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Biocontrol Potential of Lipopeptide Bio Surfactant Produced by *Bacillus sp.* from Mangrove Ecosystem at Vellar-Coleroon Estuarine Complex against Early Blight of Tomato

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Abstract:- Biosurfactant producing microorganisms *Bacillus sp,* isolated from the Rhizosphere soil area Mangorve ecosystem at Vellar –Coleroon Estuarine complex, Killai backwater, Chidambaram, Tamilnadu, India. The biosurfactant producing ability was tested in crude oil as the hydrocarbon in a Minimal Salt Medium (MSM). The isolate found to produce Biosurfactant which is evident from the rapid screening test. The biochemical and Fourier transform infrared spectroscopy studies reveal the Biosurfactant is a lipopeptide. The biocontrol potential of the crude Biosurfactant tested against Early blight of tomato (*Solanum lycopersicum L.*) is caused by *Alternaria solani* (Ellis & Martin), which is one of the most devastating diseases in tomatoes. Currently, the only way to stop the illness from spreading is to apply fungicides. In vitro experiments revealed that at a concentration of 3.00 g l^{-1}, the biosurfactant generated by this bacterial strain inhibited the growth of *A. solani* by 72 per cent. In the pot culture, a concentration of 1.50 g l^{-1} crude biosurfactant was adequate to completely inhibit *A. solani*.

Keywords:- *Biosurfactant, Biocontrol, Bacillus sp, Lipopeptide, Alternaria solani (Ellis & Martin).*

I. INTRODUCTION

Surfactants area unit a category of chemical compounds possessing amphiphilic (both hydrophobic and hydrophilic) moieties that distribute themselves between two incompatible fluids, with an impact of reducing the surface/interfacial tensions and inflicting the solubility of polar compounds in non-polar solvents (1). They show properties such as solubilization, and lubrication; have stabilizing and foaming capacity; and dispersion (2). Surfactants area unit either derived synthetically or biologically. Naturally derived surfactants area unit denominated biosurfactants since they're made from biological entities, particularly microorganisms. Fungi, bacteria, and yeast (3). Amongst the bacterium domain, genera of genus *Pseudomonas, Bacillus,* and *Acinetobacter* dominate the literature house as producers of biosurfactants (2).

The species among these genera that are extensively studied area unit genus *Bacillus sp, Bacillus subtilis,* and *Acinetobacter calcoaceticus,* amongst alternative species (1, 4). They have a deliquescent portion that is drawn to the majority medium and a hydrophobic section with very little affinity for it. Industrial applications for biosurfactants include lubricants, penetrants, flocculating, wetting, and foaming agents, as well as biocontrol for fungi. (5).

Early blight of tomato caused by *Alternaria solani* (Ellis & Martin) is taken into account in concert of the for most ruinous flora diseases throughout the world. The causative organism is an air borne and soil inhabiting flora that is additionally accountable for collar rot and fruit rot of tomato besides the first blight (6). The disease symptomatic on leaves, stems, petiole, twig and fruits under neath favorable conditions that ends up in defoliation, drying off of twigs and premature fruit drop that causes close to 50–86 cognitive state in fruit yield (7). The application of agrochemicals has definitely belittled the eruption of flora diseases, however at identical time has contributed to the event of resistant pathogens. Moreover, such chemicals are often fatal to helpful microorganisms within the rhizosphere and helpful soil insects, and that they may additionally enter the organic phenomenon and accumulate within the build as undesirable chemical residues (8, 9). To beat the issues, a non-hazardous different like biological management has been extensively studied, and numerous microorganisms and their metabolites are according to be sensible biocontrol agents against phytopathogenic fungi (10).

Microbial chemical agent (i.e., biosurfactant) are extensively tried as antifungal, antiviral, antitumor, insecticidal, and antimycoplasma activities. Biosurfactant area unit surface active microbe metabolites and belong to numerous categories together with lipopeptides, lipopeptide-fatty acids, phospholipids, neutral lipids and lipopolysaccharides (11). The distinctive properties of biosurfactant have recently attracted the eye of industries to become a doable replacement to the artificial chemical pesticides. Biosurfactant have special advantage over the chemicals as they're less carcinogenic, value effective, and extremely perishable with higher environmental compatibility (12, 13).

