

Journal of Biological Control, 39(2): 174-180, 2025, DOI: 10.18311/jbc/2025/49689

Research Article

Management of bacterial blight of Taro using microbe-based bio-pesticides

STAR LUIKHAM[1*], LOHIT C. BORA[1], SHERIFUL ALAM[2], JAPANI CHINIR[3], CHUMKI DUTTA[4], D. SHEPHROU HELENA[3], K. S. SABASTIAN[3] and SOLEI LUIRAM[5]

[1]*Department of Plant Pathology,* [2]*Department of Horticulture, Assam Agricultural University, Jorhat – 785013, Assam, India*
[3]*Feeds Group of Institutions, College of Agricultural Sciences, Hengbung – 795106, Manipur, India*
[4]*Assam Downtown University, Guwahati – 781026, Assam, India*
[5] *ICAR Krishi Vigyan Kendra, Ukhrul- 795142, Manipur, India*
**Corresponding author E-mail:* starluikham@gmail.com

ABSTRACT: Bacterial blight of Taro caused by *Xanthomonas axonopodis* pv. *dieffenbachia*, a quarantine pathogen, is a serious biotic stress causing damage and loss to Taro production. Contemplating the importance of the disease, four different microbe-based bio-formulations (Biofor pf-2, Biozin-ptb, Biotime, and Biogreen-5) were investigated for bio-intensive management of bacterial blight in two susceptible cultivars of Taro, *viz. Colocasia* cv. *Piyaji kochu and Colocasia* cv. *SC-1* under shade net conditions. Biotime; Biozin-ptb; Biogreen-5 @5% and Streptocycline (Streptomycin Sulphate 90% w/w and Tetracycline Hydrochloride 10% w/w) as a chemical check @100 ppm were foliar sprayed as per recommendation. Biofor pf-2 as soil treatment was applied @100 g/plant. Corm treatment was done by dipping the corm overnight with the pastes of various bio-formulations. All the treatments resulting from the combination of corm treatment, spraying, and/or soil treatment could significantly reduce the intensity of bacterial blight, increase the corm yield and other growth parameters as compared to the untreated control. The lowest disease severity (15.63%) and Area under disease progress curve (AUDPC) of 1228.13 coupled with the highest yield of 1194.47 g/plant along with other growth parameters was recorded in *Colocasia* cv. *Piyaji kochu* when treated with Biogreen-5@5%. Similarly, in *Colocasia* cv. *SC-1*, the highest yield of 1039.06 g/plant along with the lowest disease severity (16.56%) and AUDPC (1387.50) could be observed with Biogreen-5@5% treated plants.

KEYWORDS: Bacterial blight, biocontrol, consortia bio-formulation, Taro, *Xanthomonas axonopodis* pv. *dieffenbachia*

(Article chronicle: Received: 03-05-2025; Revised:27-06-2025; Accepted: 29-06-2025)

INTRODUCTION

Taro (*Colocasia esculenta* L. Schott), known as Cocoyam or Colocasia, is an important tropical and subtropical tuber crop. In terms of world production and cultivated area, Taro ranks fifth amongst tuber and root crops cultivated in the world's total area of around 2.48 million ha, with a total production of 17.72 million tons (FAOSTAT, 2022). Taro can survive in varied agro-climatic regions and saline soil because of its versatile nature (Grubben & Denton, 2004). Taro is being utilised and consumed in different ways, as it is rich in vitamins, minerals, carbohydrates, mainly starch (Huang *et al*., 2000) and also has many medicinal properties (Babu *et al*., 2010).

Xanthomonas axonopodis pv. *dieffenbachia* (Xad), the causal organism of bacterial leaf blight of Taro (Asthana, 1946; Tomlinson, 1987; Opara *et al*., 2013), is one of the main biotic stresses that causes a widespread reduction in the quality and yield of the crop. It was first reported in India (Asthana, 1946) and is considered a quarantine pest (EPPO, 2009). At an early stage, the symptoms appeared to be small water-soaked spots with a yellow halo, which subsequently became uneven brown, necrotic, yellow margins and eventually destroyed the whole plant when the systemic infections took place (Constantin *et al*., 2017). Heavy rain coupled with an average temperature of 20-30°C helps in the direct entry of bacteria or through stomata that remained dormant in the cormels (Brown & Asemota, 2009). The bacteria spread through detached infected leaves, running water, and infested equipment or implements (Paulraj, 1993).

