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RESEARCH ARTICLE

In vitro evaluation for the efficacy of the chemicals/ antibiotics, botanicals and bioagents against black rot (*Xanthomonas campestris* pv. *campestris*) of cauliflower in plains of Kerala

■ S. Lakshmi Prasanna and S. Ravi

SUMMARY

Cauliflower (*Brassica oleracea* var *botrytis*) is one of the important cool season vegetable crop grown in India. One of the major disease which causes huge loss (50-70%) is black rot of cauliflower caused by *Xanthomonas campestris* pv. *Campestris*. In the present study*in vitro* evaluation of chemicals/ antibiotics, botanicals and bio agents were studied and best selected treatments were studied at field level. Two antibiotics-Streptocycline, Tetracycline, (100,200,250ppm); Two chemicals- Copper oxychloride, Copper hydroxide, (0.15%, 0.2%); one combination of antibiotic and chemical Copper oxychloride + Streptocycline (0.15%+100ppm). Four botanicals leaf extract of tea, tea waste decoction, garlic extract, turmeric extract at two different concentrations 5 and 10 per cent and two bioagents *Bacillus subtilis* and *Pseudomonas fluoresces* were tested for their efficacy against *Xanthomonas campestris* pv. *Campestris*. The results were garlic extract 10 per cent was found to be effective against the pathogen followed by tetracycline 250 ppm. The untreated control recorded the maximum disease incidence, followed by turmeric extract 10 per cent application. But there observed no significant difference among the treatments Tetracycline 250, 200 ppm, *P. fluorescens*, copper hydroxide 0.2%, garlic extract 5 per cent and turmeric extract 10 per cent are found to be on par.

Key Words : Cauliflower, Black rot, Chemicals, Antibiotics, Botanicals, Bio agents, *Xanthomonas*

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Cauliflower, is an important winter vegetable crop, widely grown in many parts of the world. Cauliflower is a good source of Vitamin C, Vitamin K and glucosinolates which are antioxidants. Antioxidants are beneficial in helping the body fight free radicals and in reducing inflammation. Cauliflower helps to increase the body's ability to detoxify itself; it boosts

the body's antioxidant system, and helps to balance the levels of inflammation to anti-inflammation in the body. With these activities cauliflower helps to reduce the risk of several types of cancer. India next to China, has larger area and production of cauliflower. About half of all cauliflower is raised in China and one fourth in India.

Cauliflower black rot was first reported by (Patel *et al.,*1949). Cauliflower black rot losses may exceed 50 per cent due to the rapid spread of the disease. A crop loss upto 50 to 70 per cent in cauliflower has been recorded in India (Guptha and Thinol, 2006). In Himachal Pradesh, curd rot of cauliflower has been a menace to the seed crop and is the cause of huge losses to farmers in India (Shyam *et al.,*1994). Black rot appears annually in Manipur near the end of February and is severe (upto 50% losses) in susceptible cultivars (Gupta, 1991). Losses to the tune of 100 per cent are not uncommon (Roberts *et al.,* 2007).

In view of the potential crop losses, the economic significance of the disease *invitro* evaluation studies were conducted against the pathogen with the selected chemicals-tetracycline and Streptocycline @ 250, 200,100 ppm, copper hydroxide and copperoxychloride @ 0.15%, 0.2%, combination copper oxychloride (0.1%) + streptocycline 100 ppm (Kumud and Shyam, 2003 and Beura *et al.*, 2006), Botanicals- turmeric, garlic, tea leaf and tea decoction extracts (Satya *et al.,* 2007; Bharadwaj and Laura, 2009 and Dhanya and Mary, 2006) and bio agents- Pseudomonas and Bacillus (Jalali and Parashar, 1995 and Chuaboon and Prathuangwong, 2008).