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The lipopeptide encompasses a protest on the cell surface structure. It will effectively cause lysis within the cell wall. The matter is accountable for the alteration of the organic structure of the membrane because of interruption in super molecule conformation that successively alters the very important functions like transport and energy production within the infective agent (14). Further, anti-adhesive property that may be a novel means of preventing the organization of pathogens on the surfaces (15). In addition, (16) have incontestable that lipopeptide at low concentrations act as typical elicitor of plant defense. Lipopeptide induces plant resistance by triggering production of reactive gas species, Ca^{2+} inflow and microbe associated molecular patterns activation in plants. Numerous studies were conducted to ascertain lipopeptide as a possible agent to guard several economically necessary crops (17, 18, and 19). This investigation aimed to test biocontrol potential of Biosurfactant from *Bacillus sp*, isolated from the Rhizosphere soil area Mangorve ecosystem at Vellar – Coleroon Estuarine complex, Killai backwater, Chidambaram, Tamilnadu, India.

II. MATERIALS AND METHODS

Biosurfactant producing microorganisms *Bacillus sp*, isolated from the Rhizosphere soil area Mangorve ecosystem at Vellar –Coleroon Estuarine complex, Killai backwater, Chidambaram, Tamilnadu, India. The Biosurfactant production ability is tested using the following tests: Rapid screening bioassay for biosurfactant production is a Glass plate assay described by (20). Blood agar was prepared according to the method described by (21). Surface tension was measured with a du Nouy Tensiometer (Kruss Digital-tensiometer 10, Hamburg, Germany) at room temperature (28 ± 2°C). Surface tension measurement values were recorded and expressed as mN/m. Between each measurement, the platinum wire ring was rinsed three times with water, followed by acetone and was allowed to dry. The surface tension value shown is the average of three replicates from the same culture. Control consisted of a sterile culture medium plus an inoculum, at initial at 28 ± 2°C. Distilled water and isopropanol were used as standards (22). Drop-collapse test is done as per (23). Oil spreading test was done according to (24). BATH assay was carried out as described by (25). The emulsifying activity of the biosurfactant was determined by using the cell free culture broth (26)**.** Bacterial isolates were grown after that the cells were then resuspended in the same buffer (8ml) prior to the measurement of the initial density of cell suspension spectrophotometrically at the wavelength of 400nm. The bacterial cell suspension (8ml) was then mixed with hexadecane (2ml) in tissue culture tubes (15 x 2.5 cm) and incubated at room temperature (28 ± 2°C) for 10 minutes prior to vigorous mixing by vortex for about 2 minutes.

The mixture was vortexed, then allowed undisturbed for 15 minutes to allow the hexadecane to separate from the aqueous phase. The aqueous phase (bottom layer) was then carefully removed and the cell density remained in the aqueous phase was measured spectrophotometrically at 400nm. Hydrophobicity was expressed as the percentage of cell adhered to hydrocarbon a calculated as follows:

$$\text{Hydrophobicity Index}(\%) = 100\ (1\text{-}Od_a/OD_c)$$

- ODc - initial density of the cell suspension
- (ODa) - Cell density remained in the aqueous phase after 15 minutes

The dry cell biomass of a hydrocarbon-grown microbe was evaluated by centrifuging 50 ml of culture broth for 20 minutes at 19,300 g. The sediment cells were then extracted with a mixture of acetone/hexane (3:1) to remove the adhering hydrocarbon. This was followed by centrifugation with hexane (10 ml) and drying at 80°C overnight in an oven to obtain a concordant value of dry biomass. When the culture was grown on a water-miscible substrate, DCBM was determined by centrifuging of the culture broth at 7,740 × *g* for 15 min. The cell pellet obtained was dried overnight at 60°C and weighed. The dry cell biomass was expressed in g/l.

A. Production of crude Biosurfactant

5ml of x 10^8 *Bacillus* sp. seed culture was inoculated into a 500 ml conical flask containing 100 ml MSM containing 2 percent crude oil as hydrocarbon source. For 72 hours, the flask was shaken at 35°C with 150 rpm in a shaking incubator. The culture supernatant was obtained from centrifuging the culture broth for 20 minutes at 4 °C at 10,000 rpm. The supernatant was deproteinized by boiling it for 15 minutes at 110 °C. It was acidified to pH 3.0 after cooling by adding 2 N HCl. Further, at room temperature, biosurfactant was extracted continuously with ethyl acetate. For phase separation, the mixture was violently agitated and then left static. After solvent evaporation at 40 °C under reduced pressure, the organic phase was transferred to a rotary evaporator and recovered as a viscous honey coloured product (27).

