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Evaluation of different agrochemicals/ antibiotics under *in vitro* against *Pectobacterium carotovorum* subsp. *carotovorum* (*Erwinia carotovora*) causing tip over disease of banana

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Abstract
Bacteria cause significant impacts on bananas globally and management practices are not always well known or adopted by farmers. Overall, the adoption of management practices at the farm and landscape level is considered as the most critical factor to manage bacterial disease causing tip over disease of banana after pathogen incursions are confirmed in a given production area. Hence the success of control strategies, however, strongly relies on the use of agro chemicals/ antibiotics. The *in vitro* studies conducted reveals that among the different agrochemicals, 2-Bromo-2 nitropropane-1, 3-diol (8.33, 8.67, 10.33, 15.67mm) showed highest inhibition at 200, 300, 400 and 500 ppm followed by cristocycline (3.33, 5.33, 7.00, 14.33mm). Among the other agrochemicals of concentrations, copper hydroxide (3.67, 4.00, 7.67, 12.67mm) and copper oxy chloride (3.00, 3.33, 6.33, 10.33mm) recorded highest inhibition at 1000, 1500, 2000 and 2500 ppm.

Keywords: Evaluation, agrochemicals/antibiotics, *Pectobacterium carotovorum,* tip over disease

Introduction
The use of agro chemicals to control plant diseases is an integral component of crop management. Although agrochemicals have been used to good effect in agriculture since the 1940s, the introduction of new agrochemicals is an essential element to provide sustained control of major crop diseases. The need for new and innovative agrochemicals is driven, among other factors, by resistance management, regulatory hurdles, and increasing customer expectations. New agrochemicals can be discovered either within established mode of action groups, ideally with low resistance risk or in areas with completely novel modes of action. Compounds having a novel mode of action are of course of special interest, since they play a key role in resistance management strategies, but equally important are new agrochemicals with enhanced characteristics such as systemicity, curativity, and longevity of disease control. With the background of increasing registration hurdles, increasing costs, and increasing market needs, the current market position of major crop protection agrochemicals needs to be reviewed, along with the consideration of current and future market needs. Hence present investigation was aimed at evaluating commercially available antibiotics to assess their efficacy against the growth of *Pectobacterium carotovorum* subsp. *carotovorum* (formerly *Erwinia carotovora*) under *in vitro* conditions.

Materials and Methods
Antibiotics/ agrochemicals were evaluated under *in vitro* against the growth of bacterium causing tip over or rhizome rot of banana at different concentrations by inhibition zone assay by paper disc method. Following were the list of agrochemicals evaluated with three replications in each treatment as given in table 1.
The bacterium was multiplied by inoculating the culture into 20 ml of nutrient broth taken in 100 ml conical flask. The inoculated flasks were incubated at 30ºC for 48 hours. The bacterial suspension was then seeded to the lukewarm nutrient agar medium (1000 ml). The seeded medium was poured into the sterilized petriplates and plates were allowed to solidify.

The chemicals and antibiotics were prepared at different concentrations as mentioned in the list. The filter paper discs (Whatman No. 42) measuring 5mm in diameter were soaked in the respective chemical solution for 5-10 minutes and transferred onto the surface of the seeded medium in petriplates. The inoculated plates were kept in the refrigerator at 4 ºC for 30 minutes to allow the diffusion of chemicals into the medium. Then plates were incubated at 28±2 ºC for 24 hr and observed for the production of inhibition zone around the filter paper discs. The results obtained were analyzed statistically.

Results and Discussion

In vitro evaluation of chemicals provides preliminary information about the efficacy of particular chemical in the shortest period of time and therefore, it serves as a basis for further field assay. Present investigation was aimed at evaluating commercially available antibiotics to assess their efficacy against the growth of *Pectobacterium carotovorum* subsp. *carotovorum* (formerly *Erwinia carotovora*) under *in vitro* conditions.