Control of Taro bacterial blight is challenging and cumbersome because of its systemic infection, chances of resistant development to the antibiotics. Management of the disease has been advocated with various chemicals, plant extracts, and bio-control agents. So, the present investigation has been explored with the consortia of some bacterial and fungal biocontrol agents.

MATERIALS AND METHODS

In vivo management of bacterial blight of Taro using microbial biopesticides

The experiment was carried out in a green/ shade net house, Department of Plant Pathology, Assam Agricultural University (AAU), Jorhat, India, during 2018-2019. The experimental pots were arranged in a Completely Randomised Design (CRD) with 4 replications. In each replication, 10 numbers of plants were taken for the experiment. Two susceptible germplasms of Taro, *viz. Colocasia* cv. *Piyaji kochu and Colocasia* cv. *SC-1* (Luikham *et al*., 2024) were selected for the pot experiment to evaluate the antagonistic efficacy of different bio-control agents against the bacterial blight of Colocasia. Streptocycline (Streptomycin Sulphate 90% w/w and Tetracycline Hydrochloride 10% w/w) @ 100 ppm were applied as a foliar spray for comparison, along with absolute control. Biopesticide products procured from the biocontrol unit, AAU, Jorhat, used for the pot experiments were Biofor pf-2 (*Pseudomonas fluorescens* + *Trichoderma viride*); Biozin-ptb (talc-based) (*P. fluorescens* + *T. viride*); Biotime (*P. fluorescens* + *T. viride* + *Metarhizium anisopliae*) and Biogreen-5 (*P. fluorescens* + *T. viride* + *M. anisopliae* + *Bacillus thuringiensis*+ *Beauveria bassiana*).

Colocasia was sown in an earthen pot @ one corm per pot, following all the recommended agronomic practices. Corms were treated separately with different biopesticides along with Streptocycline. Application methods and doses were made as per recommendations. For corm treatment, pastes of Biofor pf-2 @ 150g/kg of corm; Biotime, Biozin-ptb and Biogreen-5 @ 5%; Streptocycline @100 ppm were prepared. Corms were washed properly with clean water and dipped overnight in the paste solution. The coated corms were then removed and spread in a cool and dry place (under shade overnight) before sowing. Soil treatment of Biofor pf-2 @ 100 g/plant was done five times at monthly intervals. Various bio-consortia, *viz*. Biozin-ptb, Biotime and Biogreen-5 @ 5% were sprayed at an interval of 15 days for 6 sprays, followed by two sprays with 30 30-day interval. Streptomycin @ 100 ppm was sprayed 5 times at 30-day intervals. The soil near the base of the plant was well-drenched while spraying.

Per cent Disease Incidence (PDI) was calculated following the formula:

$$PDI = \frac{Number\ of\ plants\ \text{inf}ected}{Total\ number\ of\ plants\ sampled} \times 100$$

Per cent Disease Severity (PDS) was evaluated using the formula given by Wheeler (1969) as

$$PDS = \frac{Sum\ of\ individual\ disease\ scores}{Total\ number\ of\ leaves\ observed \times Maximum\ disease\ score} \times 100$$

Per cent Disease Reduction (PDR) was calculated by the formula as,

$$PDR = \frac{Percent\ disease\ severity\ of\ control - Percent\ disease\ severity\ of\ respective\ treatments}{Percent\ disease\ severity\ of\ control} \times 100$$

The Area Under the Disease Progress Curve (AUDPC) was calculated from the pooled mean of the disease severity according to the formula given by Shaner and Finney (1977).

$$AUDPC = \sum_{i=1}^{n-1} [\{(Y_i + Y_{i+1})/2\} \times (t_{(i+1)} - t_i)]$$

where Y_i = mean disease severity on the i^{th} day, t_i = time (days after planting) on which Y_i was recorded, and n = total number of observations.

The scores of disease severity assessment were based on the first five youngest open leaves. Yield attributing characters were also recorded starting from the second month until 180 days after planting at an interval of 30 days. The number and weight of corms and cormels/plants were recorded at the time of harvesting.