B. Biosurfactant characterization

Molisch's test was used to check for the presence of carbohydrate group in the biosurfactant. Molisch's test consisted of mixing 3 ml of culture supernatant with 1 ml of 10% α-naphthol, then adding 1 ml of concentrated sulfuric acid. The carbohydrate content of the biosurfactant was determined as per (28, 29). After centrifugation, a 333 μl sample of the culture supernatant was extracted with two volumes of 1 ml diethyl ether. The ether portions were blended and evaporated to dryness before being mixed with 0.50 ml of distilled water. The sample was cooled to 25 °C after being heated at 80°C for 30 minutes, and its absorbance was measured in a spectrophotometer at 421 nm. By comparing with standard curve of pure L-lipopeptide (Sigma grade), the lipopeptide concentration was determined. The presence of lipid in the extract was confirmed using an emulsion test. After dissolving the extract in ethanol, it was decanted into distilled water.

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Foaming property was studied by hand shaking a 5 g/l of crude biosurfactant solution from the isolate for several minutes. The stability of the foam was monitored by observing them for 2 h duration.

A Fourier transform infrared spectrometer (FTIR. Nicolet 6700, USA) in attenuated total reflectance (ATR) mode in the range of 339 –71cm^{-1} was used to identify lipopeptide in *Bacillus sp.* The FT-IR spectra were obtained using potassium bromide (kBr) solid cells in a Thermo Niocolet AVATAR 330 FT-IR system, Madison, WI 53711-4495 in the spectral range of 4000-400 cm 1.The analysis was done in the Department of Chemistry, Annamalai University, India. To reduce scattering effects from big crystals, the air dried biosurfactant sample was crushed using a refined potassium bromide salt (Sigma). This powdered combination is then mechanically pressed to produce a transparent pellet through which the spectrometer beam may travel. The spectra were recorded and analyzed using the standard methods described by the previous authors (30).

C. Preliminary screening of biosurfactant producer for potential antifungal activity against A. Solani

5ml of x 10^8*Bacillus* sp seed culture was transferred to a 500 ml conical flask containing 100 ml MSM for this experiment. It was incubated for 72 hours at 35 °C. The culture supernatant was collected by centrifuging the whole culture medium for 20 minutes at 4 °C at 10,000 rpm. PDA powder was sterilized and put into 90 mm Petri plates after being dissolved in culture supernatant. *A. solani* mycelia plugs of six mm were aseptically placed into the center of the plates from a seven-day old culture. After that, the plates were incubated at 24 ± 2 °C. After 12 days of inoculation, the diameters of *A. solani* mycelial growths were measured, and percentage inhibitions were computed. All of the experiments were replicated thrice.

D. Evaluation of antagonism of crude Biosurfactant in pot assay

For the experiment, the susceptible commercial tomato cv. Rocky (Syngenta, India) was used. Seedlings were grown for two weeks in plastic trays (45 × 35 cm). The two-week-old seedlings were then transplanted into clay pots (15 ×12 cm) with 2 kg of soil and the required doses of fertilizer NPK (3:4:6) and micronutrients. For five weeks, the plants were kept in their natural habitat before being treated with fungal spore, fungicide, and crude biosurfactant. The experiment was carried out twice between November and February of 2018–2019, while the temperature and humidity were between 18 and 24 °C and 75–80 %, respectively. The *Alternaria solani* fungus was cultivated in a 90-mm PDA plate and cultured for nine days at 24± 2 °C. The spores were extracted from fully developed cultures using a cold distilled water spray, as described by (31) and the spore count was adjusted to 10^6 spores ml^{-1} using distilled water using a haemocytometer. In the months of November and December (2018) a field trial was conducted with five-week-old plants. The experiment included three replicates of each healthy plant (Control), fungicide-treated FT (Bavistin 50 % WP carbendazim), and crude biosurfactant treatment at concentrations at 1, 1.5, 2, 2.5, 3.00 and 3.5 g l^{-1} of sterile distilled water. The treatments were set up in a full Randomized Block Design, with spore suspensions of *A. solani* (50 ml each of 10^6 spores ml^{-1}) sprayed to run-off over all but the healthy plant (control). The fungicide was made in distilled water at the required dosage of 1.00 g l^{-1}. The crude biosurfactant solutions were made by dissolving the extract in sterile distilled water to achieve the appropriate concentrations. The approach outlined was used to design the marijuana trial experiment. To increase the possibilities of disease development, the spore injected plants were covered with transparent plastic bags for 24 hours following inoculation (32). For the control plants, distilled water was sprayed on them and they were covered with bags for 24 hours. After 1, 8, 15, and 22nd days of spore inoculation, the fungicide solution and biosurfactant concentrations were sprayed according to treatments. On the 29th day after fungal spore injection, the disease incidence was determined by dividing the number of infected/wilted leaves by the total number of leaves in the plant. During the months of January–February (2019) a second pot experiment was conducted to determine the lowest biosurfactant concentration necessary to prevent *A. solani* infection in plants under field circumstances. The experiment was conducted using the same methodology as the first.

