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Efficacy of biocontrol agents and bactericides for the management of bacterial blight incited by *Xanthomonas axonopodis* pv. *dieffenbachiae* in *Anthurium andraeanum*

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ABSTRACT

Bacterial blight of *Anthurium* incited by *Xanthomonas axonopodis* pv. *dieffenbachiae* (XAD) is one of the most serious and devastating disease causes severe losses to cut flower production. *In vitro* screening of antagonistic *B. mojavensis* strain KA3 inhibited the growth of *X. axonopodis* pv. *dieffenbachiae* over an area of 3730 mm^2. It was followed by *B. subtilis* isolate BSD4, which inhibited the pathogenic bacteria to an extent of 3430 mm^2. *In vitro* screening with bactericides and fungicides against *X. axonopodis* pv. *dieffenbachiae* reflected that streptomycin sulphate was most effective in inhibiting the bacterial blight pathogen at 2000 ppm which confers an area of inhibition of 1810 mm^2, which was significantly superior over all other treatments and succeeded by 1000 ppm of streptomycin sulphate, which recorded 1254 mm^2 area of inhibition against XAD. Screening with gentamycin, indicated that the mean maximum area of inhibition of the bacterial pathogen XAD was 1054 mm^2 at 2000 ppm against XAD under *in vitro*. However, comparison of the efficacy between streptomycin sulphate and gentamycin, indicated that, streptomycin sulphate was highly effective rather than gentamycin. Similarly, fungicides such as copper oxychloride, alliete, isotianil and bromopol (2-bromo 2-nitro propane 1, 3-diol) which had antibacterial activity were tested against XAD under *in vitro*.

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INTRODUCTION

Anthurium (*Anthurium andraeanum*) is the second largest tropical cut flower crop cultivated throughout the tropics as well as in temperate regions. It is an excellent flower crop commercial cultivated throughout the world. In India it is grown in Wyanad, Idukki and Nelliyampathy of Palghat districts in Kerala, Nilgiris, Yercaud, Thovazhai and Palani Hills in Tamil Nadu, North-Eastern States

and Bastar district in Madhya Pradesh, Madikeri of Coorg district in Karnataka, Goa and Maharashtra. In recent years, anthurium is grown in more than 50 hectares of poly houses in India (Nair, 2004).The flowers of anthurium are popular among flower arrangers because of their attractive colours, increased vase life, bold effect and long lasting qualities (Bhatt and Desai, 1989). Anthurium produces cut flowers throughout the year which predisposes to bacterial blight during cool climate.

The anthurium cut flower production is hampered by numerous diseases. Among this bacterial blight incited by *Xanthomonas axonopodis* pv. *dieffenbachiae* (McCulloch and Pirone) is one of the most serious and devastating disease which directly causes considerable yield losses to the cut flower production. It was first reported from Brazil in 1960 (Nishijima, 1988) and is prevalent in almost all anthurium growing regions of the world. The management of anthurium bacterial blight is the major challenge to minimize the yield losses. However, the potential use of bactericides and biocontrol agents with combination in an integrated manner needs to be exploited for the management of anthurium blight.

MATERIAL AND METHODS

Collection and isolation of pathogen :

Samples of typical bacterial leaf blight symptoms on *Anthurium* leaves were collected from different *Anthurium* growing regions of Tamil Nadu, India during 2013-14. The infected portion were cut into small pieces and surface sterilized by dipping in 0.1 per cent $HgCl_2$ for 1 min, and rinsed three times with sterile distilled water and transferred onto the surface of Nutrient Agar medium. The pure cultures of the *Xanthomonas axonopodis* pv. *dieffenbachiae* were obtained by single colony isolation and purification of bacteria.

***In vitro* screening of antagonistic *Bacillus* spp. against *X. axonopodis* pv. *dieffenbachiae* :**

The antibacterial activities of different *Bacillus* spp. were tested against *Xad* by dual culture technique using Nutrient Agar medium. The bacterial antagonists *Bacillus* spp. were collected from, Trichoderma lab, Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore. The bacterial antagonists were inoculated in nutrient broth and incubated for 24hrs. Nutrient agar was poured in to the sterile Petri plates and allowed to solidify. A well (5 mm in diameter) was made by punching the nutrient agar with a sterile cork borer on the corner of the plate in four places by leaving a distance of 1cm from the periphery of the plates. Bacterial antagonists were poured into the wells at the rate of 20 µl per well and incubated for 24 h at 28±2°C. Each treatment was replicated thrice. The Nutrient Agar plates without antagonist served as a control. The one ml of 72h old *X. axonopodis* pv *dieffenbachiae* maintained in nutrient broth consisting of 10^6 cells/ml was added into nutrient agar medium. The Nutrient media seeded with the pathogen were poured over the plate's which was pre-inoculated with the antagonistic *Bacillus* spp. The efficacy of the bacterial antagonists against the *Xad* was assessed by measuring the area of inhibition zone in mm^2.

