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Rhamnolipid biosurfactant as robust inhibiting agent for *Colletotrichum gloeosporioides*-pathogen of *Capsicum chinense* and evaluation of general welfare of the host plant

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The king chilli, *Capsicum chinense* var. Jacq is a chilli variant well-known as one of the world's hottest chillies. It is also well-reported to have various biomedical prospects. To control one of the major fungal pathogen, *Colletotrichum gloeosporioides* of this economically important chilli variety, rhamnolipid biosurfactant treatment was carried out. Bacterial strain *Pseudomonas aeruginosa* SR17 was employed to produce rhamnolipid biosurfactant using glycerol as the carbon source. The rhamnolipid exhibited inhibition against the tested fungal pathogen in both lab-scale and in-planta investigations. Rhamnolipid showed promising result in post harvest management of king chilli fruits. It also aided in general welfare of the host plant.

Keywords: *Capsicum chinense* , *Colletotrichum gloeosporioides*, *Pseudomonas aeruginosa*, Rhamnolipid.

INTRODUCTION

Biosurfactants are a plethora of newly emerging bio-metabolites studied for their versatile applications ranging from bioremediation (Karlapudi *et al*. 2018) to antimicrobial activity. They are sustainable alternative against synthetic pesticides and fungicides (De *et al*., 2015) due to biodegradability and low toxicity. They work as adjuvants, solubilizers and wetting agents in agrochemical formulations (Thavasi *et al*. 2014) and help in even distribution of fertilizer in the soil. Biosurfactants consist of a hydrophobic and a hydrophilic chain joined together by ester or amide or glycosidic linkage (De *et al*. 2015) and are categorised into glycolipids, lipopeptides and lipoproteins, fatty acids, neutral lipids and phospholipids, polymeric surfactants and particulate surfactants. Rhamnolipid is the most extensively and thoroughly investigated biosurfactant for their role in bioremediation and agriculture. It acts as an internal immunity booster to plant thereby eliciting defence response in it (Sanchez *et al*. 2012).There are a few scientific evidences regarding agricultural activity of rhamnolipid as a fungicide (Borah *et al*. 2016; Lahkar *et al*. 2018).

This research studied the antifungal efficacy of rhamnolipid extracted from *Pseudomonas aeruginosa* against phytopathogen of king chilli (*Capsicum chinense* Jaqc.), an endemic crop plant to north east India. King chillies, being widely cultivated in the regions like Assam, Manipur, Mizoram and Nagaland are pertaining to an important spice in the local cuisines and also widely favoured in internal market. Fungal infections are responsible for imparting a second highest loss in the production of king chilli next to viral infections causing a reduction in its market value. *Colletotrichum gloeosporioides* causes die-back in the host plant (Talukdar *et al*. 2015), which is also responsible for causing anthracnose in almost 470 different plants across the globe (Sharma and Kulshrestha, 2015). There is so far no published data against the phytopathogens of king chilli by using rhamnolipid. This research investigated *in vitro* and *in planta* effect of rhamnolipid extracted from *P. aeruginosa* against *C. gloeosporioides*. The general welfare in king

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chilli by treating with rhamnolipid was also investigated. This research is one of the pioneer study on antifungal efficacy of rhamnolipid against *C. gloeosporioides* and impact of rhamnolipid on general growth of king chilli.

MATERIALS AND METHODS

Production of biosurfactant from strains of Pseudomonas aeruginosa

Three biosurfactant producing strains *Pseudomonas aeruginosa* SR17 (GenBank Accession Number KR028434), *P. aeruginosa* JS29 (GenBank Accession Number KC862289) and *P. aeruginosa* SS14 (GenBank Accession Number KC866140) collected from germplasm of Institute of Advanced Study in Science and Technology (IASST, Guwahati, Assam, India) were used for rhamnolipid production. Bacterial inocula (5%) from active cultures of SR17, JS29 and SS14 were inoculated into mineral salt medium (MSM) with carbon source glycerol (pH 7.0±0.2) under sterile condition and incubated in shaking incubator at 150 rpm for 72 hrs at a temperature of 35±1°C. The bacterial cultures were then centrifuged for 15 mins at 10,000 rpm in a cooling Centrifuge at 4°C. The cell free supernatant (CFS) of cultures of SR17, JS29 and SS14 obtained were deproteinized at 110°C for 15 mins followed by acidification with 6N HCl and kept at 4°C for whole night for precipitation of rhamnolipid. Precipitated CFS was mixed with ethyl acetate in a ratio 1:1 and shook vigorously. The solvent was evaporated using rotary evaporator (Equitron 63R-D) under reduced pressure at 60°C (Goswami *et al*. 2015) to get the crude rhamnolipid.