E. Statistical analysis

Using PASW statistics 18, the data were submitted to one-way analysis of variance (ANOVA) and pair-wise least significant difference (LSD) (SPSS Inc., IL, USA). At the 5% level, the variations between the means were judged significant.

III. RESULTS

Bacillus sp. was isolated from the rhizosphere soil area mangrove system at Vellar- Coleroon Estuarine complex, killai backwater, Chidhambaram, Tamilnadu, India. Biosurfactant producing microorganisms *Bacillus sp*. is analyzed. Biosurfactants are naturally occurring surface active compounds derived from the microbes from the extracellular excretion by bacteria and fungi. It is composed of generally glycolipids or lipopeptides in which Rhamnolipids, Trehalolipids and Sophrolipids compounds are dominant. Biosurfactant have both surface tension reduction and emulsification activity. It has the advantage of high efficiency in broad range of pH and salt concentrations, thermo-stability, low toxicity, good biodegradability, ecological acceptability when compared over chemical surfactants (33).

Biosurfactant producing ability was tested in crude oil as the hydrocarbon in a Minimal Salt Medium (MSM). The biochemical and Fourier transform infrared spectroscopy studies reveal the Biosurfactant is a lipopeptide. Lipopeptides are microbial biosurfactants and it is used successfully in enhancing the oil recovery under extreme environmental conditions. Cyclic Lipopepetides produced by different genus of the *Bacillus* can be classified into three families viz., surfactin, iturin and fengycin based on their chemical

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nature they have antifungal, antimicrobial and high surfactant activity.

The isolate found to produce Biosurfactant which is evident from the rapid screening test emulsification activity, CMC, blood agar test, oil spreading test, CTAB test, surface tension analayzing, drop collapse, hydrophobicity index, lipid, protein and food stable were checked and presented in table 1.

A. Haemolytic activity

Biosurfacant producing strain is identified by the blood agar haemolysis method. In this haemolytic activity the blood agar was prepared according to the method described by (13). Bacterial strain was streaked onto blood agar plates containing (Tripticase soy agar medium & 5% fresh sheep blood sample) and incubated at 28° c for 72 h during incubation, the formation of halo zones was observed the plates were visually inspected for clearing zone around the colonies, indicative of biosurafcant production. The diameter of the clear zones depends on the concentration of biosurfacant (34). The zones of haemolysis indicate the biosurfacant production.

A du Nouy Tensiometer (Kruss Digital-tensiometer 10, Hamburg, Germany) was used to measure the surface tension. A platinum wire ring submerged in the solution was slowly pulled through the liquid-air interface, to measure surface tension (mN/m). Surface tension measurement values were recorded and expressed as 29.11 mN/m. The surface tension value shown was the average from the same culture. Control consisted of a sterile culture medium plus an inoculum, at initial at 28 ± 2°C. Distilled water and isopropanol were used as standards (22).

B. Drop collapse test

According to this test polystyrene led of a 96-microwell titre plate was taken in that 2µl of crude oil applied in the well region and equilibrate for 24 hours. After equilibrate, 5 µl of cell free culture was transferred over the oil coated regions and the drop size was observed one minute later by using magnifying glass. If the drop size (diameter) is 1mm larger than that produced by deionised water (negative control) and it can be considered as positive biosurfactant production. The drop collapse technique was performed by following procedure described (20, 23).

C. Bacterial adherence to hydrocarbons (BATH)

- BATH assay was carried out as described based on the degree of cell adherence to liquid hydrocarbon following a brief period of mixing (25).
- Hydrophobicity was expressed as the percentage is 71.56%.
- Dry cell biomass of a hydrocarbon grown in microorganism was determined. The dry cell biomass was expressed in g/l.