A Completely Randomised Design (CRD) was used for the statistical analysis (Snedecor & Cochran, 1967) to analyse the experiment data by employing Fisher's method of Analysis Of Variance (ANOVA). The data obtained for both years were pooled together since the mean differences were non-significant. To compare the different treatments, critical differences were calculated. The significance of variance among the data was calculated by calculating 'F' values and comparing them with the appropriate values of 'F' at a 5 per cent probability level. The data on per cent values were transformed into corresponding angular values or log values wherever necessary.

RESULTS

Effect on Taro bacterial blight intensity

The effect of bacterial blight intensity with the application of various microbial consortia and an antibiotic on *Colocasia* cv. *Piyaji kochu* is presented in Table 1. The data revealed that all the treatments could reduce the disease severity significantly over the control. The disease symptoms could be observed in all the experimented pots, with the highest disease control in the Biogreen-5 application (71.42%), followed by the Biotime-treated plants (59.99%). The highest per cent disease incidence and disease severity could be seen in untreated plants, i.e., absolute control (64.59% and 54.69%, respectively), while the lowest was

observed in the treatment with Biogreen-5 (37.50% and 15.63%, respectively). AUDPC was calculated from the disease severity per cent, where the lowest was observed in Biogreen-5 treated plants (1228.13), followed by Biotime (1828.13) and the highest in untreated plants (4514.06).

Similarly, disease intensity on *Colocasia* cv. *SC-1,* shown in Table 2, revealed that all the treatments could reduce the disease significantly over the control within the range of 19.08% (Biozin-ptb) to 72.69% (Biogreen-5). Plants treated with Biogreen-5 showed the least per cent disease incidence and disease severity of 41.67% and 16.56%, respectively, followed by Biotime-treated plants (45.83% and 20.63%). The lowest AUDPC was also observed in the treatment with Biogreen-5 (1387.5), and the highest in the control (4950).

Effect on growth parameters and yield

Colocasia cv. *Piyaji kochu* treated with various microbe-based formulations and an antibiotic were statistically significant in plant growth characteristics and yield (Table 3). Plant height could be seen varying from 80.38-99.63 cm, with the least in absolute control and highest in Biogreen-5 treated plants. Similarly, the Biogreen-5-treated plants gave a higher number of leaves (3.75), leaf length (42.75 cm), and leaf width (35.50 cm). The number of corms varied from 1-1.38, with the highest for Biogreen-5 and Biotime-treated plants. The weight of corms (g/plant) varied from 519.38-698.22 g and was found to be highest for Biogreen-5 treated plants. Similarly, the highest number (36.13) and weight of cormels (496.25 g) were recorded with Biogreen-5-treated plants. Yield (g/plant) varied from 931.63 (control) to 1194.47 g (Biogreen-5 @ 5%).

Similarly, plant growth characteristics and yield for *Colocasia* cv. *SC1* was statistically significant among all the treatments (Table 4). Plant height varied from 78.38 to 89.13 cm, with the highest in Biogreen-5-treated plants. The number of leaves, leaf length, and leaf width were found to be highest for Biogreen-5 treated plants (3.88, 36.75 cm, 28.13 cm, respectively) and the lowest for untreated plants (2.75, 29.50 cm, 21.88 cm, respectively). The highest number of corms was also recorded with Biogreen-5 (1.38) treated plants, and the lowest for absolute control (1). The weight of corms was found to be highest for treatment with Biogreen-5 (621.06 g), while for the other treatments, it ranged between 466.75-610.94 g. Similarly, the highest number (28.25) and weight (418.00 g) of cormels/ plant were recorded when treated with

Table 1. Effect of different microbe-based bio-formulations on disease incidence/ disease severity in pot-grown *Colocasia* (cv. *Piyaji Kochu)*