Assessment of antifungal activities of rhamnolipid producing strain against phytopathogen

The present study used phytopathogen *Colletotrichum gloeosporioides* ITCC 6434 procured from Indian type culture collection (ITCC) centre, Indian Agricultural Research Institute (IARI New Delhi, India). For assessing antifungal activity of the rhamnolipids against the phytopathogen, Fungal plugs of size 4 mm was incubated on potato dextrose agar (PDA) amended with crude rhamnolipids prepared in a concentration range of 100, 500 and 1000 mg/L from SR17, JS29 and SS14. Fungal plugs inoculated on PDA plates without adding rhamnolipid served as controls. All the plates were incubated at 28±1°C for 7 days until the control plates were fully covered with fungal mycelia. The experiment was carried out in triplicates and repeated twice. The result was expressed as mean ±SD of two independent experiments. The percentage of inhibition of mycelial growth was calculated as follows:

$$\% \text{ Inhibition} = (1\text{-}A / B) \times 100$$

(A - Diameter of the fungal mycelia grown on media with biosurfactant, B- Diameter of the fungal mycelia grown on media without biosurfactant which served as control).

The rhamnolipid with lowest IC_{50} (inhibitory concentration that inhibits 50% of the pathogen) value against *C. gloeosporioides* was used in further experiments.

Efficacy of crude and column purified biosurfactant against C. gloeosporioides

Crude biosurfactant was column purified by dissolving 1 g crude biosurfactant in 5 ml of methanol (ACS Mark) and loaded in a glass column (length 500 mm, diameter 25 mm) packed with silica gel of mesh size 60-120. A solvent system of methanol and chloroform with the gradient 3:50, 5:50 and 50:50 was used for elution. The eluted mixture was evaporated in rotary evaporator at 40°C under reduced pressure and the extracted column purified biosurfactant was dried (Borah *et al*. 2015).

The crude and column purified biosurfactants were assayed against the mycelia and spores of *C. gloeosporioides*. In order to assess mycelial inhibition, mycelial plug of 4 mm diameter from the pathogen was inoculated on media amended with biosurfactant concentrations 100, 200, 300, 400, 500, 600, 800 and 1000 mg/L and the percentage inhibition was calculated after conducting the experiment through previously described method. For assaying the efficacy of biosurfactant against spores of *C.*

gloeosporioides, column purified biosurfactant was dissolved in dimethyl sulfoxide (DMSO) and solutions of biosurfactant with the concentrations 25, 50, 100, 200, 300, 400, 500, 600, 800 and 1000 mg/L were prepared. The fungal spores (spore count 1×10^5 spores/ml adjusted in haemocytometer) were inoculated on PDB. A 96-well flat bottom microtitre plate was loaded with biosurfactant solutions where fungal spores were inoculated and DMSO (2% final concentration) was added. The wells inoculated with spores without adding DMSO were considered as controls. The plate was incubated at 28±1°C for 48 hrs. The MIC of biosurfactant was considered as its lowest concentration that completely inhibited the growth of the phytopathogen transferred to a hole of 0.5 mm^2 diameter on the solid fungal media, PDA (Onlamool *et al.* 2023). The IC_{50} value was calculated statistically from inhibition percentage at different concentrations.

In vivo assessment of antifungal activity of biosurfactant on king chilli seeds

For investigating antifungal efficacy of rhamnolipid on seeds, the seeds were surface sterilized with 70% ethyl alcohol with vigorous shaking for 3 minutes followed by dipping in 4% sodium hypochlorite for 5 minutes and washed 3 times with Sterile distil water (Younesikelaki *et al*. 2016). Surface sterile seeds were soaked for 1 hour in rhamnolipid solutions prepared in the concentrations 50, 100, 250, 500 and 750 mg/L^- and in commercial fungicide bavistin (50% WP carbendazim) prepared in recommended dose (1 g/L). Three surface sterile seeds were placed at equidistant on the plate containing water agar media (2% w/v). Fungal spore suspension of 100 µl (10^6 spores /mL, adjusted in haemocytometer) was added on each seed. Un-inoculated and untreated seeds were kept as positive controls while inoculated seeds without treating with biosurfactant were kept as negative controls. The experiment was conducted in triplicates for 14 days at 25±1°C in dark. The germination percentages, root length, shoot length and vigour index were calculated for the seeds. Root length was measured from protrusion point of the seed to root tip while shoot length was measured from protrusion point of the seed to leaf tip. Percentage of germination (after 14 days from day 1) and Vigour Index were calculated as (Ananthi *et al*. 2014)

$$\% \text{ Germination} = \frac{(\text{Number of germinated seeds} / \text{Total number of seeds sown})}{} \times 100$$

