Efficacy of biocontrol agents, plant extracts and organic amendments against black rot of cabbage caused by *Xanthomonas campestris* pv. *campestris*

Deivamani, M.[1*] and M. Muthamilan[2]

[1]Tapioca and Castor Research Station, Tamil Nadu Agricultural University, Yethapur, Tamil Nadu, India.
[2]Department of Plant Pathology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai, Tamil Nadu, India. *Corresponding author's E-mail: deivamanimariyappan@gmail.com

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ABSTRACT

Cabbage is one of the most popular winter cruciferous vegetables grown in India. It is mostly cultivated in cool places and hence, the crop is mostly affected by *Xanthomonas campestris* pv. *campestris* (*Xcc*) (Pammel) causes black rot disease worldwide. Increased usage of different chemicals based products to control these pathogens have resulted in problems like residual effect of chemicals in agri-based products and also increased resistance for chemicals in target pathogens and environmental pollution. The crude extracts of some well-known botanicals, biocontrol agents and organic amendments are used to control some of the plant pathogens. The results of botanicals the leaf extract of *Datura stramonium* (10%) was maximum inhibition and minimum inhibition of *Ocimum basilicum* were recorded. Whereas, the biocontrol agents of *Streptomyces* spp and *Trichoderma viride* showed maximum inhibition zone were effective against the growth of *Xcc*.

Key words: Black rot of cabbage, antibacterial activity, plant extracts, biological agents

Cabbage (*Brassica oleracea* var. *capitata* L.) is one of the most popular winter cruciferous vegetables grown in India. Cabbage is mostly cultivated in cool places and hence, the crop is commonly affected by various fungal, bacterial and viral diseases. Black rot caused by *Xanthomonas campestris* pv. *campestris* is one of the most important diseases of cabbage. *Xanthomonas* is a very important phytopathogenic bacterium, which causes plant disease throughout the globe. The hosts of this bacterial genus infect 124 monocotyledonous and 268 dicotyledonous plants, amid them rice bacterial blight, black rot of cabbage and citrus blight disease are the most serious diseases and causes huge loss on agricultural production (Hauben, 1997). The yield losses due to black rot of cabbage may increase up to 50 per cent during warm and wet conditions by Sain *et al.,* (2005). This disease is prevalent in almost all the cabbage growing areas of India. The bacteria invade the host plant through water pores (hydathodes) at the leaf margins (Babadoost, 1999). Symptoms first appear as yellow 'V' shaped areas with the open part of the 'V' along edges of the leaf. The diseased areas then become brown and brittle, and the affected leaf veins turn dark brown to black (Seebold *et al.*, 2008). The increased reflection on environment concern over pesticide use has been instrumental in a large upsurge of biological control. Development of fungicide resistance among the pathogens, ground water and foodstuff pollution and the development of oncogenic risks have further encouraged the exploitation of potential antagonistic microflora in disease management. Management of plant diseases through plant extracts and biological method envisages the use of antagonistic organisms like, rhizobacteria, bacteriophages and avirulent strains of the pathogen and bacterial metabolites. Garg and Kasera (1984) reported the antibacterial activity of essential oil of *Anacardium occidentale* L. against this pathogen. Grainge and Alvarez (1987) found that the leaf extract of *Artabotrys hexapelatus* L. was most inhibiting to the *Xcc*. Garcia *et al.*, (1994) reported that black rot of crucifer caused by *Xcc* can be controlled by using various plant extracts. Among

the 22 plant extracts / oils tested against *Xcc in vitro*, datura leaf extract was the most inhibitory to the pathogen followed by leaf extracts of keezhanelli, polyalthia and parthenium. Actinomycetes represent a high proportion of the soil microbial biomass. They have the capacity to produce a wide variety of extracellular hydrolyses that give them an important role in the decomposition of organic matter in the soil. They appear to have a high degree of importance among the microbial flora of the rhizosphere. Actinomycetous bacteria have been recognized as sources for several secondary metabolites, antibiotics and lytic enzymes of medical and industrial value but only a few taxa mainly *Streptomyces* spp. have been studied as potential biocontrol agents against fungal phytopathogens (Sabaratnam and Traquair, 2002).