Table 1

Organism	Blood agar lyis	Drop collapse test	Oil spreading (cm)	Surface tension (mN/m)	Hydrophobicity index (%)	CTAB agar plates	Lipase activity	Emulsification activity	CMC Value (mg/l)	Lipid test	Protein test	Foam stability y
Bacillus sp	+	+	**3.2**	**29.11**	**71.** 56	+	+	75. 56	**74**	+	+	**Very good**

Oil spread test, in this test adding of 20 µl of crude oil to a petri plate containing 50 ml of distilled water and then add 10 µl of cell-free bacterial supernatant was added to the oil surface. The diameter of the clear zone on the oil surface was measured and expressed positive value is 3.2 cm. In this test the distilled water use as negative control.

D. Cetyl Trimethyl Ammonium Bromide (CTAB) plate assay procedure

CTAB plate assay procedure was carried out according to (62). The cultures were grown in orbital rotary shaker using 500 ml shaker flasks at 34°C for 24 h with the Siegmund and Wagner Medium (SWM). The SWM contained 20 g glucose, 0.7g KH_2PO_4, 0.9g Na_2 HPO_4, 2g $NaNO_3$, 0.4g $MgSO_4H_2O$, 0.1g $CaCl_22H_2O$, distilled water - 1000ml and 2 ml of a trace element solution, which contained, per liter, 2 g $FeSO_4.7H_2O$, 1.5 g $MnSO_4.H_2O$ and 0.6 g $(NH_4)_6$ $Mo7O_2.4H_2O$. The SWM agar plates were prepared by adding 0.2 g CTAB, 15 g agar to 1 litre of the above medium. Following the improved procedure of Gunther *et al.* (2005), shallow wells were cut on the agar plate surface using the gel puncher. Ten µl of the inoculum was already prepared and added into each well. The plates were incubated for 48 h at 34°C. In control plates only medium without inoculum was added in the wells. The blue halo zones indicated**:** '+'; whereas '_', as no halos. Three replicates for each isolate was maintained and clear zones in each plate was analyzed**.** These plates are indicated blue halo zones (positive) the production of biosurfactant.

E. Emulsification avtivity

The assay was carried out by adding kerosene (3ml) to the cell free culture liquid (3ml) in a test tube. The tubes were then vigorously vortexed for 2 minutes and allowed to settle for 24 h before the % of volume occupied by the emulsion was determined. The emulsifying activity of the biosurfactant was determined by using the cell free culture broth (26). The equation used to determine the emulsion index *E24* (%) is as follows:

$$E_{24}(\%) = \frac{\text{The height of emulsion layer}}{\text{The height of total solution}} \times 100$$

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The equation used to determine the emulsion of emulsification activity index *E24* (%) value is 75.76%.

F. CMC value of the biosurfactant

This method involved the measurement of the surface tension of a series of dilutions. The CMC was determined from the break point of the Surface tension versus dilution times curve. The dilution reduces the biosurfactant levels below the CMC value at a point in which the surface tension of the media increases. The *Bacillus sp.* grown in the MSM containing glucose 2 per cent for 72 h in an orbital shaker at 120 rpm. Then, the surface tension of the media was determined as described earlier (section 3.6.2) after making dilutions of $10^{-1,}$ 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}. The dilution at which the surface tension suddenly increases value is 74 **(mg/l)** the break point (CMC).

For the experiment is carried out biosurfacant producing microorganism and strain is identified during, the lipase activity, lipase test and protein test was observed the plates were visually inspected, report on positive the **indicative of biosurafcant production**. Foam stable was determined very good.

G. Estimation of macromolecules

The concentrated extract of the isolate *Bacillus sp.* was subjected to estimation of protein, carbohydrate and lipid by the methods said in procedure. Total protein was estimated as 65.29 µg/0.1ml and lipid as 286.2 µg/0.1ml.