The *in-vitro* evaluation of different antibiotics in inhibiting the growth of *Pectobacterium carotovorum subsp. carotovorum* were assessed by paper disc method and results are significantly different which are presented in table 2. Among the different agrochemicals, 2-Bromo-2 nitropropane-1, 3-diol (8.33, 8.67, 10.33, 15.67mm) showed highest inhibition at 200, 300, 400 and 500 ppm followed by cristocycline (3.33, 5.33, 7.00, 14.33mm), Kasugamycin (3.33, 4.33, 5.33, 10.33), plantamycin (3.00, 3.67, 6.33, 11.00mm), streptocycline (2.33, 2.67, 4.67, 8.00mm) and validamycin (0.33, 1.33, 2.67, 4.33, mm). The concentrations of agrochemicals tested were concentrations tested were higher than the previously used chemicals. Among them, copper hydroxide (3.67, 4.00, 7.67, 12.67mm) and copper oxy chloride (3.00, 3.33, 6.33, 10.33mm) recorded highest inhibition at 1000, 1500, 2000 and 2500 ppm respectively. Whereas copper sulphate recorded average values of 2.00, 2.67, 4.33 and 6.00mm respectively. However, carbendazim, mancozeb and untreated control recorded no inhibition zone in all the concentrations tested.

Results indicated that, among all the antibiotics evaluated, streptocycline was found significantly superior over rest of the antibiotics in suppression of the growth of the pathogen at all the three concentrations. The antibiotic streptocycline recorded highest mean inhibition zone of 53.9 mm followed by K-cycline with an average inhibition zone of 49.1 mm. The antibiotic bionol-100 exhibited superior efficacy at the higher concentration of 500 ppm. Significantly poor efficacy was exhibited by bacterinashak. All the antibiotics and their concentrations were significantly effective from lower to higher concentrations with greater efficacy at higher concentrations. The findings of the present investigation are strengthened through the reports of earlier workers. Sensitivity of *E. carotovora* to the antibiotics *viz*., streptomycin, tetracycline, ampicillin and chloramphenical was reported by Farag *et al.* (1984) [3].

Tsuyumu (1982) [14] found that carboxylic acid was a pectinase inhibitor and its compounds tested, inhibited both maceration and disease development by *E. carotovora* sub sp. *carotovora.* Results of the present *in vitro* evaluation of antibiotics are strongly supported by the findings of Thammaiah *et al.* (2005c) [12], that streptocycline (1000 ppm) in combination with copper oxychloride (2000 ppm) had maximum inhibition of *E. chrysanrhami* followed by copper oxychloride alone. Thammaiah *et al.* (2005c) [12] made an *in vitro* evaluation of chemicals against *Erwinia chrysanthemi* by modified paper disc method with seven treatments. The results revealed that the combination of streptocycline 1000 ppm + copper oxychloride 2000 ppm recorded the maximum inhibition of *Erwinia chrysanthemi* (24.00 mm) followed by copper oxychloride 4000 ppm (23.33).

Munnecke (1960) [8] noticed the partial control of *E. chrysanthemi* of *Dieffenbachia* by dipping in agrimycin-100. Miller and McFadden (1961) [7] observed the inhibition of *E. chrysanthemi* by 5, 10 and 30 µg each of aereomycin, chloramphenicol and terramycin and tetracycline. The materials containing streptomycin was more effective in reducing disease incidence followed by agrimycin @ 200 ppm and phytomycin @ 400 ppm. Sakurai *et al.* (1976) [11] found the antibiotic streptomycin 100 µg/ml effective against the bacterium. Chase (1990) [2] studied the bactericide agribrom through an overhead mist system to determine efficacy against the bacterial pathogen and observed that agribrom gave a minimum of 40 per cent control of *Erwinia* blight on *Philodendron selloum* at 55 ppm. Gomez- Caicedo *et al.* (2001) [4] reported the positive reaction for tetracycline and streptomycin and negative for penicillin against *Erwinia* species causing vascular rot and wilt in plantain banana. Hseu *et al.* (2008) [5] found tetracycline as most effective against *E. chysanthemi* at different concentrations.