Seed treatment and seedling dip with *Trichoderma harzianum* isolate proved best option along with *T.harzianum* TH-3 and captaf, where the studies showed captaf to be the safest fungicide for *Trichoderma* (Tronsmo, 1986). The use of yeasts as biocontrol agents of plant diseases was a strategy with great potential (Reis *et al.,* 1996), mainly because of their ability to compete for nutrients (Blackeman 1992) and colonization sites. Twenty yeast isolates, obtained from cabbage phylloplane, were evaluated for antagonistic activity against *Xcc* in field (Assis *et al.,* 1999). In the present study, attempts were made under *in vitro* screening to identify the effective antagonists as well as plant products against *Xcc* in cabbage.

MATERIALS AND METHODS

Collection of isolates of *Xanthomonas campestris* pv. *campestris* (*Xcc*)

Black rot disease infected leaves were collected from the major cabbage growing areas of Tamil Nadu. The infected portion of the leaf was cut into 3 mm bits separately and they were surface sterilized with 0.1 per cent mercuric chloride solution and washed sterile distilled water. These leaf bits were submerged in test tubes each containing three ml of sterile distilled water and incubated for 6 h at room temperature (28±2^{o}C) and thereby prepared bacterial suspension from each sample and finally it was streaked onto the centre of sterile petriplates each containing 10 ml of nutrient agar medium separately. After 48 h of incubation, yellowish bacterial growth was appeared and these ten cultures were further sub cultured on nutrient agar slants separately and purified by the dilution plate technique (Waksman, 1952).

Pathogenicity of *Xcc* isolates on cabbage leaves

The suspension of isolates of *Xanthomonas campestris* pv. *campestris* (*Xcc*) were prepared separately by suspending 48 h old axenic culture (log phase) of each isolate in sterile distilled water. The optical density (OD) of suspension of each isolate was adjusted in a spectrophotometer to 0.40 to 0.45 so as to obtain about 10^8 cfu ml^{-1}. Cabbage plants (Kirti) were raised from surface sterilized seeds in pots of 30 cm dia. each containing three kg of pot mixture, fertilized with the calculated amount of 50: 125: 25 kg (N: P: K) ha^{-1}. When the cabbage seedlings were 35 d old, they were uprooted and transplanted in pots (one seedling / pot) and regularly watered fifteen days after planting. The cell suspension of each isolate of *Xcc* was inoculated by spraying method separately using an atomizer during cool hours or late in the evening. Water congestion was provided both 24 h prior to 24 h after inoculation by covering the plants with moist polythene bags. The plants were maintained inside the glass house by regular and judicious watering. Three replications were maintained for each isolates separately. The plants inoculated with sterile distilled water alone served as control. The symptoms of the disease were recorded 15 days after inoculation. Then, the bacterium was reisolated from the artificially infected plants separately and compared with the original isolates. Twenty days after inoculation, the disease incidence was recorded by grading the three opened inner leaves in each of the plants on a scale of 1 to 9 (Jayaraj *et al.,* 1987) as detailed below;

Grade		Leaf area affected (%)
0	-	No visible symptoms
1	-	Less than 1
3	-	1 to 10
5	-	11 to 25
7	-	26 to 50
9	-	More than 50

The per cent disease index (PDI) was calculated using the following formula (Mckinney, 1923)
PDI=[Sum of all numerical ratings/ (Total number of leaves graded × maximum grade)] x 100.

On French bean pods

The virulence of ten isolates were assessed using french bean pod separately as a method described by Starr and Dye (1965) *in vitro.* Uniformly matured, straight and blemish-free green French bean pods were selected and thoroughly washed in sterile distilled water. Under aseptic conditions, the pods were pinpricked with sterilized entomological needle and a loopful of actively growing 48 h old culture of each bacterial isolate was placed to a depth of 2 mm separately and incubated at 24 – 30°C in humid chamber for 4 days. The pods pinpricked with sterile distilled water were kept as control. The lesion type and size (dia) developed in each pod were recorded, separately.

Efficacy of extracts of plant products against the growth of isolates of *Xcc*

Leaves of plants having antimicrobial activity were collected afresh separately and ground with sterile distilled water @ one ml/g in a sterile pestle and mortar. The extracts of each plant were collected separately by squeezing the macerate with sterile cotton wool. Each extract was strained separately through two layers of muslin cloth and finally through Whatman No. 1 filter paper. This was again passed through seitz filter separately to free the extract from the bacterial contaminants. Each extract was further diluted to a concentration of ten per cent separately in sterile distilled water. The efficacy of above extracts was tested against the growth of the isolates of bacterial pathogen *in vitro* (Shekhawat and Prasad, 1971).