Treatment		Disease incidence (%)	Disease severity (%)	Disease Reduction (%)**	AUDPC
T_1	Bioforpf-2 @100g/ plant	54.17(47.42)*	36.81(38.54)*	32.69(34.91)*	3041.25
T_2	Biotime @5 %	41.67(40.23)*	21.88(27.91)*	59.99(50.80)*	1828.13
T_3	Biogreen-5 @5%	37.50(37.76)*	15.63(23.29)*	71.42(57.69)*	1228.13
T_4	Biozin-Ptb@5%	60.42(51.02)*	45.63(42.91)*	16.57(24.04)*	3951.56
T_5	Streptocycline @100 ppm	45.83(42.62)*	26.25(30.84)*	52.00(46.15)*	2100.00
T_6	Control (absolute)	64.59(53.52)*	54.69(47.73)*	0.00	4514.06
S.Ed. ±		**0.55**	**0.36**	**0.69**	
CD ($_{P=0.05}$)		**1.43**	**0.93**	**1.77**	

Data are the pooled mean of three replications for the years 2018 and 2019.
* Figures in parentheses are angular transformed values
**Per cent reduction of disease severity (%) over control

Table 2. Effect of different microbe-based bio-formulations on disease incidence/ disease severity in pot-grown *Colocasia* (cv. *SC-1*)

Treatment		Disease incidence (%)	Disease severity (%)	Disease Reduction (%)**	AUDPC
T_1	Bioforpf-2 @100g/ plant	56.25(48.61)*	35.94(36.85)*	40.72(39.66)*	3131.25
T_2	Biotime @5%	45.83(45.83)*	20.63(27.02)*	65.97(54.34)*	1818.75
T_3	Biogreen-5 @5%	41.67(40.23)*	16.56(24.02)*	72.69(58.53)*	1387.50
T_4	Biozin-Ptb@5%	64.59(53.52)*	49.06(44.49)*	19.08(25.92)*	4307.81
T_5	Streptocycline @100 ppm	47.92(43.82)*	21.25(27.47)*	64.95(53.72)*	2081.25
T_6	Control (absolute)	72.92(58.65)*	60.63(51.15)*	0.00	4950.00
S.Ed. ±		**0.38**	**0.31**	**0.51**	
CD ($_{P=0.05}$)		**0.98**	**0.79**	**1.32**	

Data are the pooled mean of three replications for the years 2018 and 2019.
* Figures in parentheses are angular transformed values
**Per cent reduction of disease severity (%) over control

Table 3. Effect of different microbe-based bio-formulations on growth parameters and yield in pot-grown *Colocasia* (cv. *Piyaji Kochu)*

Treatment		Plant height (cm)	No. of leaves/ plant	Leaf length (cm)	Leaf width (cm)	No. of corms	Weight of corms (g/plant)	No. of cormels	Weight of cormels (g/plant)	Yield (g/ plant)
T_1	Bioforpf-2 @100g/ plant	87.13	3.00	36.50	28.25	1.13	567.50	29.38	450.63	1018.13
T_2	Biotime @5%	96.88	3.63	41.00	34.00	1.38	686.63	34.63	491.38	1178.00
T_3	Biogreen-5 @5%	99.63	3.75	42.75	35.50	1.38	698.22	36.13	496.25	1194.47
T_4	Biozin-Ptb @5%	84.00	2.88	35.00	27.38	1.00	525.13	27.50	415.25	940.38
T_5	Streptocycline @100 ppm	94.25	3.38	40.13	32.88	1.38	670.56	33.38	486.13	1156.69
T_6	Control (absolute)	80.38	2.50	33.50	27.00	1.00	519.38	26.38	412.25	931.63
S.Ed. ±		**0.05**	**0.05**	**0.10**	**0.09**	**0.02**	**3.57**	**0.14**	**1.86**	
CD ($_{P=0.05}$)		**0.14**	**0.12**	**0.25**	**0.22**	**0.06**	**9.17**	**0.36**	**4.78**	

*Data are the pooled mean of four replications for the years 2018 and 2019.