IV. MOLECULAR COMPOSITION AND STRUCTURAL ANALYSIS FOURIER TRANSFORM INFRARED (FT-IR) SPECTROSCOPY FOR CRUDE BIOSURFACTANT

The molecular composition and structural analysis of the isolated biosurfactant was evaluated by FT-IR. The important peaks, which were located at 3359, 1635, 1559, 1456, 1407 cm^{-1} confirmed the presence of lipopeptide type biosurfactant (Fig. 3). The peak appearing at 3359 cm^{-1} denoted the presence of –OH stretching carbohydrate group. The characteristic peak appearing for 1653 (C═O-NH) compound was observed at cm^{-1}, conforming –O=C stretching vibrations of glycoprotein. The important adsorption peak, suggested the presence of lipopeptides in *Bacillus sp.*

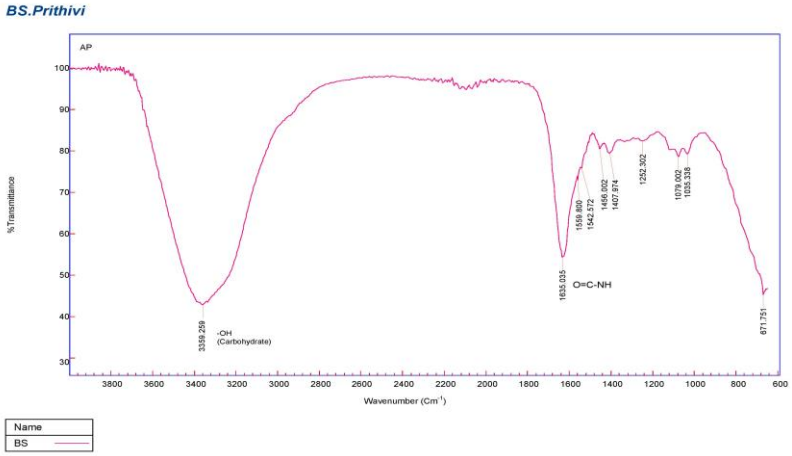

Fig. 3: Fourier Transform Infrared (FT-IR) spectroscopy for crude biosurfactant

A. Microtiter plate assessment of antifungal activity against A. solani spore

The antifungal effects of crude and column purified biosurfactant against *A. solani* spores were tested in a microtiter assay. The reference standard was commercial lipopeptide (Sigma Aldrich, USA). Inoculated 96-well microtiter plates containing 100 µl of crude biosurfactant, column purified biosurfactant, and R-95 at concentrations of 25, 50, 100, 200, and 300 µg ml^{-1} in PDB with a 20 µl spore solution of *A. solani* (10^6 spore's ml^{-1}). There was also a positive control (just PDB) and a negative control (PDB + spore suspension) in the tests.

For 48 hours, plates were incubated at 24 2 °C. The percentage of inhibition was measured for all three treatments by measuring optical density (OD) at 600 nm in a Varioskan Flash Multimode Reader (Thermo Scientific, Waltham, MA, USA).

B. Microtiter plate assay

The antifungal activity of the crude and column purified biosurfactant, as well as R-95, against *A. solani* spore was found to be significant ($p < 0.05$, F14, 45 = 2.79) at all concentrations. After 48 hours of incubation of fungal spore in various treatments, the percentage inhibition was calculated. At concentrations of 25, 50, 100, 200, and 300 mg l^{-1} (w/v), crude, column purified, and commercial biosurfactants were used. With crude, percentage inhibitions were 26.60, 44.66, 50.09, 55.72, and 58.64; with column purified, 22.82, 47.47, 55.95, 58.98, 69.49; and with commercial biosurfactant R-95, 42.71, 53.39, 61.35, 69.90, 74.95. The antifungal activity of R-95 against the phytopathogen *A. solani* is considerably superior (p 0.05, F1, 38 = 4.35) when compared to the crude biosurfactant, according to the findings of an analysis of variance between crude and commercial lipopeptide R-95. The results also showed that the effects of crude and column purified biosurfactant were similar, as there was no significant

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difference between the two treatments ($p > 0.05$, F1, 38 = 0.78).

C. Pot culture trial

The crude biosurfactant generated by strain AP7 was found to have equivalent efficacy to the column purified chemical and was chosen for testing bio control activity against *A. solani* growth in a pot culture trial because it would be a cost-effective way for treating the disease in the field. Between November and February of 2013–2014, two separate pot experiments were undertaken to determine the concentration necessary to suppress the phytopathogen *A. solani* in tomato plants. The concentration of biosurfactant utilized in the first pot trial was 3.00 g l^{-1}, which was the highest concentration of biosurfactant employed in the in vitro studies. The results of the pot trial revealed that plants treated with biosurfactant at 3.00 g l^{-1} may fully prevent pathogen growth in the plant, but plants treated with 0 g l^{-1} of biosurfactant had a disease incidence of 77.00 %. During the whole study period, the plants inoculated with 3.00 g l^{-1} of crude biosurfactant of AP7 displayed entirely healthy development, with healthy leaves and perfect disease inhibition. In the second pot testing, the lowest crude biosurfactant concentration effective against *A. solani* infection in the plant was determined.