Efficacy of leaf extracts against the growth of *Xcc in vitro*

The leaf extracts (10%) of fifteen plants belonging to twelve botanical families were tested against cabbage black rot bacterial pathogen by following paper disc assay. Three number of sterilized filter paper disc of 10 mm dia were dipped in the respective leaf extracts for five min separately, uniformly mopped the excess extract present in the disc by touching the same on the wall of the container and these were air-dried. Then these discs were placed onto the petriplates which contained previously seeded (with *Xcc*) nutrient agar medium separately by using a sterilized forceps at equidistance @ three discs per petriplate. The filter paper discs dipped in sterile distilled water and placed on to the petriplates were served as control. The petriplates were incubated at room temperature (28±2°C) and the inhibition zone was measured after 72 h (Smale and Keil, 1966).

Efficacy of extract of organic amendments against the growth of *Xcc in vitro*

Required quantity of each oil cake and compost was taken and made into powder separately. It was soaked in sterile distilled water @ 1 g in 1.25 ml of sterile distilled water separately and kept overnight. Each material was ground using a pestle and mortar separately and filtered through a muslin cloth and each filtrate was collected separately and centrifuged at 10,000 rpm for 15 min. The supernatant of extract of each oil cake and compost was served as the standard solution (100%) (Dubey, 2002).

Testing the antimicrobial activity of extract of compost and oil cakes against *Xcc in vitro*

The efficacy of extracts of composts and oil cakes were tested against *Xcc* using paper disc assay. The standard solution of each oil cake and compost were diluted further and thereby prepared 10 per cent concentration of each with NA medium separately and sterilized. The paper discs were dipped in actively grown culture of *Xcc* (10^8 cfu ml^{-1}) for five min and were placed on the petriplate containing NA medium which was already incorporated with extracts of organic amendments and incubated at room temperature. The NA medium without extract of either oil cake or compost served as control. The radial growth (mm) of *Xcc* was recorded two days after incubation.

Screening of antagonistic bacteria against *Xcc* isolate (I_6) *in vitro*

The bacterial antagonists viz., *Streptomyces spp*., *Bacillus subtilis* and *Pseudomonas* sp were screened against *Xanthomonas campestris* pv. *campestris* (I_6) by the method explained by Chakarvarthi *et al,*. (1974). A loopful of twenty four

h old culture (2×10^8 *cfu*/ml) of each antagonistic sterilized petriplate containing nutrient agar (NA). The pathogen *Xcc* (I_6) was also immediately streaked in the opposite side of all the petriplates and incubated for five d at room temperature 28±2°C. The extent of inhibition of pathogen growth was measured after five days of incubation.

Efficacy of bacterial antagonists against the growth of *Xcc in vitro*

The bacterial antagonists *viz*., *Streptomyces exfoliatus, Streptomyces violaceusniger, Bacillus subtilis* and *Pseudomonas fluorescens* (Pf 1, Pf 2, Pf 3) were tested against *Xcc in vitro*. The *Streptomyces* spp., *B. subtilis* and *P. fluorescens* were grown on yeast extract malt extract agar medium, nutrient agar medium and King's B medium separately. The cell suspension of (2×10^8 cfu ml^{-1}) each above mentioned bacterium was prepared separately and they were used for paper disc assay as described by Smale and Keil, (1966).

Efficacy of fungal antagonists against the growth of *Xcc in vitro*

Four species of *Trichoderma viz., Trichoderma viride, T. harzianum, T.koningii, T. hamatum* and *Saccharomyces cerevisiae* were maintained on PDA slants and were multiplied separately in petriplate containing PDA medium. The PDA culture discs were prepared by using a 10 mm sterilized cork borer. A culture disc was inoculated into each of the sterilized conical flasks containing 25 ml of PDA broth and incubated at room temperature for seven d. The mycelia mat of each species of *Trichoderma* were collected separately by removing the mat by filtered through Whatman No. 1 filter paper. Then, spore suspension of each species (2×10^8 cfu ml^{-1}) was prepared separately and the cell suspension of *Saccharomyces cerevisiae* (2×10^8 cfu ml^{-1}) was also prepared. The suspension of each of above organisms was used separately for assessing their efficacy against *Xcc* on paper disc assay as described by Smale and Keil, (1966).