Table 4. Effect of different microbe-based bio-formulations on growth parameters and yield in pot-grown *Colocasia* (cv. *SC-1*)

Treatment		Plant height (cm)	No. of leaves/ plant	Leaf length (cm)	Leaf width (cm)	No. of corms	Weight of corms (g/ plant)	No. of cormels	Weight of cormels (g/ plant)	Yield (g/plant)
T_1	Bioforpf-2 @100g/ plant	82.50	3.00	32.13	22.75	1.00	528.63	21.38	374.19	902.81
T_2	Biotime @5%	88.00	3.75	35.88	27.38	1.38	610.94	27.13	412.88	1023.81
T_3	Biogreen-5 @5%	89.13	3.88	36.75	28.13	1.38	621.06	28.25	418.00	1039.06
T_4	Biozin-Ptb @5%	80.50	2.88	30.25	22.13	1.00	503.13	20.13	352.94	856.06
T_5	Streptocycline @100 ppm	86.88	3.13	35.25	26.63	1.25	608.50	25.00	401.00	1009.50
T_6	Control (absolute)	78.38	2.75	29.50	21.88	1.00	466.75	19.00	342.31	809.06
S.Ed. ±		**0.12**	**0.02**	**0.10**	**0.02**	**0.02**	**2.37**	**0.17**	**1.14**	
CD ($_{P=0.05}$)		**0.30**	**0.06**	**0.27**	**0.05**	**0.06**	**6.10**	**0.44**	**2.92**	

*Data are the pooled mean of four replications for the years 2018 and 2019.

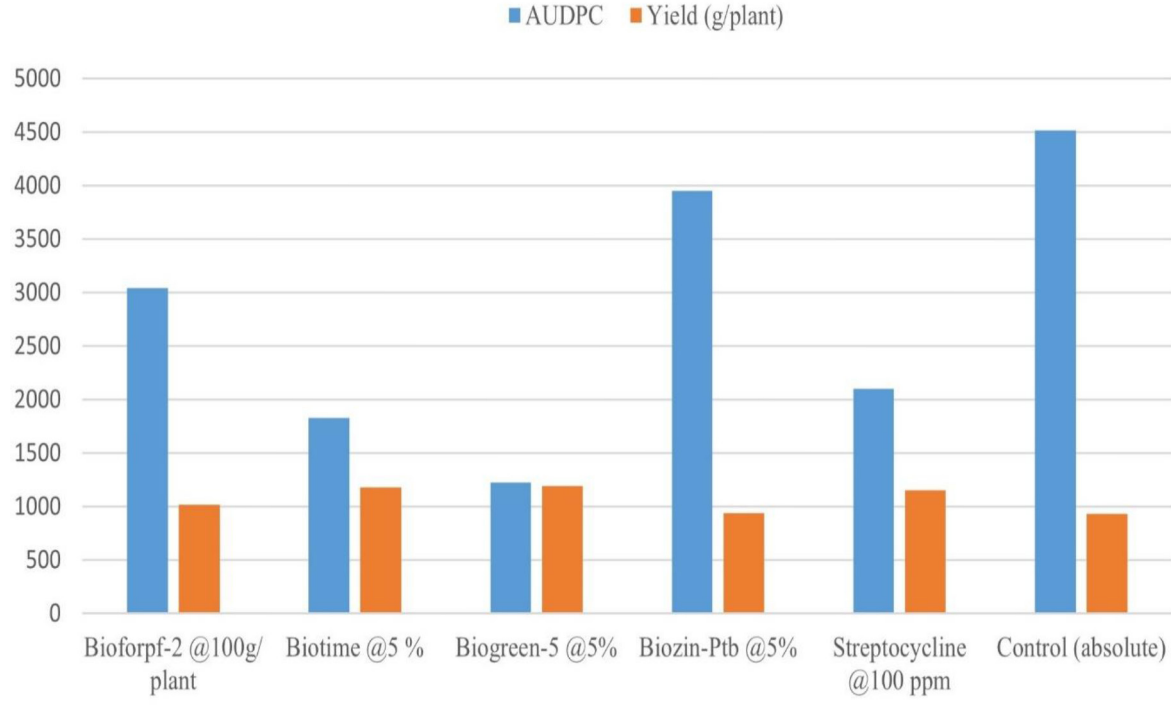


Fig.1. Inverse relationhsip betweenAUDPC and Yield of Taro (CV. *Piyachi Kochu*) under different bioformulations and chemical treatment