Vigour Index = Germination Percentage x (Root lengt + Shoot length)

Plant bioassays

For assaying the effect of biosurfactant on disease infected king chilli plants, earthen pots (diameter 25 cm × height 28 cm) were prepared with 4 kg sterile soil planted with a 28 days old seedling and kept at a temperature of 25-28°C and relative humidity 75-85%. The experiment was conducted in the period of October 2019-January 2020, for 120 days where plants were arranged in the randomized block design.

The *in planta* experiment of evaluation of the biosurfactant efficacy against *C. gloeosporioides* was conducted in two sets viz. preventive (Set 1) and curative (Set 2) comprising of 3 replicas in each case and were compared against the commercial biosurfactant bavistin. Solution of biosurfactant was prepared in the concentration of 50, 500 and 1000 mg/L while the solution of bavistin was prepared in recommended dose of 1000 mg/L. The experimental plants comprised of plants treated with biosurfactant, plants treated with bavistin and controls (negative control-inoculated with fungal spore and treated with sterile distil water, positive control-without inoculation of spores and biosurfactant treatment). Set 1 was uniformly sprayed with biosurfactant all over the shoot 24 hrs prior to infection with 20 µl fungal spore (spore count 10^6/ ml). The plants in Set 2 (Curative Set) were treated with biosurfactant after 72 hrs of inoculation. Plants were covered with moist polythene bags for 24 hrs of post-inoculation to ensure maximum disease development. The percentage of disease was calculated from anthracnose infected chilli harvested on 21^{st} day of post fungal spore inoculation.

Post harvest disease management of king chilli fruit

Management of post harvest decay of ripe king chilli fruit by applying biosurfactant was assayed

through this experiment. Ripe king chilli fruits of same size were surface sterilized with 70% ethyl alcohol for 2 mins, 4% sodium hypochlorite for 5 mins and rinsed three times with sterile distil water (Younesikelaki *et al*. 2016). The fruits were immersed in solutions of crude biosurfactant (prepared of the concentration 50, 100, 250, 500, 750 and 1000 mg/L) and bavistin (1000 mg/L) for 20 mins. Spore suspension of 10 µl (10^6/ml concentration) was inoculated in the fruits by piercing a wound of 2 mm (Lahkar *et al*. 2018). The control was dipped in sterile water. The experiment was carried out in triplicates. All the fruits were placed in sterile moist chamber for 9 days at temperature of 25°C and relative humidity 75-80%. The disease severity was measured on a scale of 0-5 where 0 = No symptom, 1 = 1-10 % disease infection, 2 = 11-25 % disease infection, 3 = 26-50 % disease infection, 4 = 51-75 % and disease infection 5 = > 75 % disease infection. The disease reduction percentage was calculated by comparing the inhibition against the control. The Per cent Disease Index (PDI) was calculated as

$$PDI = \frac{\text{(Sum of all disease ratings/ Total number of chilli examinated x Maximum disease rating)}}{} \times 100$$

General welfare experiment

The set of general welfare experiments was conducted for 90 days. The parameters examined were germination percentage of seeds, seedling vigour indices, days to flowering, days to fruiting and chlorophyll content.

Germination percentage of seeds and seedling vigour indices

For this experiment, two sets of sterile seeds (sterilized by previously described method) were soaked in biosurfactant solutions (500 mg/L) (set A) and sterile distilled water (set B) for 2 hrs. Both the sets, each comprising of 20 numbers of seeds, were sowed in 2 separate earthen pots (10 cm height × 8 cm diameter) filled with 1½ kg of sandy loam garden soil and kept at green house for germination. The parameters assayed after 30 days from sowing were percentage of germination, root length, shoot length and seedling vigour indices.

