Bangladesh J. Bot. **52**(4): 965-970, 2023 (December) DOI: https://doi.org/10.3329/bjb.v52i4.70578

EVALUATION OF ANTIBIOTICS AND BIOAGENTS FOR THE MANAGEMENT OF BLACK ROT DISEASE OF CABBAGE AND THEIR EFFECT ON EXTRACELLULAR POLYSACCHARIDE SECRETION

POOJA SANGWAN*, RAKESH SANGWAN, VINOD MALIK* AND MANJEET SINGH

Department of Plant Pathology, CCS Haryana Agricultural University, Hisar-125 004, Haryana

Keywords: Cabbage, Black rot, Antibiotics, Bioagents

Abstract

Black rot disease caused by bacterium *Xanthomonas campestris* pv. *campestris* has been identified as major cause for low cabbage yields. The present study was conducted to evaluate the efficacy of various antibiotics and bioagents for controlling black rot disease and also their effect on extracellular polysaccharide secretion (EPS) of *Xanthomonas campestris* pv. *campestris*. Under *in vitro* conditions, bacterial proliferation and EPS production were examined on yeast peptone agar medium containing sucrose or trehalose with or without antibiotics. Number of *Xanthomonas campestris* pv. *campestris* on yeast peptone agar medium with Validamycin-A was less as compared to control. Extracellular polysaccharide secretion (EPS) was also inhibited on yeast peptone agar medium with antibiotic Validamycin-A. *In vivo*, disease intensity was recorded minimum in seed treatment + root dipping + foliar spray with Validamycin-A @ 500 µg/ml followed by seed treatment with Validamycin-A @ 500 µg/ml in comparison to control. Similarly, 45.61% higher cabbage yield was recorded in seed treatment + root dipping + foliar spray with Validamycin-A @ 500 as compared to untreated control. However, the antibiotic Validamycin-A which was found effective can be recommended to manage the disease.

Introduction

Cabbage (*Brassica oleracea* var. *capitata* L.) belonging to *Brassicaceae* is one of the most important cole crops cultivated worldwide (Hall *et al.* 2002, Arthur 2012).The genus *Brassica* comprises of 40 different species, of which several are annual and biennial foliage and root vegetables. It is a leafy winter vegetable grown for its edible enlarged terminal bud. Among cole crops cultivated in India, it ranks next to cauliflower in acreage and first in terms of production. The cabbage crop draws attention due to its nutritional and antioxidants benefits. The cabbage is also cultivated for industrial production of vegetable oil (Zhao *et al.* 2005, Bong *et al.* 2012, Kim *et al.* 2013, Lee *et al.* 2015). In the cabbage head, substantial amount of protein, carbohydrate, minerals, phosphorus and ascorbic acid are present. Cabbage is an excellent source of Vitamin-C (44%) and other mineral nutrition's, containing more than 20% of the daily value for each of these nutrients per serving (Terefa 2017).

Cabbage crop is affected by many diseases both in the nursery and in the field which reduce the yield as well as quality of the produce. Amongst all diseases, black rot caused by bacterium *Xanthomonas campestris* pv. *campestris* (Pam.) Dowson, is one of the most destructive disease of the cabbage and other cruciferous crops worldwide. The disease occurs in all parts of temperate and subtropical zones of the world where rainfall or heavy dews are plentiful and average temperatures ranged between 25 to 30°C. This disease not only affects the yield but also responsible for post-harvest spoilage. This disease is one of the most widespread and destructive in the world, with an economic impact in many geographical areas (Singh *et al.* 2018). In India, the disease causes considerable yield loss upto 50 to 70 per cent and about 50 per cent disease severity was recorded in cabbage (Vinayak 2012).

*Author for correspondence: <poojasangwan@hau.ac.in>; <vmexcel@rediffmail.com>.

The main route of *Xanthomonas* spp. transmission is *via* contaminated seeds, although weeds and infected plant debris are also potential sources of inoculum (Gitaitis and Walcott 2007). Initially, bacteria grow epiphytically (on leaf surfaces), and then enter into the host through either hydathodes or wounds to spread systemically through the vascular system or through stomata and then finally colonise the mesophyll parenchyma (Ryan *et al.* 2011).

The main symptoms of the disease are yellow, V-shaped, or U-shaped areas extending inward from the leaf margin and black veins in the infected areas. Systemic infections resulted in stunted growth and the death of young plants. The head of infected plants remain small and quality is reduced which makes it unfit for marketing. Secondary infection by other bacterial species can also contribute to further development of severe rotting of vegetative tissues.