The illness incidence was only 16.60 % at a concentration of 0.75 g l^{-1}, according to the results of this trial. At a concentration of 1.50 g l^{-1}, disease signs did not emerge in any area of the plant. The results were comparable for higher doses, with 100 % disease inhibition (Fig. 3). There was no significant difference between healthy plants (control) and plants receiving crude biosurfactant of 1.50 g l^{-1} or more, according to the findings. Furthermore, because there was no significant difference between the treatments, the effects of chemical fungicide and plants receiving 0.75 g l^{-1} crude biosurfactant were comparable.

V. DISCUSSION

According to the findings of this study, the crude biosurfactant generated by *B. sp* AP7 may effectively suppress the growth of the early blight causative phytopathogen *A. solani*. Lipopeptide was discovered as the crude biosurfactant. After 30 days of inoculation with *Alternaria solani*, it has been widely reported that lipopeptide can be a potential antifungal agent to control various diseases inhibition (percent)activity and disease incidence (percent diseased leaves) at various concentrations of crude biosurfactant extracted from *Bacillus sp* and fungicide treated FT (Bavistin 50 % WP carbendazim). SD is shown by the error bars. Different letters show substantially different values of multiple crop plants according to LSD at a = 0.0C. (19, 35). To resist infections, biosurfactants have developed distinct processes, and numerous research have been performed to clarify those mechanisms. *Pseudomonas sp*. has been shown to generate a variety of heterocyclic aromatic compounds, such as quinoline, as well as secondary metabolites, such as antibiotics, Fe-chelating siderophores, and cyanide, that are primarily responsible for fungal suppression (36, 37, 38, 39, and 40).

Furthermore, other enzymatic activities, such as cellulolytic and chitinolytic activity, may have a role in disease inhibition (21, 41). Lipopeptides, which interact directly with the lipid bilayer of the plasma membrane and cause plasma membrane instability, activate plant defense signaling pathways (16).The biosurfactant was generated in this work by a strain of *Bacillus sp*, which is a common soil microflora resident. Biosurfactants are environmentally friendly since they are biodegradable and do not produce hazardous byproducts. Because the performance of living microorganisms for biocontrol has been found to be variable, and the cell free culture supernatant may result in an uneven distribution of metabolite in the system, crude biosurfactant was employed for the current experiment (42, 43).

The fungal mycelia were inhibited by 73.00 and 75.26 % in an in vitro experiment employing crude biosurfactant and column purified biosurfactant, respectively. The activity of biosurfactant rises as the purity of the biosurfactant increases. The antifungal activity of column purified biosurfactant was found to be equivalent to that of crude biosurfactant in the current study (44).

The plants were infected 24 hours before fungicide and biological agent treatment using a spore solution in the pot test. For a single disease cycle, a wetness of approximately 24 hours would enable almost the maximal infection state (32). The in vitro test indicated that the spore germination may be successfully inhibited by the biosurfacant. Treatments have therefore been used to prevent spores and the consequent manifestation of disease symptoms upon inoculation of the fungus fungal spores on the host surface. In an earlier research by lipopeptides have similarly reported an overwhelmingly preventative impact of the suppression of development of illness during germination (45). However, the impact of lipopeptide following the onset of the illness was limited (curative effect). Also present in this study was minimal lipopeptide impact after the onset of the disease, since numerous uses of lipopeptide at B 0.60 g l^{-1} do not seem to have an important influence on the progression of disease symptoms. The many mechanisms of antagonistic interaction between biosurfactants and microbial membranes were addressed by reports that biosurfactants had substantial antifungal effects on mycelia and other structural structures (10). The surface activity of lipopeptide in nature may have a detrimental impact on the expanding hyphae generated at the first stage of spore germination, therefore preventing disease symptoms from developing or developing. Thus the activity of lipopeptide seems preventive and fully inhibited at the concentration is 1.50 g l^{-1} lipopeptide as fungal hyphaes at the initial stage are totally destroyed, limiting the development of typical symptoms. At the tomato *A. solani* pathosystem, we have seen a decrease in the disease incidence to 16.66 % in the concentration of 0.75 g l^{-1} of crude biosurfactant and total fungal pathogen inhibition above 1.50 gl^{-1} levels. The incidence of the illness reported was 12.35% with 1.00 g l^{-1} of commercial fungicide. The crude biosurfactant and

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chemical fungicide both had the same efficacy at a concentration of 0.75 g l^{-1}, as the difference was not statistically significant.

