Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(3): 726-734
Received: 05-03-2020
Accepted: 08-04-2020

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Production and characterization of extracellular compounds from *Pseudomonas aeruginosa* and their impact on *Dendrocalamus asper* under *in vitro* stress condition

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DOI: https://doi.org/10.22271/phyto.2020.v9.i3l.11360

Abstract
Abiotic stress factors affecting plant growth and production around the world. In this experiment, *in vitro* multiplication of Dendrocalamus asper has been standardized. Pseudomonas aeruginosa producing metabolites are improved the plant immunity, altered the signaling pathway and produce stress tolerance plants. *Pseudomonas aeruginosa* was isolated from soil and identified using 16S rRNA gene sequencing**.** The pyocyanin production medium were optimized and successfully extracted their extracellular metabolites using chloroform extraction method. Bamboo was cultivated with MS medium supplement with BAP (1, 2 & 3mg/l) were maintained for mass multiplication. The media was optimized with BAP 1mg/l is produced multiplication of shoot and mass culture. To study the effects of pyocyanin on stress in bamboo (*Dendrocalamus asper*), the explants were treated with varying concentrations of pyocyanin (0.2, 0.4, 0.6 & 0.8mg/l) under *in vitro* condition. The physiological and morphological responses of the crop against pyocyanin stress has been identified. The Chlorophyll pigment present in the plant was decreased due to stress condition. Pyocyanin induced an accumulation of phytohormones in the leaves under *in vitro* stress condition. In pyocyanin treated plants are significantly increased the proline content. Pyocyanin and was found to be effective against abiotic stress with the help of Morphological and Physiological test.

Keywords: Pyocyanin, 1-hydroxy phenazine, *Dendrocalamus asper*, *in vitro*, redox potential

Introduction
Abiotic stress is one of the major growth limiting factors, decreasing propagation rate of plant under natural as well as artificial conditions. Abiotic stress are induce slow growth, stomatal closure and therefore reduces photosynthesis [20]. Plants have evolved to live in environments where they are often exposed to different stress factors in combination. Today, agricultural land area is losing its fertility because of soil salinity, drought and fertilizer increasing with the passage of time in arid to semi-arid regions of the world [27]. Normal plant growth is assumed, when cultured under normal and balance nutritional conditions. While, if any of the nutritional components (may be inorganic or organic) is not properly supplied (deficient or exceeded) than it leads to abnormal expression in them [24]. Pseudomonas aeruginosa producing pyocyanin and play a major role in food, agriculture and textile industries. Many research, focuses on replacing the synthetic pigments with eco-friendly natural pigments and bio pigments. Pigments are naturally found in plant, microorganisms, ores and insects. Among these, microbial pigments and surfactants are highly favored due to their around the year availability, and for the level of control and reproducibility of the quantity and quality. Most microorganisms are even characterized and named based on the colour they produce [17]. Among those pigment producing microbes, *Pseudomonas species* is a highly researched and explored microbe. Different species of *Pseudomonas* has been found to produce different pigments such as pyocyanin (Bluish green), pyorubin (red), pyoverdine (Yellow-flourescent), pyomelanin (brown) and biosurfactant (rhamnolipid) [7]. But all these pigments and biosurfactant are very low in quantity and its extraction is a high resource consuming process [17].

In short *Pseudomonas* is a microbe that is able to produce phenazine and their derivatives. Most *Pseudomonas* has a characteristic yellowish green colour due to two compounds, phenazine-1-carboxylic acid and chlororaphin which is an amide of phenazine-1-carboxylic acid. Though these pigments and bio surfactant have been explored and exploited for their biological activities [5].

Pyocyanin acts as a virulence factor produced by Pseudomonas aeruginosa, a plant and animal pathogen. In this study, we evaluated the effect of pyocyanin on growth and development of tissue culture plant. Pyocyanin treated plants are modulates root system architecture, inhibiting primary root growth and promoting lateral root and root hair formation without affecting meristem viability or causing cell death [18]. Pyocyanin treated plant root growth was likely independent of auxin, cytokinin, and abscisic acid. Pyocyanin is an important factor modulating the interplay between reactive oxygen species (ROS) production and root system architecture [27].

Plant growth depends on proper metabolic processes. Metabolic dysfunction is caused by ion toxicity, osmotic stress and nutritional deficiency [23]. Under stressed conditions, plants have to synthesis and accumulate various compatible solutes such as free amino acids (proline, betaine etc.), sugars and polyols. The fluctuations in metabolites are observable in growing Dendrocalamus asper through micro-propagation [8]. Utilization of tissue culture techniques for quantifying stress tolerance of various crops has been increasing rapidly. Also, *In vitro* culture techniques minimize environmental variations due to defined nutrient media, controlled conditions and homogeneity of stress application. Plants activate a specific and unique stress response when subjected to a combination of multiple stresses [54]. So plant tissue culture system has been acting as a useful tool to assess effectiveness of various stress factors on plant growth related characters. However, abiotic tolerance mechanisms involved at the whole plant level growing under natural conditions could be quite different from *In vitro* cultures [4]. Therefore, the objectives of this study were to determine the effect of pyocyanin on the some morphological and physiological exchanges in bamboo. Whether application of pyocyanin to bamboo might be a strategy for increasing the abiotic tolerance.