(a) Rate of Germination

Seeds geminated from day-2 up to day-30 were taken into count. The rate of germination was counted as (Ananthi *et al*. 2014):

$$\text{Rate of Germination} = \frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + \ldots + \text{(Xn-Xn-1) / Yn}$$

X_1 – total no of seeds germinated on day-2, X_2 – total no of seeds germinated on day-3, X_n – total no of seeds germinated on day (n+1)

Y_1 – total no of days from sowing to day-2, Y_2 – total no of days from sowing to day-3, Y_n – total no of days from sowing to day -(n+1)

(a) Percentage of Germination

For calculating percentage of germination of seeds, the phenotypically normal seeds taken into count after the period of 30 days. It was calculated as

$$\text{\% Germination} = \text{(Number of germinated seeds/ Total number of seeds sown)} \times 100$$

(a) Seedling Vigour Tests

Seedling vigour index-I (SVI-I), seedling vigour index-II (SVI-II) and speed of germination index (SGI) were evaluated after 30 days from sowing the king chilli seeds. The parameters were calculated by the following formulae (Ananthi *et al*., 2014)

SVI-I = Germination (%) x (Average root length + Average Shoot length)

SVI-II=Germination Percentage x Average seedling dry weight (mg)

Dry weight of seedling was recorded after keeping the seedlings at 80°C for 24 hrs.

$$\textbf{SGI} = \frac{\text{Number of germinated seed +... + Number of germinated seeds}}{\text{Days of first count + ...+ Days of final count}}$$

Foliar spray and root treatment of biosurfactant

Experimental plants were prepared in 2 sets of namely set A (for root treatment) and set B (for

foliar spray) and treated with biosurfactant solutions of concentrations 100, 500 and 1000 mg/L. Root dip treatment was applied to 14 days old seedlings for 30 mins in set A. In set B, biosurfactant solution was sprayed on 14 days old seedlings for 90 days after an interval of every 15 days. Seedlings treated with sterile distil water were considered as controls in both sets. 3 replicates were used in each concentration and days of flowering and days of fruiting were measured.

Estimation of Chlorophyll content

For studying chlorophyll content, mature leaves of same size were treated with biosurfactant (type A) and without biosurfactant (type B). 100 mg from each type was homogenised in 10 ml of 90% (v/v) acetone and filtered with Whatman number 1 filter paper. The final volume of the filtrate was made 10 ml by adding 95% acetone and OD was measured at 645, 652 and 663 nm in UV-vis spectrophotometer. The amount of chlorophyll in both type A and type B plants were compared where the data was considered as means± standard deviation of three replicates. The total chlorophyll, chlorophyll a and chlorophyll b were calculated as (Richardson *et al*. 2002).

Chl a (μg/ml) = (12.7 xO.D. at 663 nm) – (2.69 xO.D. at 645 mm)

Chl b (μg/ml) = (22.9 xO.D. at 645 nm) – (4.68 xO.D. at 663 mm)

Chl (μg/ml) = (20.2 xO.D. at 645 nm) + (8.02 xO.D. at 663 mm)

RESULTS

Selection of the most potent rhamnolipid producing strain inhibiting Colletotrichum gloeosporioides

The rhamnolipids extracted from strains of *P. aeruginosa* SR17, SS14 and JS29 lowered the surface tension from 64 of MSM media up to 27-29 N/m within 24 hrs. The crude biosurfactant extracted from all the 3 strains showed antifungal efficacy against *C. gloeosporioides*. Among them, the biosurfactant extracted from SR17 exhibited maximum inhibition of mycelia. The fungal mycelia were inhibited up to 68.2% by biosurfactant of SR17, 65.1% by biosurfactant of JS29 and 50.4% by biosurfactant of SS14. The IC_{50} values against mycelia of pathogen were recorded as 631.28 mg/l by SR17, 802.51 mg/l by JS29, 927.43 mg/l by SS14 (Table 1). As IC_{50} conferred by the biosurfactant extracted from SR17 was lowest against the mycelia of *C. gloeosporioides*, this was used in the further experiments. The study found no correlation between yields of biosurfactant with the extent of inhibition conferred by them as highest yield of biosurfactant was found in case of JS29 while the biosurfactant produced by SR17 showed maximum inhibition against the tested phytopathogen.

Efficacy of crude and column purified biosurfactant against mycelia and spores of C. gloeosporioides

When the concentration of crude biosurfactant was increased, the percentage of inhibition ($F_{7, 23}$ = 1747.79, p< 0.05) also increased significantly against the fungal mycelia. There was no significant difference between the percentage of inhibition conferred by the crude concentrations 300 and 400 mg/L. Similarly, when column purified biosurfactant was applied against the mycelia of *C. gloeosporioides*, there was significant increase in the inhibition percentage ($F_{7, 23}$ = 1419.69, p< 0.05) except for the concentrations 500 and 600 mg/L. There was significant percentage of inhibition ($F_{15, 47}$ = 898.88, p< 0.05) when same concentration of crude and column purified biosurfactant was assayed against mycelia of *C. gloeosporioides*. The IC_{50} values of crude and column purified biosurfactant were 640.83 mg/l (r^2=0.98) and 525.99 mg/l (r^2=0.95) respectively. The crude and column purified biosurfactant inhibited spore germination of *C. gloeosporioides* with MIC were 400 mg/L and 300 mg/L respectively. The IC_{50} values for crude and column purified biosurfactant were calculated statistically as 381.37 mg/L and 271.95 mg/L which were significantly different from each other (Table 2). The inhibiting activity of both crude (r^2=0.84) and column purified (r^2=0.72) biosurfactant increased against the fungal spores as the concentration increased.