RESULTS AND DISCUSSION

Cabbage leaves showing the typical black rot disease symptoms were collected from different cabbage growing areas *viz.,* Ottanchathiram, Kodaikanal, Palamedu, Batalagundu, Uthamapalayam, Gudalur, Andipatti, Palakombai, Thadiyankudisai and Bhodi of Tamil Nadu with a view to identify the variability of the pathogen, if any. The disease incidence was ranged from 39.16 to 59.16 per cent. The affected cabbage plants collected from the Gudalur recorded the maximum disease intensity (59.16 PDI) whereas Bhodi were recorded the minimum disease intensity (39.16 PDI). The pathogen was isolated, purified and the isolates were maintained on Nutrient Agar (NA) medium at room temperature for further use.

The isolates were reinoculated on the susceptible cabbage leaf and French bean pods separately in the glass house under ideal conditions to study the pathogenicity. In french bean pods inoculated isolate I_6 produced the maximum lesion size of 4.6 mm dia while I_{10} produced the least lesion size of only 2.1 mm dia on french bean pods by indicating that I_6 was the most virulent while I_{10} was the least virulent. The isolates I_5 and I_8 were statistically on par with each other.

Table 1. Pathogenicity of *Xcc* isolates on French bean pods and cabbage leaves

Isolates	Mean lesion diameter on French bean pods [2 DAI (mm)]*	Disease incidence on cabbage leaves [20 DAP - PDI*]
I_1	4.0	45.83 (42.61)**
I_2	3.4	40.83 (39.72)
I_3	2.7	39.16 (37.76)
I_4	3.8	41.66 (40.20)
I_5	2.4	35.00 (35.76)
I_6	4.6	54.16 (47.39)
I_7	4.3	46.66 (43.09)
I_8	2.5	34.16 (36.27)
I_9	2.9	37.50 (38.74)
I_{10}	2.1	32.50 (34.76)
CD (P=0.05)	0.2	1.34

Whereas on cabbage leaves The isolate I_6 recorded the maximum mean PDI of 54.16 while I_{10} showed the minimum PDI of 32.50 and it was followed by the isolates I_7 and I_1 by recording 46.66 and 45.83 PDI respectively. The isolates I_4 and I_2 were on par with each other in virulence.

Table 2. Efficacy of plant products against the growth of *Xcc* (I_6) *in vitro*

Sl. No.	Common name	Botanical name	Conc. (%)	Inhibition zone [2 DAI (mm)]*
1	Thuththi	*Abutilon indicum* L.	10	1.6
2	Kuppaimeani	*Acalypha indica* L.	10	2.0
3	Vilvum	*Aegle marmelos* L.	10	2.3
4	Neem	*Azadirachta indica* A.	10	1.9
5	Eruku	*Calotropis gigantea* L.	10	1.7
6	Omavalli	*Coleus forskohlii* (Poir.)	10	2.1
7	Thorn apple	*Datura stramonium* L.	10	6.9
8	Neerium	*Neerium oleander* L.	10	1.5
9	Thiru neetru patchi	*Ocimum basilicum* L.	10	1.4
10	Thulasi	*Ocimum sanctum* L.	10	1.8
11	Parthenium	*Parthenium hysterophorus* L.	10	2.8
12	Keezhanelli	*Phyllanthus niruri* L.	10	5.2
13	Polyalthia	*Polyalthia longifolia* L.	10	4.5
14	Seemai karuvel	*Prosopis juliflora* L.	10	2.3
15	Nochi	*Vitex negundo* L.	10	1.4
16	Control		-	0.0
CD(P=0.05)				0.1