Xanthomonas campestris pv. *campestris* produce various virulence factors, including exopolysaccharides, extracellular enzymes, iron-chelating siderophores, and type III secretion-dependent effectors (Ray *et al.* 2000, Jha *et al.* 2007, Liang *et al*. 2016). The production of large amounts of an exopolysaccharide called xanthan is one of the most important virulence factors of this species. Small amounts of xanthan are produced early after the bacteria infect their plant hosts. However, copious amounts of xanthan are produced at later stages of the Xcc infection (Vojnov *et al.* 2001). At early stages xanthan seems to be necessary for the infection of mesophyll tissue and the vascular system.

The management of black rot disease involves the use of resistant varieties; pathogen free seed; crop rotation with non-brassicas; removal of crop residues; and, chemical control but the control is partial (Trench *et al.* 1992, Mishra and Arora 2012, Vicente and Holub 2013). Considering into account the importance of the crop and disease occurrence, the present study was carried out to evaluate the efficacy of various antibiotics and bioagents for controlling black rot disease and also for their effect on Extracellular polysaccharide secretion (EPS).

Material and Methods

Infected leaves showing typical symptoms of black rot disease were collected for isolation of the pathogen. The infected leaves were disinfected with 0.1% mercuric chloride solution and washed repeatedly with sterile distilled water. Small bits of diseased portion were cut with the help of sterilized blade and placed in sterile water drops on sterilized glass slide. In order to obtain bacterial ooze, each bit was incised with sterile blade. A loopful of bacterial suspension was streaked on nutrient agar (NA) plate under aseptic conditions and incubated at 30°C for 48 hrs and observed for colony. Circular, yellow and mucoid colonies were picked and transferred to Petri plates containing NA. These cultures were further purified by streak plate method and maintained on Nutrient agar slants at 4 ± 2°C for further studies.

For the proliferation of bacteria and quantification of extracellular polysaccharide, the experiment was conducted with ten treatments, T_1- Control, *Xcc* grown on Yeast peptone agar medium without antibiotics, T_2- Control, *Xcc* grown on Yeast peptone trehalose agar medium without antibiotics, T_3- Control, *Xcc* grown on Yeast peptone sucrose agar medium without antibiotics T_4- *Xcc* grown on Yeast peptone agar + Validamycin-A @ 10 µg/ml, T_5- *Xcc* grown on Yeast peptone trehalose agar + Validamycin-A @ 10 µg/ml, T_6- *Xcc* grown on Yeast peptone sucrose agar + Validamycin-A @ 10 µg/ml, T_7- *Xcc*grown on Yeast peptone agar + Kasugamycin @ 10 µg/ml, T_8- *Xcc* grown on Yeast peptone trehalose agar + Kasugamycin @ 10 µg/ml, T_9- *Xcc* grown on Yeast peptone sucrose agar + Kasugamycin @ 10 µg/ml, T_{10}- *Xcc* grown on Yeast peptone agar + Agrimycin-100 @ 10 µg/ml, T_{11}- *Xcc* grown on Yeast peptone trehalose agar + Agrimycin-100 @ 10 µg/ml and T_{12} - *Xcc* grown on Yeast peptone sucrose agar + Agrimycin-100 @ 10 µg/ml. The treatments were laid down in Completely Randomized Design with four

replications of each treatment. The bacteria were grown for 5 days at 25 ± 2°C and the colonies were enumerated.Bacteria from all the colonies were washed with 100 ml of sterilized distilled water. The number of bacteria in a suspension was counted by dilution plating method.

For extracellular polysaccharide purification, bacterial colonies from all Petridish with or without antibiotics were washed separately with 100 ml of 0.2 M NaCl containing 5 mM Na_2-EDTA. The suspension was centrifuged at 20000 × g for 20 minutes and the supernatant was collected. Extracellular polysaccharide Secretion (EPS) was precipitated with 3 times the volume of ethanol and rinsed with 70% ethanol and then dissolved in distilled water. The quantity of EPS was estimated using the phenol-sulfuric acid method (Dubois *et al.* 1956) and the value was converted to µg per ml.

The field experiment was conducted at Research Farm of Department of Plant Pathology CCS Haryana Agricultural University Hisar during rabi season of 2019-2020. The experiment was laid out in Randomized Block Design (RBD) with eleven treatments which were replicated thrice. The cabbage variety 'Golden acre' was transplanted in the end of November with a spacing of 60 × 30 cm and plot size was 3 × 2.4 m.