***In vitro* screening of bactericides and fungicides against *X. axonopodis* pv. *dieffenbachiae* :**

Seventy two hours old bacterial pathogen *X. axonopodis* pv. *dieffenbachiae* (10^6 cells/ml) maintained in nutrient broth was added into nutrient agar at the rate of 1ml/100ml of the Nutrient agar medium. Nutrient agar was poured in to the sterile Petri plates and allowed to solidify. A well (5 mm in diameter) was made by punching the nutrient agar with a sterile cork borer on the corner of the plate in four directions by leaving a distance of 1cm from the periphery of the plates. Each well was poured with 50µl of various chemicals with different concentrations *viz.*, 50 ppm, 100 ppm, 250 ppm, 500 ppm, 750 ppm, 1000 ppm, 1500 ppm and 2000 ppm. The efficacy of the product was assessed by measuring the area of inhibition zone (mm^2) after 48h of incubation at 28±2°C.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

***In vitro* screening of antagonistic *Bacillus* spp. against *X. axonopodis* pv. *dieffenbachiae* :**

The antibacterial activity of different *Bacillus* spp. such as *B. subtilis* isolate BS2, *B. subtilis* isolate BS7, *B. mojavensis* isolate KA3, *B. amyloliquefaciens* isolate VB7, *B. subtilis* isolate BSD4, *Ochrobactrum* sp isolate BSD5, *B. cereus* isolate BSC4, *B. subtilis* isolate B2, *B. amyloliquefaciens* isolate B5, *B. amyloliquefaciens* isolate KA1 and *B. cereus* isolate BSC11 were screened

against *X. axonopodis* pv. *dieffenbachiae in vitro*. Among the various antagonistic *Bacillus* spp., screened the antagonistic bacteria *B. mojavensis* isolate KA3 inhibited the growth of *X. axonopodis* pv. *dieffenbachiae* over an area of 3730 mm^2. It was followed by *B. subtilis* isolate BSD4, which inhibited the pathogenic bacteria to an extent of 3430 mm^2 (Table 1).

In vitro screening of bactericides and fungicides against *X. axonopodis* pv. *dieffenbachiae* :

Bactericides and fungicides were screened for the antibactericidal activity against *X. axonopodis* pv. *dieffenbachiae* under *in vitro*. The results indicated that, streptomycin sulphate inhibited *X. axonopodis* pv. *dieffenbachiae* at 250 ppm, 500 ppm, 750 ppm, 1000 ppm and 2000 ppm. However, the maximum area of inhibition was noticed at 2000 ppm in the Nutrient agar media seeded with XAD. The area of inhibition was 1810mm^2 which was significantly superior to all the other treatments in the study. It was succeeded by 1000 ppm of streptomycin sulphate, which recorded 1254 mm^2 area of inhibition of XAD1.

Likewise, screening with gentamycin, the broad spectrum antibacterial antibiotic, indicated that the mean maximum area of inhibition of the bacterial pathogen XAD1 was 1054 mm^2 at 2000 ppm against XAD under *in vitro*. However, comparison of the efficacy between streptomycin sulphate and gentamycin, indicated that, streptomycin sulphate was highly effective rather than

Table 1 : Antibacterial activity of *Bacillus* spp., against *X. axonopodis* pv. *dieffenbachiae*

Sr. No.	Isolates of *Bacillus* spp.	Mean area of inhibition (mm^2)
1.	*Ochrobactrum*sp (BSD5)	3320^{c} (57.62)
2.	*B. cereus* (BSC11)	2600^{g} (50.99)
3.	*B. subtilis* (BSD4)	3430^{b} (58.57)
4.	*B. cereus* (BSC4)	2360^{h} (48.58)
5.	*B. mojavensis* (KA3)	3730^{a} (61.07)
6.	*B. subtilis* (B2)	440^{k} (20.99)
7.	*B. amyloliquefaciens* (B5)	1070^{j} (32.72)
8.	*B. amyloliquefaciens* (VB7)	3080^{d} (55.50)
9.	*B. amyloliquefaciens* (KA1)	2330^{i} (48.27)
10.	*B. subtilis* (BS7)	2840^{f} (53.30)
11.	*B. subtilis* (BS2)	2970^{e} (54.50)
12.	Control	0.00^{l} (0.70)

*Values are mean of three replications;
Values in parentheses are square root transformed values In a column, means followed by a common letter are not significantly different at the 5 per cent levels by DMRT.