Nagaraj *et al.* (2002) [9] conducted two field trials to evaluate the efficacy of various bactericides and antibiotics. Three times drenching with streptocycline either alone or in combination with copper sulphate completely suppressed the disease (100%) and increased the yield by 143.37 per cent. In another field trail, norfloxin plus copper sulphate gave very good control of disease (100%) followed by streptocycline plus copper sulphate. Similar results were obtained in a study which reported that sucker dip in COC at 4g/1 for 45 minutes followed by spraying of streptocycline (0.03%) and suckers dip in *P. fluorescens* (1:1) for 45 minutes recorded better growth and lower disease incidence (Anonymous, 2008) [1]. Kannan *et al.* (2006) [6] reported that soil drenching of sodium hypochorite (0.5 g) and streptomycin sulphate (500 ppm) performed well and reduced the rhizome rot incidence.

The results obtained by Nagrale *et al*. (2013) [10] are found similar with the results of the present investigation. The effectiveness of different agrochemicals against *E. chrysanthemi* pv. *paradisiaca* were studied under *in vitro* and *in vivo* conditions. All the test agrochemicals *viz.*, kanamycin, gentamycin, streptomycin, captan, bordeaux mixture, agrimycin, tetracycline, tetracycline + Copper oxychloride, streptocycline, copper oxychloride, mancozeb, kasugamycin and tribasic copper sulphate showed inhibition zone against the bacterium in *in vitro* studies. The higher inhibition zone 23.0-25.7, 22.3-23.7 and 20.3-22.3 mm was noticed for streptocycline (200 ppm) + copper oxychloride (0.1%), tetracycline (200 ppm) and streptocyline (200 ppm) respectively. The next agrochemicals in order of superiority were agrimycin (200 ppm), bordeaux mixture (0.1%) gentamycin (200 ppm), captan (0.2%) and copper oxychloride (0.25%) where the inhibition zone was 14.8-28.2, 13.3-14.0, 12.7-14.7, 12.0- 13.3, 11.3-14.7 and 10.7-12.3 mm respectively. The agrochemicals *viz*., kasugamycin (200 ppm) kanamycin (200 ppm), mancozeb (0.2%) and tribasic copper sulphate (0.15%) showed inhibition zone of 8.7-10.0, 5.0-7.0, 5.0-7.3 and 4.3-5.0 mm respectively. These agrochemicals were further evaluated under *in vivo* condition in glass house experiment against the collar rot bacterium. The higher concentrations of these agrochemicals were prepared as these were used for rhizome treatment and as soil drench. These agrochemicals were drenched individually in the pot soil upto soil saturation point where previously bacterial inoculated and agrochemical treated rhizomes were planted and the observation on appearance of disease symptoms were noted. The agrochemicals Bordeaux mixture (0.6%), tetracycline (1000 ppm), streptocycline + copper oxychloride (1000 ppm + 0.1%) and streptocycline (1000 ppm) exhibited 100 per cent control of the disease over control. Other chemicals were not effective in controlling the disease although the period required for appearance of the disease and its development was delayed.

Rhizome treated with agrimycin (1000 ppm) and copper oxychloride (0.6%) took 43 and 40 days respectively for appearance of the disease and collapse of the plant whereas captan (0.3%), kasugamycin (1000 ppm), streptomycin (1000 ppm), gentamycin (1000 ppm), kanamycin (1000 ppm), tribasic copper sulphate (0.6%) and mancozeb (0.6%) treated rhizome took 41, 39, 37, 35, 28, 23 and 22 days respectively for the appearance of the symptoms and collapse of the plant. These results indicated that the disease can be controlled effectively by treating banana rhizomes in Bordeaux mixture (0.6%) or tetracycline (1000 ppm) or streptocycline (1000 ppm) before plantation.