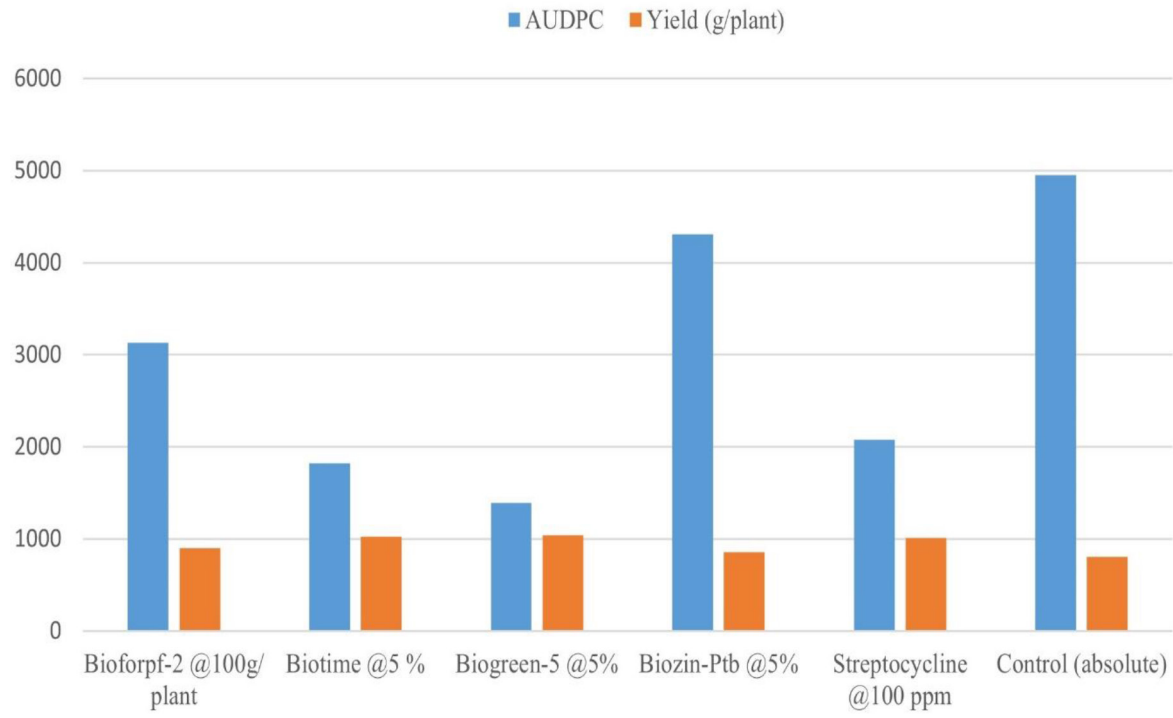


Fig.2. Inverse relationhsip betweenAUDPC and Yield of Taro (CV. *SC-1*) under different bioformulations and chemical tretment

Biogreen-5. The highest yield (g/plant) was observed with the application of Biogreen-5 (1039.06 g). The yield for the other treatment varied from 809.06- 1023.81 g/plant.

A negative correlation between AUDPC and Yield of two Taro cultivars was observed (Figures 1 and 2), indicating the effectiveness of various bioformulations in disease suppression and enhancing crop productivity.

DISCUSSION

Effect on Taro bacterial blight intensity

Biocontrol of several plant pathogens with various antagonistic microorganisms proved to be an alternative to synthetic pesticides because of the environmental and health concerns. Effects of different microbe-based biopesticides on disease reduction of bacterial blight and yield of Colocasia have not yet been reported. However, the use of microbial consortia and their formation is effective in controlling several bacterial and fungal pathogens.

The AUDPC shows the disease reaction of different biopesticide treatments with the progress in time, whereby the treatment showing the least AUDPC is the best treatment to control the disease and *vice versa.* AUDPC was calculated from the disease severity percentage observed at different DAT (Days After Treatment). AUDPC directly relates to the economic yield, where the treatment with the least AUDPC had the highest yield and lowest disease intensity in the due course of time.