Table 1: IC_{50} values of rhamnolipid produced by *Pseudomonas aeruginosa* SR17, *Pseudomonas aeruginosa* JS29 and *Pseudomonas aeruginosa* SS14 against the mycelia of *C. gloeosporioides*

Pathogen	Rhamnolipid from different strains of *Pseudomonas aeruginosa*		
	SR17	JS29	SS14
C. gloeosporioides	631.28[a]*	802.51[b]	927.43[c]

* In a same row different letters stand for significant differences (p<0.05)

Table 2: IC_{50} values of crude and column purified biosurfactant produced from *Pseudomonas aeruginosa* SR17 against the mycelia and spores of *C. gloeosporioides*

Biosurfactant	*C. gloeosporioides*	
	Mycelia	Spores
Crude	640.83[a]*	381.37[a]*
Column Purified	525.99[b]	271.95[b]

* In a same column indicating same parameter, different letters stand for significant differences (p<0.05)

Table 3 : Effect of different concentrations of crude biosurfactant and bavistin on germination and growth parameters of king chilli seeds following application and inoculation with spores of *C. gloeosporioides*

Treatments	Concentration mg/L	Germination percentage	Shoot length (cm)*	Root length (cm)*	Vigour Index*
Negative Control	-	0	-	-	-
Positive Control	-	40	1.04±0.02[a]**	0.61±0.04[a]**	64.2
Biosurfactant	50	45	1.79±0.04[b]	0.77±0.04[b]	111.5
	100	48	2.07±0.03[c]	1.12±0.04[c]	149.5
	250	52	2.51±0.06[d]	1.33±0.05[d]	194.5
	500	60	2.99±0.11[e]	1.80±0.07[e]	276.8
	750	64	2.80±0.05[f]	1.46±0.03[f]	267.5
Bavistin	1000	65	2.89±0.05[f]	1.47±0.03[f]	278.7

* Values were expressed as mean ±SD of three replicates
** In a same column indicating same parameter, different letters stand for significant differences (p<0.05)
*** Bavistin was used as standard during experimental trials

In vivo assessment of antifungal activity of biosurfactant on king chilli seeds infected with C. gloeosporioides

C. gloeosporioides infected king chilli seeds showed more germination percentage (r^2=0.97) and vigour index when treated by biosurfactants with different concentrations as compared to positive control. The germination percentage in positive control was 40% whereas in disease infected seeds treated with 750 mgL^{-1} biosurfactant it was 64% and in disease infected seeds treated with bavistin it was 65% (Table 3). The germination percentage of the seeds is greatly impacted by applying biosurfactant than the non treated infected seeds and it increased along with the increase in concentration of biosurfactant. The shoot length ($F_{4, 14}$ = 181.41,

Table 4 : Assessment of disease severity in king chilli plants infected with *C. gloeosporioides* following treatment with crude biosurfactant.

Treatments	Concentration of biosurfactant (mg/L)	Percentage of disease**** Preventive	Curative
Positive Control	-	-	-
Negative Control	-	78±2.65*	-
Biosurfactant	50	77.3±2.08[a**]	73±2.65[a**]
	500	65±1[b]	61.3±1.53[b]
	1000	53±1.73[c]	50.7±2.08[c]
Bavistin***	1000	57±1[d]	54±2[d]

* All values are in percentage and expressed as mean ±SD of three replicates
** In a same column indicating same parameter, different letters stand for significant differences (p<0.05)
*** Bavistin was used as standard during experimental trials
**** After 90 days of treatment with crude biosurfactant

Table 5: Evaluation of disease reduction in infected king chilli fruit after treatment with crude biosurfactant

Treatment	Concentration (mgL^{-1})	Percentage disease index (PDI)	Disease reduction over control (%)
Control	-	93.3	0
Biosurfactant	50	70.0	25.7[a]*
	100	66.6	35.3[b]
	250	45.5	49.3[c]
	500	39.9	60[d]
	750	25.0	66.7[e]
	1000	20.0	69.7[e]
Bavistin**	1000	25.0	67.7[e]