Thiyagarajan (2016) [13] conducted an experiment based on the performance of antibiotics, antibacterial chemicals and bio agents obtained through *in vitro* evaluation. Field trial was conducted with antibiotics, antibacterial chemicals and biocontrol agents to ascertain their efficacy against the disease. The performance of the treatments revealed that streptocycline (500 ppm) in combination with copper oxy chloride (2000 ppm) exhibited 100 per cent efficacy in controlling the disease, where all the treated plants were recovered from the infection by the pathogen. Similarly, bionol-100 in combination with copper oxychloride performed as next best effective with 83.33 per cent reduction in disease over control.

Table 1: List of agrochemicals used for *in-vitro* evaluation against *Pectobacterium carotovorum* subsp. *carotovorum* (*Erwinia carotovora*)

Sl. No.	Chemicals/ Antibiotics used	Chemical composition	Name of the manufacturer	Concentrations in ppm			
1	Bactinash – 200	2-Bromo – 2 nitropropane-1,3-diol 95% w/w	Multiplex Agricare Pvt. Ltd.	200	300	400	500
2	Bactrinashak (Immuno modulator)	2-Bromo – 2 nitropropane-1,3-diol (Immuno modulator) 25%w/w	Indofil industries Ltd	200	300	400	500
3	Omycin™	Kasugamycin 3% S.L	M/S Biostadt India	200	300	400	500
4	Plantomycin®	(Streptomycin Sulphate 9% + Tetracycline Hydrochloride 1%)	Aries agro limited	200	300	400	500
5	Valida	Validamycin 3% L	Sumitomo chemical india pvt. Ltd	200	300	400	500
6	Cristocycline	(Streptomycin Sulphate + Tetracycline Hydrochloride) 9:1 SP	Insecticide	200	300	400	500
7	Hycarb®	Carbendazim 50% W.P	Hyderabad chemical Ltd.	1000	1500	2000	2500
8	Kocide® 101	Copper hydroxide 77% W.P	E.I. DuPont de Nemours & Company	1000	1500	2000	2500
9	Blitox®	Copper oxy chloride 50% W.P	RALLIS A TATA Enterprise	1000	1500	2000	2500
10	Indofil M-45®	Mancozeb 75% W.P	Indofil industries Ltd.	1000	1500	2000	2500
11	Copper sulphate	Copper sulphate	-	1000	1500	2000	2500

Table 2: Effect of Agrochemicals against *Pectobacterium carotovorum* subsp. *carotovorum* (*Erwinia carotovora*) under *in vitro* condition

Treatments	Agrochemicals/ antibiotics	Mean diameter zone of inhibition (mm)				Average
		200 ppm	300 ppm	400 ppm	500 ppm	
T_1	Bactinash-200	8.33 (3.05)	8.67 (3.10)	10.33 (3.36)	15.67 (4.08)	10.75 (3.39)
T_2	Cristocycline	3.33 (2.06)	5.33 (2.51)	7.00 (2.82)	14.33 (3.91)	7.49 (2.82)
T_3	Kasugamycin	3.33 (2.06)	4.33 (2.30)	5.33 (2.50)	10.33 (3.36)	5.83 (2.55)
T_4	Plantamycin	3.00 (2.00)	3.67 (2.15)	6.33 (2.70)	11.00 (3.46)	6.00 (2.57)
T_5	Streptocycline	2.33 (1.82)	2.67 (1.91)	4.67 (2.37)	8.00 (3.00)	4.41 (2.27)
T_6	Validamycin	0.33 (1.13)	1.33 (1.52)	2.67 (1.91)	4.33 (2.30)	2.16 (1.71)
T_7	Control	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
SEm±		0.11	0.07	0.09	0.06	-
CD (0.01)		0.34	0.22	0.28	0.20	-
Agrochemicals		**1000 ppm**	**1500 ppm**	**2000 ppm**	**2500 ppm**	**Average**
T_1	Carbendazim	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
T_2	Copper hydroxide	3.67 (2.15)	4.00 (2.23)	7.67 (2.94)	12.67 (3.69)	7.00 (2.75)
T_3	Copper oxychloride	3.00 (1.98)	3.33 (2.07)	6.33 (2.70)	10.33 (3.36)	5.74 (2.52)
T_4	Copper sulphate	2.00 (1.73)	2.67 (1.90)	4.33 (2.30)	6.00 (2.62)	3.75 (2.13)
T_5	Mancozeb	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
T_6	Control	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
SEm±		0.06	0.07	0.04	0.09	-
CD (0.01)		0.21	0.23	0.14	0.30	-