For the preparation of inoculum, the bacterium was grown to mid log phase in 25 ml nutrient broth and culture supernatants were removed by centrifuging at 5000 × g for 10 min. The cell pellets were washed with sterile 0.03 M $MgSO_4$ and then resuspended in phosphate buffer (pH 7.0) and the optical density was adjusted to 1.00 ± 0.02.

One month old seedlings of cabbage were transplanted in the field and after four days, seedlings were inoculated by vein inoculation method. In this method, drops of bacterial suspension was placed on leaves surface at five sites on veinlets and then entomological pins were used to gently prick the veins through bacterial drops

The following treatments of antibiotics and bioagents were imposed to test the efficacy in managing black rot disease. T_1: Seed treatment with Validamycin-A @ 500 µg/ml, T_2: Seed treatment with Kasugamycin @ 500 µg/ml, T_3: Seed treatment with Agrimycin-100 @ 500 µg/ml, T_4: Seed treatment with *Trichoderma viride* @ 10g/l, T_5: Seed treatment with *Pseudomonas fluorescens* @ 5g/l, T_6:Seed treatment + Root dipping +foliar spray with Validamycin-A @ 500 µg/ml, T_7: Seed treatment +Root dipping+foliar spray with Kasugamycin @ 500 µg/ml, T_8: Seed treatment +Root dipping +foliar spray with Agrimycin-100 @ 500 µg/ml, T_9: Seed treatment +Root dipping +foliar spray with *Trichoderma viride* @ 10g/l, T_{10}: Seed treatment +Root dipping +foliar spray with *Pseudomonas fluorescens* @ 5g/l, T_{11}: Control (Spray with plain water). First spray was done at the initial appearance of the disease followed by two more sprays at fifteen days interval. Data were recorded on per cent disease intensity using 0 – 5 scale (Kashyap and Dhiman 2010) and per cent disease control was calculated. Yield data and per cent yield increase over control was also calculated.

For computing per cent disease intensity, 0-5 scale was used as follows: 0 = No disease, 1= Up to 20 per cent leaf area infected, 2= Between 20-40 per cent leaf area infected, 3= Between 40-60 per cent leaf area infected, 4= Between 60-80 per cent leaf area infected, 5= > 80 per cent leaf area infected

Results and Discussion

Biofilm is multilayer matrix-enclosed structure generally tolerant to host defense responses, antibiotics, and environmental stress. It helps in adherence of bacteria, facilitate colonization, and disease progression in the host. Biofilms allow the production of xanthan pigment and EPS that protect the bacteria from photochemical damage. In the present study Yeast peptone agar medium

was prepared containing trehalose or sucrose with or without antibiotics. Bacterial proliferation, EPS production and per cent EPS inhibition over control was calculated.

Under *in vitro c*onditions, bacterial proliferation and EPS production were examined and significant difference was observed between the numbers of bacteria with or without antibiotics. The persual of data presented in Table 1 which revealed that amongst all antibiotics, Validamycin-A was most effective followed by Kasugamycin. The bacterial populations were minimum (1.2×10^6 per ml) on yeast peptone agar medium with Validamycin-A as compared to control (4.1×10^6 per ml). On yeast peptone trehalose agar media with Validamycin-A, the bacterial population was 1.5×10^6 per ml as compared to 3.8×10^6 per ml in yeast peptone trehalose agar media without any antibiotics.

Table 1. Effect of antibiotics on bacterial population and Extracellular Polysaccharide Production by *Xanthomonas campestris* pv. *campestris* (Xcc) causing black rot of cabbage under *in vitro* conditions.

Treatments	Cfu (1×10^6)/ml	Exopolysaccharide (µg/ml)	% EPS Inhibition
T_1	4.1	104.28	-
T_2	3.8	98.99	-
T_3	3.7	94.08	-
T_4	1.2	41.26	60.43
T_5	1.5	50.36	49.12
T_6	1.8	56.01	40.46
T_7	2.0	63.82	38.79
T_8	2.4	80.15	19.03
T_9	2.8	81.91	12.93
T_{10}	3.0	83.79	19.64
T_{11}	3.2	86.41	12.7
T_{12}	3.4	92.99	1.15

The extracellular polysaccharides (EPS) production was weighed on yeast peptone agar medium containing trehalose or sucrose and it was found that Xcc grown on yeast peptone agar without antibiotics and carbohydrates produced 104.28 µg/ml EPS followed by 98.99 µg/ml on yeast peptone trehalose media without any antibiotics (Table 1). On yeast peptone agar media, Validamycin-A reduced EPS about 60.43% over control followed by 49.12% reduction on yeast peptone trehalose media with antibiotic Validamycin-A. Results obtained in the present study are in agreement with the findings of Ishikawa *et al.,* 2004 who reported that the antibiotic Validamycin-A inhibit the production of extracellular polysaccharide (EPS) on yeast-peptone (YP) agar containing carbohydrates. The antibiotic Validamycin - A reduced the number of *Xanthomonas campestris* pv. *campestris* (*Xcc*) in leaves.