* In a same column indicating same parameter, different letters stand for significant differences (p<0.05)
***Bavistin was used as standard during experimental trials

Table 6 : Effect of crude biosurfactant on percentage germination, root and shoot length and vigour indices of king chilli seeds

Treatment	Rate of Germination	Germination %	Shoot length (cm)*	Root length (cm)*	Average seedling dry weight (mg)*	SVI-I	SVI-II	SGI
Set A (500 mg /L biosurfactant)	1.55	85	9.06± 0.6[a**]	6.34± 0.55[a**]	2.22± 0.33[a**]	1309	189.04	0.57
Set B (distilled water)	1	70	7.04± 0.41[b]	4.17± 0.52[b]	1.5± 0.22[b**]	784.7	105.42	0.44

* Values were expressed as mean ±SD of three replicates
** In a same column indicating same parameter, different letters stand for significant differences (p<0.05)

Table 7: Days taken for flowering and fruiting in biosurfactant treated king chilli plants during 90 days experimental trials

Treatments	Concentration of biosurfactant	Days for flowering	Days for fruiting
Control		83±1[a*]	87.7±0.57735[aa**]
Root treatment	50	81.7±0.57735[a]	86±1[aa]
	500	78.7±0.57735[b]	84.3±0.57735[ba]
	1000	75.7±0.57735[c]	81.3±0.57735[c]
Foliar spray	50	77±1[dbc]	82.7±0.57735[dbc]
	500	72.7±0.57735[e]	79±1[e]
	1000	69.3±0.57735[f]	77.3±0.57735[fe]

* Values were expressed as mean ±SD of three replicates
** In a same column indicating same parameter, different letters stand for significant differences (p<0.05)

Table 8: Cholorophyll contents of biosurfactant treated plants

King chilli plant	Total chl	Chl a	Chl b
Biosurfactant treated	21.03±0.64[a*]	6.14±0.11[a]	14.9±0.54[a**]
Control	6.64±0.31[b]	2.92±0.18[b]	3.71±0.17[b]

* Values were expressed as mean ±SD of three replicates
** In a same column indicating same parameter, different letters stand for significant differences (p<0.05)

p< 0.05) of germinated seeds in positive control was 1.03 cm, 2.80±0.05 cm in biosurfactant (750 mg/L) treated seeds which was significantly similar with bavistin treated seeds (2.89±0.05) cm. There was an increase in root ($F_{4, 14}$ = 202.31, p< 0.05) and shoot length among the infected seeds treated with 50 mg/L to 500 mg/L biosurfactant. Root length in pathogen infected germinated seed was 1.46±0.03 cm, a significantly similar value with bavistin treated infected seed (1.47±0.03 cm). Vigour index was highest (276.8) at the biosurfactant concentration 500 mg/L while it was 278.7 in infected seeds treated with commercial fungicide.

Plant bioassays

The crude biosurfactant displayed a better curative (r^2= 0.99) and preventive (r^2= 0.99) effect over the anthracnose infected control. Biosurfactant when applied in a concentration of 50 mg/L, the percentage of anthracnose infected plants was 77% and 73% in preventive and curative treatments respectively which was lower than the control (78%). The preventive (53%) and curative (50%) treatment of the highest concentration of biosurfactant showed significantly lower percentage of disease than the commercial fungicide bavistin in both the cases (Table 4). The disease percentage decreased as the dose of biosurfactant was increased in both set A and set B as biosurfactant has the potential of conidial lysis by altering the permeability of the cell membrane. Curative effect of biosurfactant was better than the preventive one. Biosurfactant was more efficient than the commercial fungicide bavistin which makes it a better and sustainable fungicide.

Post harvest disease management of king chilli fruit

The percentage of disease reduction increases (r^2=0.72) along with the increase in concentration of biosurfactant in king chilli fruits (Table 5). The disease reduction percentages after treating with biosurfactant of concentration 750 (66.7%) and 1000 mg/L (69.7%) respectively were not significant with that (67.7%) conferred by bavistin ($F_{7, 23}$ =641.29). The IC_{50} value was 486.34 mg/L

