most effective, achieving the lowest PDI of 5.86, which represented a 93.37% reduction in PDI compared to the control. Additionally, it increased plant height to 9.7 cm, compared to 5.8 cm in the control. Following streptomycin sulfate, Bactrinashak was effective with a PDI of 6.97, a 92.1% reduction in disease, and a plant height of 9.4 cm. Copper oxychloride and cuprous oxide were next in effectiveness, with PDI values of 13.06% and 17.43%, corresponding to reductions of 85.23% and 80.28%, respectively. Their respective plant heights were 8.6 cm and 8.1 cm. Mancozeb was the least effective, providing only a

Table 3. Effect of different treatments on inhibition of *Xanthomonas campestris* pv. *campestris*

Treatment	Concentration (ppm)	Inhibition zone (cm)
Streptomycin sulphate	25	1.94
	50	2.03
	100	2.20
Bactrinashak	250	1.28
	500	1.42
	1000	1.61
Mancozeb	250	0.00
	500	0.00
	1000	0.68
Copper oxychloride	250	0.00
	500	0.78
	1000	1.43
Cuprous oxide	250	0.00
	500	0.00
	1000	0.73
Control	-	0.00
CD (p≤0.05)		0.053

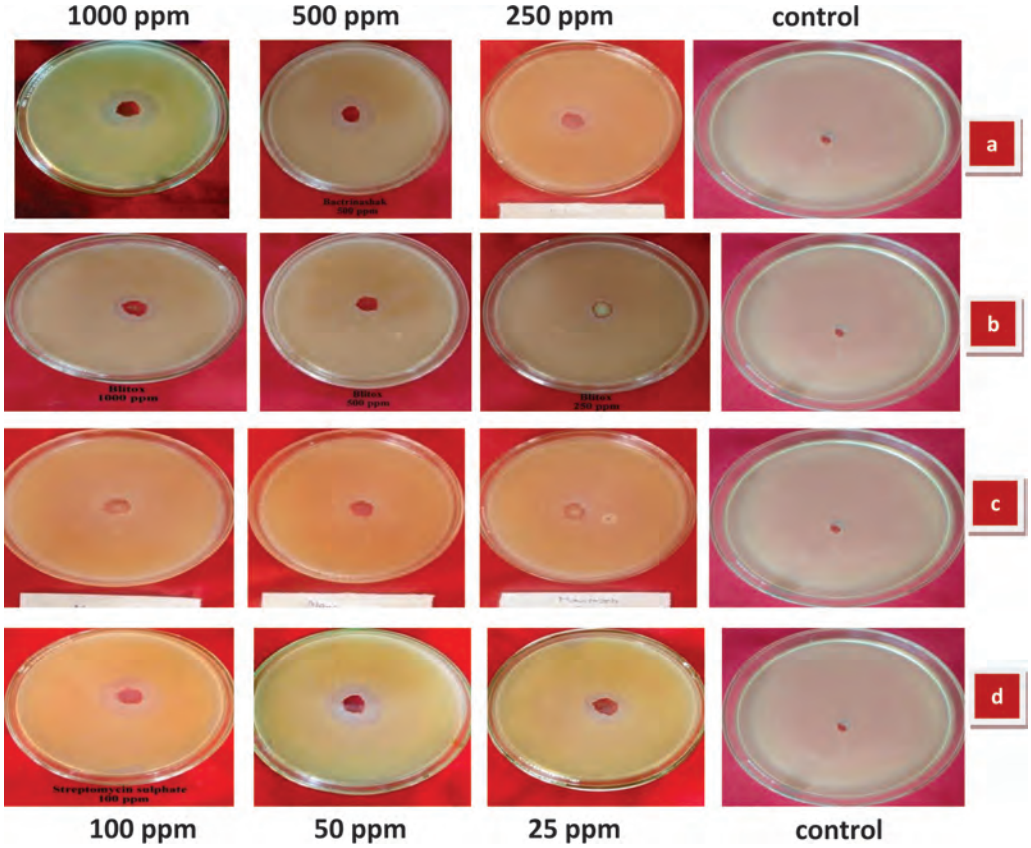

Fig. 4. Effect of chemicals on growth of *Xanthomonas campestris* pv. *campestris*. a) Bacterinashak, b) copper oxychloride, c) mancozeb, d) streptomycin sulphate

56.20% reduction in PDI and resulting in a plant height of 7.2 cm. Although streptomycin sulfate proved to be the most effective treatment, other chemicals such as Bactrinashak, copper oxychloride, and cuprous oxide also demonstrated significant efficacy, with over 80% disease reduction. These alternatives could potentially be used in place of streptomycin sulfate for managing black rot in cabbage.