MATERIAL AND METHODS

Xanthomonas campestris pv. *campestris* (*Xcc*) was isolated from cauliflower curds showing characteristic symptoms of black rot collected from the field (grown at Agriculture Research Station, Mannuthy) on PSPA medium (Potato sucrose peptone agar medium; potato 300g, peptone, 2g, sucrose, 20g, KH_2PO_4, 0.2g, $NaHPO_4$, 0.5g, calcium nitrate, 0.5g, KCl, 0.05g, ferrous sulphate, 0.05g, agar 20g per litre). Bio 7 agents were grown at 30^0C on nutrient agar (beef extract 3g, peptone 6g, sucrose 10g. agar 15g (Guo *et al.*, 2004). Botanical extracts with required concentration were prepared from the fresh samples after proper sterilization. All these were evaluated by well method (Perez *et al.*, 1990) and the efficacy was expressed as per cent inhibition using the formulae put forward by (Vincent, 1927).

In vitro evaluation of chemicals and antibiotics against black rot causing pathogen :

For *in vitro* evaluation, 20 ml medium was transferred into sterilized Petri dishes. After solidification of medium, 0.1 ml of 48 h old bacterial suspension (one loop full in 10 ml water, from that 0.1 ml) was poured on it and was spread with spreader. Required quantity of chemicals were mixed with the sterile water to get desired concentrations and poured in to the well made in the center of the plate. Five replications were maintained for each concentration of the chemicals. The plate with sterile water in the well served as control. The inoculated Petri dishes were incubated at 28 ± 2^0C. The diameter of the inhibition zone was recorded until its effect was lost. The per cent inhibition was calculated as per the method of (Vincent, 1927). The chemicals selected and the concentrations used for *in vitro* evaluation are presented in Table A.

Table A : Details of chemicals

Sr. No.	Chemical	Concentration
1.	Streptocycline	100,200,250 ppm
2.	Tetracycline	100,200,250 ppm
3.	Copper oxychloride	0.15%, 0.2%
4.	Copper hydroxide	0.15%, 0.2%
5.	Copper oxychloride + Streptocycline	0.15%+100ppm

In vitro evaluation of selected botanicals against pathogen :

Four botanicals at two different concentrations were used for *in vitro* evaluation against the pathogen. The list and the concentrations are furnished in Table B.

Table B : Botanicals used for *in vitro* evaluation against pathogen

Sr. No.	Botanicals	Concentration
1.	Tea leaf extract	5%, 10%
2.	Tea waste decoction	5%, 10%
3.	Garlic extract	5%, 10%
4.	Turmeric extract	5%, 10%

Fifty gram leaves of tea, garlic and turmeric were taken separately, washed in sterile water, disinfected with 70 per cent ethyl alcohol and then exposed to U.V. light for one hour by keeping it upside down for every 15 min. The extract was prepared by macerating using sterilized pestle and mortar with 50 ml of sterile water under aseptic condition and filtered through clean, sterilized

muslin cloth. In the case of tea spent waste, 50 g material is taken without washing and surface sterilized with alcohol, as the procedure may result in leaching of antibacterial agents. They were subjected to U.V. light treatment only and the extraction procedure followed. A bacterial lawn was prepared by adding 0.1 ml suspension on the mediated plates. A well was made at the center using a cork borer and one ml of the suspension was poured into the well. Five replications were maintained for each treatment. The diameter of the inhibition zone was recorded until its effect was lost and the per cent inhibition was calculated using the formulae of (Vincent, 1927).

In vitro evaluation of bioagents against black rot causing pathogen :

Two bioagents available at the Department of Plant Pathology *viz., Pseudomonas fluorescens* and *Bacillussubtilis* were tested against *Xcc.* A well was made at the centre using a cork borer and one ml (from one loop full in 10 ml water) of the bioagent suspension was poured into the well after preparing the bacterial lawn on the solidified medium. Five replications were maintained. The diameter of the inhibition zone was recorded and the per cent inhibition was calculated using the formulae of (Vincent, 1927).

Disease management :

The effectiveness of chemicals, botanicals and bioagents against black rot pathogen were tested under laboratory conditions. All these were evaluated by well method (Perez *et al.*, 1990). The efficacy was expressed as per cent inhibition using the following formulae (Vincent 1927).

$$\text{Per cent inhibition} = \left(\frac{C - T}{C}\right) x\ 100$$

C = Growth of pathogen in control (cm)
T = Growth of pathogen in treatment (cm).