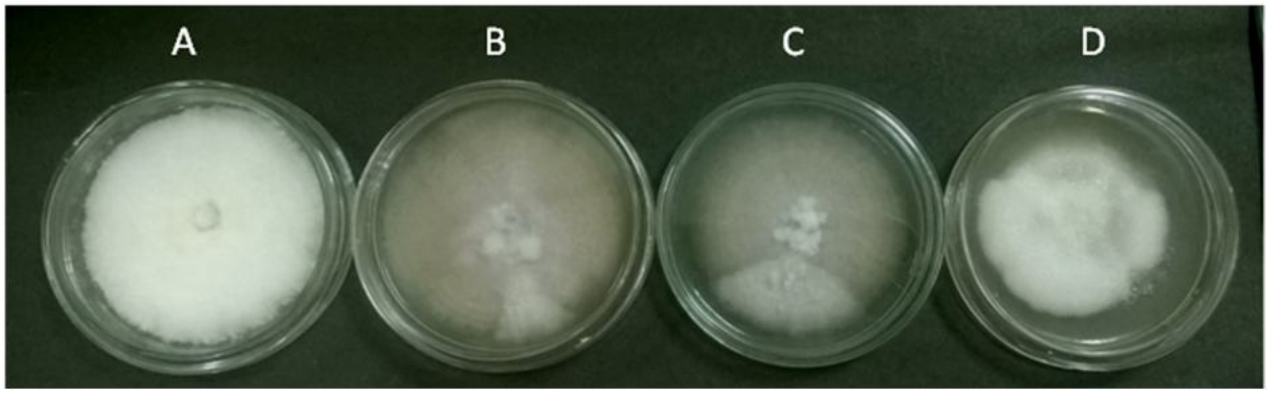

Fig. 1: (A-D). Effect of rhamnolipid on the growth of fungal mycelia of *C. gloeosporioides.*
A. *Colletotrichum gloeosporioides* growing on untreated plate (Control); **B.** *C. gloeosporioides* growing on plate treated with rhamnolipid produced by strain SS14 **C.** *C. gloeosporioides* growing on plate treated with rhamnolipid produced by strain JS29 **D.** *C. gloeosporioides* growing on plate treated with rhamnolipid produced by strain SR17

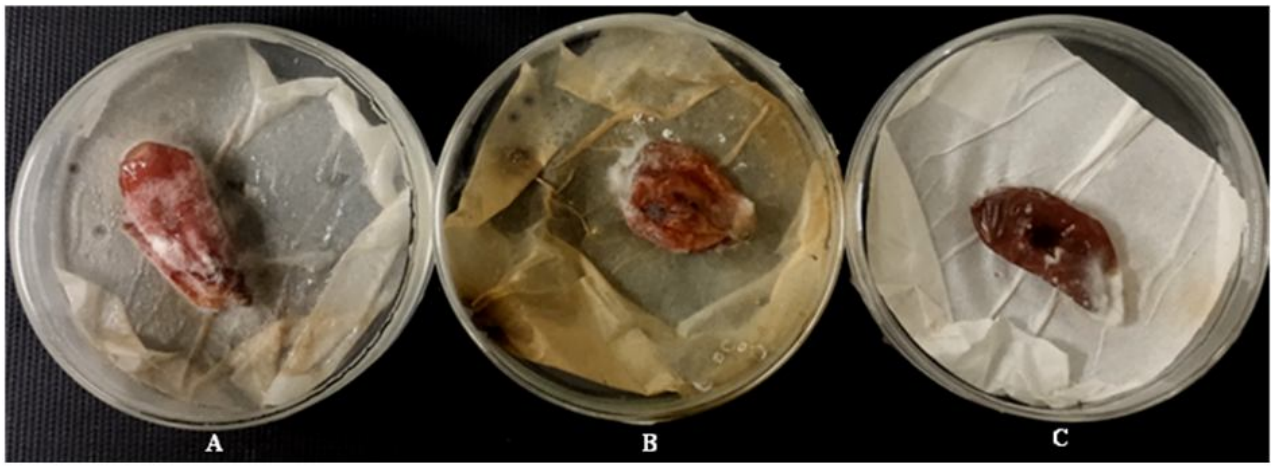

Fig. 2: (A-C). King chilli fruit infected by *C. gloeosporioides*. **A**. Control (Infected fruit without any treatment) **B**. Infected fruit treated with Bavistin (1 g/L) **C**. Infected fruit treated with biosurfactant (1 g/L)

against the fungal mycelia. The percentage disease indices (PDI) were 93.3 for control while 20 and 25 for infected chilli fruit treated with biosurfactant (1000 mg/L) and bavistin (1000 mg/ L) respectively.