The *in vitro* evaluation of plant extracts against the bacterium revealed that, regardless of concentration, *Vitex negundo* exhibited the largest mean diametric inhibition zone of 1.315 cm, followed by Eucalyptus hybrid with 1.177 cm, with significant differences between these extracts (Table 5; Fig. 5). In contrast, the extracts from *Ocimum sanctum*, *Melia azedarach*, and *Aloe vera* did not inhibit bacterial growth. In pot

Table 4. Per cent disease incidence (PDI) and plant height in different treatments

Treatment	Dose (%)	PDI	Reduction in PDI	Plant height (cm)
Streptomycin sulphate	0.01	5.86 (14.01)	93.37	9.7
Bactrinashak	0.10	6.97 (15.30)	92.1	9.4
Copper oxychloride	0.10	13.06 (21.18)	85.23	8.6
Mancozeb	0.10	38.73 (38.47)	56.20	7.2
Cuprous oxide	0.10	17.43 (24.66)	80.28	8.1
Control	-	88.43 (87.27)		5.8
CD (p≤0.05)		0.388		0.150

Figures in the parentheses are arc sine transformed values

Table 5. Evaluation of plant extracts against *Xanthomonas campestris* pv. *campestris*

Treatment	Inhibition zone (cm) at concentration (%)			
	10	25	50	Mean
Ocimum sanctum	0.000	0.000	0.000	0.000
Melia azedarach	0.000	0.000	0.000	0.000
Vitex nigundo	0.753	1.256	1.937	1.315
Aloe vera	0.000	0.000	0.000	0.000
Eucalyptus hybrid	0.503	1.190	1.840	1.177
Mean	0.251	0.489	0.755	
CD (p≤0.05)	Treatment			0.09
	Concentration			0.007
	Treatment × Concentration			0.016

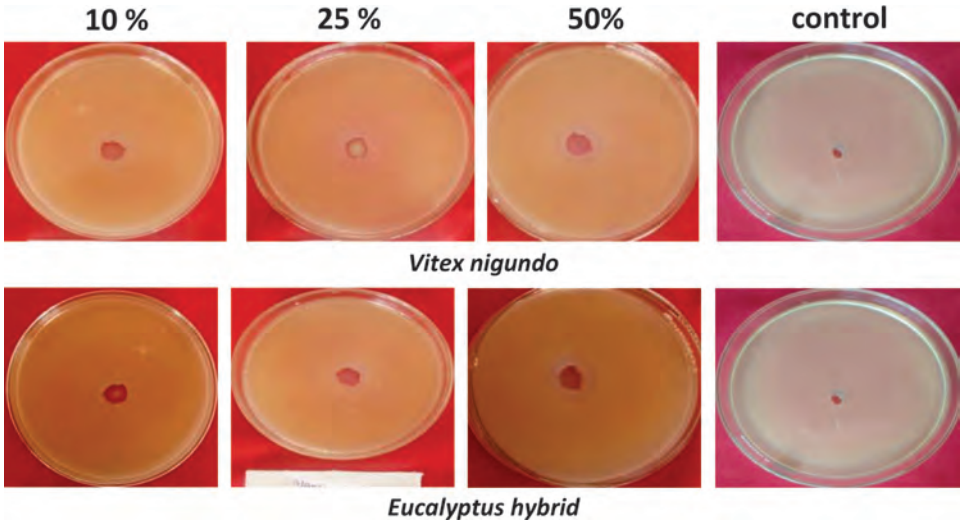

Fig. 5. Effect of plant extracts on growth of *Xanthomonas campestris* pv. *campestris*

experiments assessing plant extracts against black rot of cabbage, only two extracts demonstrated significant efficacy in reducing the percentage disease index (PDI) and increasing plant height compared to the untreated control (Table 6). *V. negundo* was the most effective, reducing the PDI to 50.03, which represents a 42.46% reduction from the 86.96% PDI observed in the control, and increasing plant height to 7.2 cm. *Eucalyptus hybrid* followed, with a PDI of 53.59, a 37.73% reduction, and a plant height of 6.9 cm. While the disease reduction with *V. negundo* and Eucalyptus hybrid extracts was less than the 93.37% reduction achieved with streptomycin sulfate, these plant extracts could serve as viable alternatives in integrated

Table 6. Evaluation of plant extracts against black rot of cabbage

Treatment	Dose (%)	PDI	Reduction in PDI	Plant height (cm)
Ocimum sanctum	50	86.04 (68.07)	1.05	6.0
Melia azedarach	50	86.91 (68.77)	0.06	5.8
Vitex nigundo	50	50.03 (44.99)	42.46	7.3
Aloe vera	50	86.95 (68.81)	0.015	5.9
Eucalyptus hybrid	50	53.59 (47.04)	38.37	7.0
Control	-	86.96 (68.81)		5.8
CD (p≤0.05)		2.158		0.693

Figures in the parentheses are arc sine transformed values

disease management strategies. *Eucalyptus* extracts contain components such as 1,8-cineole, citronellal, citronellol, citronellyl acetate, p-cymene, eucamalol, limonene, linalool, β-pinene, γ-terpinene, α-terpinol, alloocimene, and aromadendrene, which are known to contribute to their antibacterial activity (Nezhad *et al*., 2009). The bactericidal activity of *V. negundo* extract is likely attributed to the detergent-like properties of its phytoconstituents (Palaninathan *et al.*, 2022).

Authors' contribution: Conceptualization and designing of the research work (MS, AS), Execution of field/lab experiments and data collection (R), Data analysis and interpretation (R, AS), Preparation of manuscript (DS and PB), manuscript revision and editing (MS).

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