Field studies were conducted to evaluate the efficacy of different antibiotics and bioagents to control black rot disease of cabbage. Per cent disease intensity was calculated after each spray. All the antibiotics and bioagents were significantly effective in reducing the disease over control. Amongst all treatments, minimum disease intensity (14.67%) was recorded in treatment T_6 in comparison to control (31.0 %) (Table 2). The next best treatment T_1 followed by T_7 that reduced

the disease up to 47.32% and 36.19% respectively. Higher yield of cabbage (284.47 t/ha) was achieved in treatment T6 followed by T_1 (262.15 t/ha). These results are similar to the findings of Ishikawa *et al.* 2004 who reported that foliar spray of validamycin-A is effective against black rot of cabbage in pot and field trials. Similarly, Bhat *et al.* (2000) found that foliar spray of chlortetracycline and oxytetracycline resulted in least incidence of black rot.

Table 2. Evaluation of effect of antibiotics and bioagents against black rot disease of cabbage.

Treatments	% Disease Intensity					Yield (t/ha)	% Yield increase over control
	After 1st spray	After 2nd spray	After 3rd spray	Mean	% Disease control		
T_1=Seed treatment with Validamycin-A @ 500 µg/ml	9.00	17.00	23.00	16.33	47.32	262.15	40.98
T_2= Seed treatment with Kasugamycin @ 500 µg/ml	13.67	24.00	30.67	22.78	26.51	206.45	25.06
T_3= Seed treatment with Agrimycin-100 @ 500 µg/ml	14.33	25.67	33.00	24.33	21.51	171.81	9.95
T_4= Seed treatment with *Trichoderma viride* @ 10g/l	18.67	27.00	35.67	27.11	12.54	160.28	3.47
T_5= Seed treatment with *Pseudomonas fluorescens* @ 5g/l	19.33	27.33	38.33	28.33	8.61	157.52	1.78
T_6= Seed treatment + Root dipping+foliar spray with Validamycin-A @ 500 µg/ml	6.67	16.00	21.33	14.67	52.67	284.47	45.61
T_7= Seed treatment +Root dipping+foliar spray with Kasugamycin @ 500 µg/ml	10.67	21.33	27.33	19.78	36.19	220.27	29.76
T_8= Seed treatment +Root dipping +foliar spray with Agrimycin-100 @ 500 µg/ml	12.00	23.67	28.67	21.44	30.83	178.72	13.43
T_9= Seed treatment +Root dipping +foliar spray with *Trichoderma viride* @ 10 g/l	14.67	25.33	32.33	24.11	22.22	167.40	7.58
T_{10}= Seed treatment +Root dipping +foliar spray with *Pseudomonas fluorescens* @ 5 g/l	17.33	26.67	34.33	26.11	15.77	162.60	4.85
T_{11}= Control (Spray with plain water)	20.00	30.00	43.00	31.00	-	154.71	-
C.D. at 5%	4.25	2.70	7.27	-	-	36.10	-

References

Arthur GC 2012. Colewort and Cole crops. University of California.

Bhat NA, Masoodi SD and Sidique SH 2000. Chemical control of black rot of cabbage under field conditions in Kashmir valley. Appl. Biol. Res. **2**: 87-89.

Bong YS, Shin WJ, Gautam MK, Jeong YJ, Lee AR, Jang CS, Lim YP, Chung GS and Lee KS 2012. Determining the geographical origin of Chinese cabbages using multi-element composition and strontium isotope ratio analyses. Food Chemis. **135**: 2666-2674.

Dubois M, Gilles KA, Hamilton JK, Rebers PA and Smith F 1956. Ann. Chemis. **28**: 350-356.

Gitaitis R and Walcott R (2007). The epidemiology and management of seedborne bacterial diseases. Ann. Rev. Phytopathol. **45**: 371-97.