Note: Figures in parentheses indicate Square root transformed values

References

1. Anonymous. Etiology and management of tip over or rhizome rot disease of banana. Res. Rep. for Group Discussion, TNAU, Coimbatore, March. 2008, 9-12, 364.
2. Chase AR. Control of some bacterial diseases of ornamentals with Agribrom. Proc. Fla. State Hort. Soc. 1990; 103:192-193.

3. Farag NS, Lashin SM, Abdel-All RS, Shatiz HM, Seif-Elyazal AM. Antibiotics and control of potato black leg and brown rot diseases. Agric. Res. Rev. 1984; 60:149-166.
4. Gomez-Caicedo LE, Echeverry E, Gonzalez S. Evaluation of cultural, chemical and biological control of vascular rot and wilt in plantain (*Musa* AAB Simmonds). *Infomusa*. 2001; 10:17-21.
5. Hseu SH, Kuo KC, Lin CY. Bacterial stalk rot of sorghum occurred in Kimmen area caused by *Erwinia chrysanthemi*. *Pl. Pathol*. 2008; 17:257-262.
6. Kannan R, Uma Sankareswari C, Gopalakrishnan, Balamohan TN. Management of *Erwinia* rot in banana. Abstracts published In: Nation. Semi. on Int. Prod. and Post-Harvest Mgmt. Tropical Fruits. 2006, 11-12, 59.
7. Miller HN, McFadden LA. A bacterial disease of philodendron. Phytopathol. 1961; 51:826-831.
8. Munnecke DE. Bacterial stem rot of *Dieffenbachia*. *Phytopathol.* 1960; 50:697-700.
9. Nagaraj MS, Khan ANA, Ravikumar MR, Amarnanjundeswara H. Management of tip over disease of banana. Global Conference on Banana and Plantain, Bangalore, India. 2002, 28-31.
10. Nagrale DT, Suresh NGB, Shailesh P, Gawande Asit KM, Satish AR. Characterization of a bacterial collar and rhizome rot of banana (*Musa paradisiaca*) caused by strains of Erwinia chrysanthemi pv. paradisiaca. J Appl. Nat. Sci. 2013; 5(2):435-441.
11. Sakurai H, Naito H, Fujita S. Sensitivity distribution of phytopathogenic bacteria and fungi to antibiotics. J Antibiotics. 1976; 29(11):1230-1236.
12. Thammaiah N, Kanamadi VC, Shirol AM, Gangadharappa PM, Kulkarni MS. Incidence of bacterial rhizome rot of banana in northern Karntataka and *in-vitro* evaluation of chemicals, antibiotics, and plant extracts against *Erwinia chrysanthemi* causing rhizome rot or tip over disease of banana. National Seminar on Integrated production and post-harvest management of Tropical Fruits held at Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, 2005c, 58.
13. Thiyagarajan V. Investigations on tip over disease of banana caused by *Erwinia carotovora* sub sp. *carotovora* (Jones) Holland. M. Sc. Thesis submitted to College of Agriculture, Raichur, Uni. Hortl. Sci., Raichur., 2016.
14. Tsuyumu S. Non-toxic chemical control of soft rot disease, In: Proceedings of the Fifth International Conference and Plant Pathogenic Bacteria, Columbia. Centro International de Agricultura Trophical. 1982; 28:640.