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

In vitro evaluation of chemicals:

The results showed that all the antibiotics tested irrespective of the concentrations were more effective in inhibiting the *Xcc* compared to other chemicals and its combination with antibiotic. Maximum inhibition of 70.67 per cent was noticed with tetracycline 250ppm, followed by tetracycline 200ppm (61.78%), streptocycline 250ppm (56.22%), tetracycline 100ppm (54.78%), streptocycline 200ppm (46.00%) and 100ppm (34.22%). Among the chemicals, copper hydroxide 0.20 per cent recorded 29.56 per cent inhibition. Copper oxychloride 0.15 per cent had shown least inhibition among the chemicals and antibiotics tested Table 1.

In vitro evaluation of botanicals :

Garlic extract 10 per cent was found to be the best botanical with an inhibition of 67.22 per cent, followed by lesser concentration of garlic at 5 per cent level (54.56 %). Garlic has a wide spectrum of actions; antibacterial, antiviral, antifungal and antiprotozoal. Allicin, a major component of garlic was found to have antibacterial

Table 1 : *In vitro* inhibitory effects of plant protection chemicals against *Xanthomonas campestris* pv. *campestris*

Treatments	Concentration	Per cent inhibition*			
		1	2	3	4
		Days after inoculation			
Copper oxychloride	0.15%	23.67	20	17.56	17.22
	0.2%	32.89	30.56	29.44	29.44
Copper hydroxide	0.15%	25.33	22.00	21.22	21.11
	0.2%	29.56	58.44	28.11	27.33
Streptocycline	250ppm	56.22	50.67	47.67	41.78
	200ppm	46	42.78	41.11	39
	100ppm	34.22	26.78	20.89	14.56
Tetracycline	250ppm	70.67	66.44	43.11	41.11
	200ppm	61.78	60.11	57	55.56
	100ppm	54.78	51.11	46.84	40.89

Table 2 : *In vitro* inhibitory effect of botanicals against the pathogen

Treatments	Dosage	Per cent inhibition*			
		1	2	3	4
		Days after inoculation			
Turmeric extract	5%	22.56	38.11	16.22	15.56
	10%	3.33	51.67	24.	23.67
Garlic extract	5%	54.56	45.11	37	18.89
	10%	67.22 [a]	54 [a]	49.22 [a]	44.00 [a]
Tea leaf extract	5%	0	0	0	0
	10%	0	0	0	0
Tea decoction	5%	0	0	0	0
	10%	20.67	17.56	17.22	12.56

* Mean of three replications; In each figure followed by same letter do not differ significantly DAI - Days after inoculation

Table 3 : *In vitro* inhibitory effects of bioagents against pathogen

Treatments	Per cent inhibition*				
	1 DAI	2 DAI	3 DAI	4 DAI	5 DAI
P. fluorescens	31011	27.89	26.11	24.44	11.44
B. subtilis	70	67.33	64.44	61.22	52.22

* Mean of three replications; DAI-Days after inoculation

property (Harris *et al*., 2001). Turmeric extract 10 per cent (30.33%) and turmeric extract 5 per cent (22.56 %) were found to be on par and minimum was recorded by tea decoction. Tea leaf extract and tea waste decoction 5 per cent were found to have no effect on the black rot pathogen. (Table 2).

In vitro evaluation of bioagents :

It was observed that *B. subtilis* was found to be the best among the two bioagents which recorded an inhibition of 70.00 per cent (Table 3).

Conclusion:

From the above experiment we conclude that tetracycline, garlic 10 per cent and *Bacillus subtilis* were found to be effective in inhibiting pathogen*in vitro* (70% and above inhibition). A part from tetracycline, garlic extract 10 per cent (botanical), *Bacillussubtilis* (bioagent) should be tested at field level and at different locations to test their efficacy. There by reducing the use of plant protection chemicals protecting environment from its adverse effects using eco friendly materials.

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