Germination percentage of seeds and seedling vigour indices

The effect of biosurfactant on seed germination and seedling vigour indices were better than the control. The germination percentage was 85% after treating the seeds with biosurfactant (Set A) and 70% in control (Set B). The shoot and root length in biosurfactant treated seedling was found to be 9 cm and 6cm respectively after 30 days, significantly higher than shoot (7 cm) and root (4 cm) length in control. Similarly, biosurfactant treated seedlings showed higher vigour indices. The SVI-I and SVI-II were calculated as 1309 and 189.04 in set A while 784.7 and 105.42 in set B respectively. The average dry weight of 10 random seedlings in set A was as 2.22 mg and 1.5 mg in seedlings from set B. The SGIs were 0.57 and 0.44 for set A and set B respectively. All the parameters in biosurfactant treated seeds were higher than seeds without any treatments (Table 6).

Effect of biosurfactant on plant growth and chlorophyll content

The root treatment (set A) and foliar spray (set B) of biosurfactant on king chilli seedling up to a period of 90 days exhibited lesser days of flowering and days of fruiting in comparison to control which decreased along with the increase in concentration of biosurfactant in both set A and set B. The highest concentration in set A significantly reduced flowering days up to 75 from

83 days and fruiting days from 87 to 81 days. The reduction in days of flowering and fruiting were 69 and 77 respectively in set B which were significantly lower than the respective controls in both the cases (Table 7).

Application of biosurfactant caused a marked increase in total chlorophyll content than the controls (Table 8). The value of total chl observed in king chilli leaves treated with biosurfactant (21.03) was significantly higher than the total chl in leaves of control plants (6.63). The calculated values of chl a (6.14) was higher than control (2.92). Similarly, there was significant increase in chl b (14.9) than the control (3.71).

DISCUSSION

The management of the infection caused by *C gloeosporioides* can be partially mitigated by cultivating resistant cultivars. However this practice is not sustainable as the resistance can be broken in field conditions by development of mutated strain of pathogen at any point. Application of fungicide such as bavistin may help in post harvest management of fruits (Meetei *et al*. 2016). The prolonged usage of chemical fungicide is discouraged due its environmental toxicity. Another form of disease management is the application of living microbes such as *Trichoderma viride*, *Pseudomonas fluorescence* for inhibition of mycelia growth of *C. gloeosporioides* (Meetei *et al*. 2016). This kind of biocontrol is of limited success as the survival of a living microbe and its metabolytic activities are compromised under *in* situ condition (Lahkar *et al*. 2018). Biosurfactants are active bacterial metabolites that have the potential of conidial lysis as they alter the permeability of the cell membrane which is also accompanied with mycelial disruption. Biodegradability and low toxicity aided to their environmental sustainability. Literature survey of earlier studies revealed the usage of rhamnolipid biosurfactant against phytopathogens both *in vivo* and *in planta* (Borah *et al*. 2016; Lahkar *et al*. 2018). This study found antifungal efficacy of rhamnolipid extracted from SR17 against mycelia and spores of *C.gloeosporioides*. It is useful in reducing the disease infection in king chilli plant and post harvest management of its fruit without interfering with the environment.

The study found no correlation between yields of rhamnolipid biosurfactant with the extent of inhibition conferred by them as highest yield of biosurfactant was found in case of JS29 while the biosurfactant produced by SR17 showed maximum inhibition against the tested phytopathogens. The different effect of three biosurfactants against the phytopathogens was probably due to the variation in congeners present in them along with the type of the phytopathogen used for the study as the compositions of the congeners of mono- and di- rhamnolipid is accountable for its activity and efficiency (Du *et al.* 2019).

Requirement of higher concentration of rhamnolipid to inhibit the spores than mycelia is in agreement with previous work on antifungal efficacy of biosurfactant (Borah *et al*., 2016). It might be attributed by difference in cell wall of mycelia and spores of fungus. Biosurfactant treated seeds showing a better result in each parameter probably attributed by its ability to damage spores even after applying in low concentration (Borah *et al*. 2016).Usage of crude biosurfactant for treating infection and its efficiency even in low concentration makes it cost effective for field use. Curative effect of biosurfactant was better than the preventive one which was also observed in previous work on *in planta* effect of biosurfactant (Lahkar *et.al*., 2018). Biosurfactant can be used as a post harvest management agent as it has low toxicity, biodegradability and resistant nature in harsh environment (Lahkar *et al*.2018). Micronutrients in the soil become bioavailable to beneficial microbes due to action of biosurfactants (Sachdev and Cameotra, 2013) and develop the plant health. Further research on biosurfactant has a scope of incorporating it in integrated disease management as a safer and cheaper diseasing controlling biological agent.

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DECLARATION

Conflict of Interest : Author declares no conflict of interst.

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