Hall JC, Sytsma KJ and Iltis HH 2002. Phylogeny of *Capparaceae* and *Brassicacea*e based on chloroplast sequence data. American J. Bot. **89** (11): 1826-42.

Jha G, Rajeshwari R and Sonti RV 2007. Functional interplay between two *Xanthomonas oryzae* pv. *oryzae* secretion systems in modulating virulence on rice. Molecu. Plant Microbe Interac. **20**: 31-40.

Kashyap PL and Dhiman JS 2010. Eco-friendly strategies to suppress the development of Alternaria and Black rot of cauliflower. Acad. J. Plant Sci. **3**(4): 140-146.

Kim J, Jung Y, Song B, Bong YS, Ryu DH, Lee KS and Hwang GS 2013. Discrimination of cabbage (*Brassica rapa* ssp. *pekinensis*) cultivars grown in different geographical areas using H-NMRbased metabolomics. Food Chem.**137**: 68-75.

Lee J, Izzah NK, Jayakodi M, Perumal S, Joh H J and Lee H J 2015. Genome-wide SNPidentification and QTL mapping for black rot resistance in cabbage. BMC Plant Biol. **15**: 32.

Liang X, Yu X, Pan X, Wu J, Duan Y, Wang J 2016. A thiadiazole reduces the virulence of *Xanthomonas oryzae* pv. *oryzae* by inhibiting the histidine utilization pathway and quorum sensing. Mole. Plant Pathol. **19**: 116-128.

Mishra S and Arora NK (2012). Evaluation of rhizospheric *Pseudomonas* and *Bacillus* as biocontrol tool for *Xanthomonas campestris* pv. *campestris*. *World J. Microbiol Biotechno.* **28**: 693–702.

Ray S K, Rajeshwari R and Sonti RV (2000). Mutants of *Xanthomonas oryzae* pv. *oryzae* deficient in general secretory pathway are virulence deficient and unable to secrete xylanase. Molecu. Plant Microbe Interact. **13**: 394-401.

Ryan RP, Vorholter FJ and Potnis N 2011. Pathogenomics of *Xanthomonas*: understanding bacterium-plant interactions. Natural Rev. Microbiol. **9**: 344-55

Ryo Ishikawa, Mayumi Suzuki-Nishimito, Atsushi Fukuchi and Kazuho, Matsuura. (2004). Effective control of cabbage black rot by Validamycin A and its effect on extracellular polysaccharide production of *Xanthomonas campestris* pv. *campestris*. J. Pesticide Sci. **29**(3): *209-213*.

Singh S, Dey SS, Bhatia R, Batley J. and Kumar R 2018. Molecular breeding for resistance to black rot [*Xanthomonas campestris* pv. *campestris* (Pammel) Dowson] in Brassicas: Recent advances. Euphytica. **214**: 196.

Terefa F 2017. Effect of Irrigation Scheduling and Nitrogen Fertilizer Rates on Growth and Productivity of Cabbage (*Brassica oleracea* L. var. *capitata*) at Buyo Kachama Kebele Seka Woreda Jimma. M.Sc. Thesis. Submitted to School of Graduate Studies, Jimma University College of Agriculture and Veterinary Medicine, Department of Horticulture and Plant Sciences.

Trench TN, Wilkinson DJ and Esterhuysen SP 1992. South African Plant Disease Control Handbook. Farmer Support Group, University of Natal. Strachan (Pvt) Ltd, Pietermaritzburg, South Africa.

Vicente JG and Holub EB 2013. *Xanthomonas campestris*pv. *campestris* (cause of black rot of crucifers) in the genomic era is still a worldwide threat to brassica. *Molec.* Plant Pathol. **14**: 2-18.

Vinayak N 2012. Investigation of cabbage black rot caused by *Xanthomonas campestris*pv. *Campestris* (Pammel). M.Sc. thesis. Department of Plant Pathology, Univ. Agricul. Sci, Dharwad. p. 60.

Vojnov AA, Slater H, Daniels MJ, Dow JM, Centre JI, Lane C, Nr N. 2001. Expression of the gum Operon Directing Xanthan Biosynthesis in *Xanthomonas campestris* and its regulation in planta. 14: 768-774.

Zhao J, Wang X, Deng B, Lou P, Wu J, Sun R, Xu Z, Vromas J, Koorneef M. and Bonnema G 2005. Genetic relationships within *Brassica rapa* as inferred from AFLP fingerprints. Theoreti. Appl. Genet. **110**: 1301-1314.

(Manuscript received on 14 June, 2022; revised on 05 November, 2023)