The isolate I_6 was observed as virulent isolate in all the above experiments (Table 1). The similar results the virulence of ten *Xanthomonas campestris* isolates was evaluated using the percentage of lesion area of leaves in *Brassica oleraceae* host plant, comparing the dia of colonies, xanthan production and gum viscosity reported by Nitschke and Rodrigues (2000). Sharma *et al.,* (2004) reported that cabbage cultivars were highly susceptible or possessing partial resistance to black rot pathogen. Griffin and Baker (1976) tested variability in the virulence among the four isolates of *Xcc* on cabbage. The bacterial isolates obtained from seeds were produced similar symptoms as observed in naturally infected plants during pathogenicity test (Chakravarthi *et al.,* 2004). The symptom of disease on the artificially inoculated cabbage leaves was appeared on the seventh day in the form of necrotic lesions on the leaf margin. These lesions progressed towards the midrib of the leaf blade and developed in the form of 'V' shaped chlorotic lesion. The veins and veinlets were turned black and the leaf tissues became necrotic and brittle. After 15 to 20 days, the infection was systematically appeared on the vascular system and caused blackening of vascular tissues. Dowson (1939) and Seebold *et al.* (2008) were also described similar symptoms on the infected cabbage leaves. *In vitro* screening of plant products against *Xcc* revealed that leaf extract of *Datura stramonium* (10%) was effective against the growth of *Xcc* recording the maximum inhibition zone of 6.9 mm and it was followed by *Phyllanthus amarus* and *Polyalthia longifolia* which recorded the inhibition zone of 5.2 mm and 4.5 mm respectively. The leaf extracts of *Aegle marmelos* and *Prosophis juliflora* were ranking next best and they were statistically on par with each other and it was followed by *Coleus forskohlii, Acalypha indica, Azadirachta indica, Ocimum sanctum, Calotrophis gigantea, Abutilon indicum, Neerium oleander, Vitex negundo* and *Ocimum basilicum* by recording inhibition zones of 2.3, 2.3, 2.1, 2.0, 1.9, 1.8, 1.7, 1.6, 1.5, 1.4 and 1.4 mm respectively (Table 2). The leaf extract (10%) of *Vitex negundo* and *Ocimum basilicum* were recorded the least growth inhibition of 1.4 and 1.4 mm respectively. Since the leaf extracts (10%) of *Datura stramonium* was most effective against the growth of *Xcc,* this was selected for further testing. The corroborated the results obtained by Garcia *et al.,* (1994) who reported that black rot of crucifer caused by *Xcc* could be controlled by using various

plant extracts. Satish *et al.,* (1999) reported that eight plant species showed antibacterial activity, based on the zone of inhibition in a diffusion assay. Significant antibacterial activity was observed in the aqueous extracts of *Prosopis juliflora, Oxalis corniculata* and *Lawsonia inermis. In vitro* studies indicated that two leaf extracts, *Nerium oleander* and *Pithecolobium dulce* exerted the higher percent inhibition to mycelial growth (77.4, 75.1% respectively) and spore germination (80.3, 80.0% respectively) of *Bipolaris oryzae* (Harish *et al.,* 2008).

Table 3. Efficacy of extract of organic amendments against the growth of *Xcc* (I_6) *in vitro*

Sl. No.	Treatments	Conc. (%)	Inhibition zone [2 DAI (mm)]*
1	Gingelly cake	10	4.3
2	NSKE	10	8.6
3	Caster cake	10	5.9
4	Groundnut cake	10	5.3
5	Mahua cake	10	7.9
6	Coconut cake	10	4.1
7	Sugarcane waste compost	10	2.3
8	Sheep waste compost	10	1.3
9	Mushroom waste compost	10	2.6
10	Coir pith compost	10	2.8
11	FYM	10	3.2
12	Vermicompost	10	2.9
13	Control	-	0.0
CD (P=0.05)			0.2

The NSKE (10%) exerted the maximum inhibition of 8.6 mm and it was followed by mahua oil cake (10%), castor oil cake (10%), groundnut oilcake (10%) and gingelly oil cake (10%) by recording 7.9, 5.9, 5.3 and 4.3 mm respectively. The sheep waste was recorded the minimum inhibition zone (1.3 mm) against *Xcc* (Table 3). Harish et al., (2008) reported that the *in vitro* studies indicated two leaf extracts (10 %), *Nerium oleander* and *Pithecolobium dulce* exerted the higher percent inhibition to mycelial growth (77.4, 75.1% respectively) and spore germination (80.3, 80.0% respectively) of *Bipolaris oryzae.*

Table 4. Efficacy of fungal antagonists and antibiotics against the growth of *Xcc* (I_6) *in vitro*

Sl. No.	Particulars	Concentration	Inhibition zone [2 DAI (mm)]*
Antagonists			
1	*Trichoderma viride*	2×10^8 cfu ml	10.2
2	*T.harzianum*	2×10^8 cfu ml	09.2
3	*T.koninji*	2×10^8 cfu ml	09.7
4	*T.hamatum*	2×10^8 cfu ml	07.6
5	*Saccharomyces cerevisiae*	2×10^8 cfu ml	07.1
Antibiotics			
6	Streptomycin	100 ppm	18.1
7	Chloroamphenicol	100 ppm	15.3
8	Penicillin	100 ppm	15.5
9	Streptocycline	100 ppm	17.6
10	Control	-	0.0
CD (P=0.05)			0.6