Table 2 : *In vitro* screening of bactericides and fungicides against *X. axonopodis* pv. *dieffenbachiae*

Sr. No.	Different concentration of bactericides and fungicides	Area of inhibition zone in mm^2					
		Streptomycin sulphate	Gentamycin	Copper oxy chloride 50% WP	Fosetylaluminium 50% WP	Isotianil 200 SC	Bromopol
1.	50 ppm	537^{h} (23.18)	410^{h} (20.26)	0.0	0.0	0.0	0.0
2.	100 ppm	640^{g} (25.30)	530^{g} (23.03)	0.0	0.0	0.0	0.0
3.	150 ppm	790^{f} (28.12)	590^{f} (24.30)	0.0	0.0	0.0	0.0
4.	250 ppm	950^{e} (1.20)	680^{e} (26.08)	0.0	0.0	0.0	0.0
5.	500 ppm	1060^{d} (30.83)	757^{d} (27.52)	0.0	0.0	0.0	0.0
6.	750 ppm	1240^{c} (35.22)	957^{c} (30.94)	0.0	0.0	0.0	0.0
7.	1000 ppm	1460^{b} (38.21)	1030^{b} (32.10)	0.0	0.0	0.0	0.0
8.	2000 ppm	1810^{a} (42.55)	1254^{a} (35.41)	0.0	0.0	0.0	0.0
9.	Sterile water control	0.0^{i} (0.70)	0.0^{i} (0.70)	0.0	0.0	0.0	0.0

*Values are mean of three replications
Values in parentheses are square root transformed values, In a column, means followed by a common letter are not significantly different at the 5 per cent levels by DMRT

gentamycin. Smilarly, fungicides such as copper oxy chloride, alliete, isotianil and bromopol (2-bromo 2-nitro propane 1, 3-diol) which had antibacterial activity were tested against XAD1 under *in vitro*. Though several reports explain that, these fungicides have antibacterial activity, but in the present study they were not effective against XAD1 under *in vitro* (Table 2).

Bacillus spp., were found to produce array of anti microbial peptides and were found effective against various plant pathogens including fungi, bacteria and virus. Based on the potential of *Bacillus* spp., it was screened against *Xanthomonas campestris* and was found effective due to the production of antimicrobial and haemolytic activity of the antagonist (Issazadeh *et al*., 2012). The crude culture filterate of *B. amyloliquefacines*, *B. subtilis* and *B. pumilis* inhibited *X. campestris* pv. *campestris* due to the production of various antimicrobial compounds (Wulff *et al*., 2002). Monteiro *et al*. (2005) reported that *Bacillus* spp. had both antimicrobial and haemolytic activity against *X. campestris pv. campestris* the casual agent of crucifers black rot. Similarly in the present study, various species of *Bacillus* spp., like *B. mojavensis*, *B. subtilis*, *B. cereus* and *B. amyloliquefaciens* were effective against *X. axonopodis* pv. *dieffenbachiae* due to the production of antimicrobial compounds such as iturin, fengycin, surfactin and bacillomycin. This in turn might be responsible for the maximum suppression of the pathogen growth under *in vitro.*

Arturmikicinski *et al*. (2012) reported that the antibacterial activity of the fungicides was evaluated against *Erwinia amylovora*, *X. arboricola* pv. *corylina*, *X. arboricola* pv. *juglandis*, *Pseudomonas syringae* pv. *syringae*, *Agrobacterium tumefaciens*. Among the tested fungicides, metalaxyl-M with mancozeb, Mancozeb alone, and copperoxychloride inhibited all of the tested strains of pathogenic bacteria. Moreover, a study on the control of walnut bacterial blight also showed the significant efficacy of Manex a preparation based on mancozeb and copper. It was found more effective than copper alone (Buchner *et al.*, 2001). Fosetyl aluminium was not effective against *X. axonopodis*pv. *dieffenbachiae* infecting syngonium (Matheron and Matejka, 1991; Utkhede and Smith, 1991).

The present study revealed that fosetyl aluminium, copper oxy chloride, and isotianil were not effective in inhibiting the growth of bacterial blight pathogen infecting anthurium. Maximum inhibition of pathogen was observed in streptomycin sulphate and gentamycin treated agar plate under *in vitro*. Maher *et al*. (2005) reported that streptomycin sulphate, agrimycin-100, vitavax, dithane M -45 and benlate at 0.2 per cent concentration were sprayed on the field grown citrus plants and then inoculated with *X. campestris* pv. *citri* to assess the efficacy towards the control of citrus canker. Chakravarti and Hedge (1970) and Vibhute and Wadge (1975) achieved best controlof citrus canker with agrimycin-100 at the rate of 1000 ppm concentration. Krishna and Nema (1983) reported that streptomycin was effective at 500ppm with 4th spray schedule against citrus canker. Thirumalesh *et al*. (2011) reported that bacterial spot of mango caused by *X. axonopodis* pv. *mangiferaeindica* was reduced by spraying of various combination of ciprofloxacin + copper sulphate; ciprofloxacin + bactrinashak; ciprofloxacin + copper oxychloride, ciprofloxacin + tetracycline and tetracycline + bactrinashak and also gentamycin lead to reduction of disease.

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