The antagonistic activity of crude biosurfactant AP7 against Alterneria solani was determined by incorporation in PDA plates, the crude biosurfactant exhibited a significant inhibition of the growth of pathogen composed to the untreated i.e., control. biosurfactant AP7 is a produced increase in its concentration that induced significant increase in fungal growth inhibition. Bacillus species produced biosurfactant showed promising antagonistic effect against the Alterneria solani. Whwn grown in PDA plate Rhamonolipid biosurfactant has subsequently shown to be highly effective against plant pathogen. (5). Certain biosurfactant that have high antifungal activity against plant pathogen and it can be considered as promising biocontrol in sustainable agriculture.

A recent study involving the trade lipopeptide in a *Fusarium oxysporum f. sp. pisi* microtiter study shows an inhibition rate of around 84% at 0.10 grammes g l^{-1}, while the same *A. solani* lipopeptide at a similar concentration shows an inhibition rate of 61.35% in this study (46). During the same investigation, *B. sp* mono-lipopeptide with a composition of congeners distinct from R-95, with a concentration of 25 μg^{-1} against *F. oxysporum F. Sp. pisi* in pea plants was shown to be efficient. It may therefore be concluded that the effective concentration of lipopeptide may vary according on the pathogen type studied and the makeup of the lipopeptide's constituent congeners.

The biosurfactant has been sprayed four times during the plant experiment. As instance have also stated 2-3 times the use of biosurfactant to effectively control the pathogen (46, 47). Biosurfactants are degradable under the environment. By way of biochemical and spectrometric analyses, the strain producer has been identified as a lipopeptide. In the LC–MS analysis of the extracted biosurfactant seven distinct congeners consisting of 4 mono and 3 di-lipopeptides were identified. The ring lipopeptide biosurfactants may usually be discovered as blends of distinct congeners of lipopeptide, as reported by different strains of *B. sp* (48, 49, 50 and 30). The resulting antifungal action of a certain lipopeptide against a certain fungus may play a crucial role in these composing congeners and their unique combinations. The comparative antifungal investigation using lipopeptide has further supported this finding. The existence of 15 lipopeptide conger ants has been found in LC-MS analyses of R-95 (11 mono and four di- lipopeptide congeners). The discrepancy in fungus anti-testing action and commercial lipopeptide can be explained by the underlying difference in their component congeners. The *B. sp* AP7 strain of lipopeptide biosurfactant can be an effective anti-fungal agent to prevent early blight infection in tomato plants. The use of 0.75 g l^{-1} of crude biosurfactant might demonstrate the same effectiveness level as with the chemical fungicide. At concentrations of 1.50 g l^{-1} and above, the biosurfactant may suppress the illness occurrence entirely. The usage of raw lipopeptides was favoured in relation to the use of purified lipopeptide, as it offers a more cost effective alternative than the latter. The low-toxic, highly biodegradable biosurfactant can be efficiently employed as a possible option in the replacement of hazardous agrochemicals.

VI. CONCLUSION

An efficient biosurfactant producing and high antifungal activity *Bacillus sp* AP7, isolated from rhizosphere soil area mangroves ecosystem were analysed. From the study it can be concluded that high antifungal activity, high stability, high emulsifying activity it has major possibilities for its usage in environmental protection of synthetic agro chemicals. In this followed by the results of the pot trial revealed that plants treated with biosurfactant at 3.00 g l^{-1} may fully prevent pathogen growth in the plant, but plants treated with 0 g l^{-1} of biosurfactant had a disease incidence of 77.00 %. During the whole study period, the plants inoculated with 3.00 g l^{-1} of crude biosurfactant of *Bacillus sp.* AP7 displayed entirely healthy development, with healthy leaves and perfect disease inhibition. Biosurfactant that have high antifungal activity against plant pathogen and it can be considered as promising biocontrol in sustainable agriculture.

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