The growth of *Xcc* isolate (I_6) was highly (+++) inhibited by *Streptomyces* spp. and *Pseudomonas fluorescens* and it was followed by Pf 2, Pf 3 and *Bacillus subtilis* (Table4). Karthikeyan (1999) observed similar findings of *P. fluorescens* isolates effectively inhibited the growth of *Alternaria. palandui in vitro*. Actinomycetes in general and *Streptomycetes* in particular, are known to include several species that may inhibit growth activities of many plant-pathogenic micro-organisms *in vitro* (Fauth *et al.,* 1986). The bacterial antagonists were varied in their inhibitory effect against *Xcc in vitro*. *Streptomyces exfoliatus, S. violaceusniger* and *P. fluorescens-1* were exerted the maximum (13.4, 13.1 and 12.4 mm) growth inhibition against *Xcc* and these three were on par with each other. *P. fluorescens-2, Pf3 and Bacillus*

subtilis recorded inhibition zone of 9.3, 8.5 and 9.8 mm respectively and they were on par with each other. Based on the above results, the most effective *Streptomyces exfoliatus* and *P. fluorescens*-1 were therefore selected for the pot culture and field experiments (Table 5).

Table 5. Screening of antagonistic bacteria against *Xcc* isolate (I_6) i*n vitro*

Sl. No.	Antagonists	Level of Inhibition [2 DAI (mm)]*	Concentration	Inhibition zone [2 DAI (mm)]*
1	*Streptomyces violaceusniger*	+++	2×10^{8} cfu ml^{-1}	13.1
2	*Streptomyces exfoliates*	+++	2×10^{8} cfu ml^{-1}	13.4
3	*Pseudomonas fluorescens-1* (*Pf1*)	+++	2×10^{8} cfu ml^{-1}	12.4
4	*Pf2*	+	2×10^{8} cfu ml^{-1}	09.3
5	*Pf3*	+	2×10^{8} cfu ml^{-1}	08.5
6	*Bacillus subtilis*	+	2×10^{8} cfu ml^{-1}	09.8
7	Control	_	-	00.0
	CD (P=0.05)			0.6

The biocontrol agent *Streptomyces violaceusniger* YCED9 inhibited *in vitro* growth of seven fungal pathogens of turfgrass. Three different antibiotics were produced by the actinomycete, including nigericin, geldanamycin and a complex of macrocyclic lactone antibiotics. Each had a different spectrum of antifungal activity (Trejo-Estrada *et al.,* 1998). Getha and Vikineswary, (2002) reported that *Streptomyces violaceusniger* G10 showed a strong antagonism toward *Fusarium oxysporum* f. sp. *cubense* by producing extracellular antifungal metabolites. Sendhilvel (2000) studied the efficacy of *Pseudomonas* strain FP 7 and *Bacillus subtilis* in reducing the growth of soft rot bacteria of onion *in vitro.* Yamunarani (2009) reported among the antagonists tested *S. violaceusniger-violaceusniger* and *S. exfoliatus* recorded the maximum reduction of mycelia dry weight (100 per cent) followed by *P. fluorescens* and *T.viride* (GNTV1) (72.65 per cent).

The five fungal antagonists and four antibiotics (100 ppm) were tested against *Xcc in vitro*. Among these, streptomycin (100 ppm) exerted the maximum (18.1 mm) growth inhibition of *Xcc* followed by streptocycline (17.6 mm) and these two were statistically on par each other. It was followed by penicillin (15.5 mm) and chloramphenicol (15.3 mm). Among the fungal antagonists tested, the *Trichoderma viride* recorded maximum inhibition zone of 10.2 mm and it was followed by *T. koningii* (9.70 mm) and *T. harzianum* (9.20 mm) and the later two were statistically on par with each other. The least inhibition zone (7.10 mm) was recorded by *Sacchromyces cerevisiae* (Table 5). *In vitro* studies conducted by Ravikumar *et al.,* (2003) highlighted the efficacy of streptomycin sulphate at 500 ppm in arresting the growth of *X. campestris* pv. *viticola.* Manonmani (2004) demonstrated the effectiveness of streptomycin sulphate at (100 ppm) in controlling citrus canker caused by *X. campestris* pv. *citri.* The monooxygenase gene had a role in the antagonistic activity of *Trichoderma* species against specific fungal plant pathogens and was therefore a potentially important factor in biocontrol by *Trichoderma* species (Carpenter *et al.,* 2008).

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