Rachid *et.al.* (1998) evaluated the disease intensity in healthy Taro corms (cv. Negatam) infected with *Xad,* where the highest disease intensity (88.4%) was recorded in plants raised from infected corms, 5.3% disease intensity in streptocycline-treated corms (1000 ppm) and no disease incidence in healthy corms. Dhanya (2006) reported that five sprays (at weekly intervals) of both turmeric powder (0.15%) and streptocycline (100 ppm) proved to be very effective and gave 100 per cent control as against 97% for *Pseudomonas fluorescens* (1.5%), neem oil (2%), and Captan (0.3%) when tested against bacterial blight of anthurium. Opara *et. al*. (2013) reported that Cocoyam treated with plant extracts, *viz*. *Cymbopogon citrates, Piper guineense* seed, *Citrus sinensis* each @ 20 %, Streptomycin sulphate (1%) and Copper oxychloride (10%) showed disease severity of 1.83, 1.33, 1.17, 1.33, 1.67, respectively as compared to the control (3.00) while the disease incidence ranged from 65-68%. Bora *et. al.* (2015) evaluated the *in vitro* effectiveness of the aggressive strains of five different saprophytic antagonists, *Trichoderma parareesei* TPJ-S-1, *Trichoderma viride* TVJ-S-1, *Paecilomyces variotii* Isolate-1, *Bacillus thuringiensis* BTJ-S-1 and *Citrobacter farmeri* CTJ-S-1 and their consortial formulations against the bacterial wilt disease (*Ralstonia solanacearum*) of Naga chilli, where the highest inhibition (91.47%) against *R. solanacearum* was recorded in consortia of *T. parareesei, T. viride* and *B. thuringiensis*. Devi *et. al*. (2016) evaluated the *in vitro* efficacy of biocontrol agents and bactericides against bacterial blight of *Anthurium andraeanum* by using antagonists *Bacillus mojavensis* strain KA3, *B. subtilis* isolate BSD4 and

six bactericides, where *B. mojavensis* strain KA3 inhibited the growth of *X. axonopodis* pv. *dieffenbachiae* over an area of 3730 mm^2 followed by *B. subtilis* isolate BSD4 (3430 mm^2), streptomycin sulphate @2000 (1810 mm^2) and gentamycin (1054 mm^2) while others were less effective. Deb *et al*. (2017) evaluated four different microbial bioformulations (*Biosona*, *Biotime*, *Biogreen-5* and *Biogreen-L*) against major insect pests and disease of tea where the highest reduction of tea mosquito bug (89.06%), red spider mite (92.04%), looper caterpillar (91.47%), tea aphid (92.53%) and grey blight (91.20%) were recorded due to the application of Biogreen-5.

Effect on growth parameters and yield

The present study is in agreement with Opara *et al.* (2013) where they carried out pot experiment for evaluating the efficacy of various plant extracts *viz*., *Cymbopogon citrates* (lemon grass)*, Piper guineense* (black pepper) seed, *Citrus sinensis* (orange) each @ 20% along with Streptomycin sulphate @ 1% and Copper oxychloride @ 10% against *Xad* and reported that the plant height varied from 22.17 (control) to 31.50 cm (*Citrus sinensis*) while the number of corms and weight of corms ranged from 1.00 to 3.85 and 1.30 to 2.80 kg/plant, respectively. There is a slight difference in the plant height and yield, which may be because both the varieties/ cultivars used in the experiment were of medium (50-100 cm) cultivars.

The results revealed that an increase in the growth parameters and yield compared to the control might be influenced by reduced disease intensity and improvement of plant health by PGPMs and their consortia. The various bioagents used in the present study were reported to reduce plant disease intensities and improve plant growth in different crops (Windham *et al*., 1986; Kloepper *et al*., 1988; Mathivanan *et al*., 2000; Thirup *et al*., 2003). Application of compatible bioagents having different roles is more effective and preferable than individual bioagents as their cumulative effect were higher when applied alone (Camprubi *et al*., 1995; Mathivanan *et al*., 2005; Bora *et al.,* 2015*,* 2016).

CONCLUSION

Microbial biopesticides have shown significant results in controlling various pests and pathogens. The study reveals that all the treatments used for the two Colocasia cultivars were statistically significant amongst all. Biogreen-5, a consortium of five bioagents, was found to be the best treatment that can substitute the chemicals and antibiotics to manage the bacterial blight of Colocasia and improve the plant health to ensure food security.

ACKNOWLEDGDEMENT

The authors would like to thank the Department of Plant Pathology, Assam Agricultural University (AAU), Jorhat, for providing the necessary resources and inputs to carry out the research work successfully.

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