Materials and Methods

Chemicals

All the chemicals used in the experiments were of analytical grade and procured from Hi-Media, India. All the media components used were of the bacteriological grade.

Bacteria Isolation

The soil sample was collected from industrial polluted area at erode and further analysis was carried out in the laboratory. The soil sample was serially diluted and were pour plated on nutrient agar plates to obtain isolated colonies. These colonies were streaked on cetrimide agar plates for screening *Pseudomonas* from the colonies. The colonies were selected based on the characteristics of diffusible green pigment production. The selected isolate was subjected to biochemical tests and gene sequencing followed by BLAST analysis for species confirmation. Biochemical characterization was carried out based on *Bergey's manual of determinative bacteriology* [26].

Screening potential of pseudomonas aeruginosa

Ability of pigments on kings B medium

All isolates of *Pseudomonas aeruginosa* were screened for pigments production on king's B medium. After preparing the medium, it was poured in sterile condition on Petri dishes and left to solidified. All the bacterial isolates were incubated on kings B medium at 35° C for 3 days. The isolates were presumptively identified by morphology and pigment formation on selective medium.

Presumption test

Add 2-3 ml chloroform in extracted pigments. Bluish color was developed. After addition of 0.2N HCl, it gives pinkish red color which is conformation test for pyocyanin pigment [57].

Oil displacement method

100µl of castor oil was placed on the surface of 30ml of distilled water in a petri dish and 10µl of cell-free supernatant was added on the surface of oil film. The strain which caused oil spreading under visible light were considered positive [63].

Foaming activity

Isolated strain was inoculated on the nutrient medium and the flask was incubated at 37ºC in a shaking incubator for 48hrs. After incubation, based on foaming the culture was referred to be positive or negative.

Drop collapsing test

100µl of Castrol oil was placed on the surface of the slide and 50µl of cell-free supernatant and distilled water (control) was added on the surface of oil. The surface area of the drop was observed under a microscope to conform bio surfactant production [47].

Blue agar plate method

Mineral salt agar medium prepared with glucose (2%), cetyltrimethylammonium Bromide (0.05%) and methylene blue (0.02%) was used for the detection of anionic rhamnolipid bio surfactant. Wells of 4 mm diameter were made using cork borer on methylene blue agar plates and loaded with 30µl of fresh culture of individual isolate. The plates were incubated at 37ºC for 48-72 hours [56]. A dark blue halo zone around the culture was considered positive for *pseudomonas spp*.

Pyocyanin extraction and quantification

The pseudomonas aeruginosa culture broth were centrifuged to obtain cell free supernatant. To the 5ml supernatant 2.5ml of chloroform was added. The pigment dissolves in chloroform making it blue. To the 2.5ml of chloroform 0.5ml of 0.2N HCL was added and vortexes. The HCL layer turns pink. To 200µl of this pink solution 2.3ml of 0.2N HCL was added and absorbance was measured at 520nm [26].

Concentration of Pyocyanin pigment (µg/ ml) = O.D @ 520 nm x Dilution factor x 17.072

Dilution factor = Final volume/ Initial volume = 2.5/0.2 = 12.5

Pigment production medium optimization

Four types of Media including nutrient broth, nutrient broth supplemented with glycerol (2% w/v), and mineral salt media supplemented with lactose, maltose, sucrose, glycerol and glucose (2% w/v) were tested for the biosynthesis of pyocyanin for 3 days at 35 °C at 110 rpm. The purpose of the study on mineral salt media (MSM) with different carbon source supplements is to find the carbon source that best influences the pyocyanin production [32]. The parameter for screening growth media and deciding optimum growth parameters is pyocyanin quantity produced in media

Effect of pyocyanin in Different pH and Temperature

Nutrient broth supplemented with glycerol is prepared for pH 6, 7, 8, and 9. After inoculation the culture broths were incubated at 35ºC and 110 rpm. Nutrient broth supplemented

with glycerol is prepared at pH 8 and is incubated for a day at different temperatures 28, 35, 40, and 50ºC. Since the incubation period is only two days, 1 ml inoculum is taken for 100 ml media.

Antibacterial activity

The antimicrobial assay was done by a well-diffusion method using Muller-Hinton agar medium because it is a non-selective and non-differential medium. It also contains starch which absorbs toxins released from bacteria, so that there is no interference with the antibiotics. Muller-Hinton agar is a loose agar, allows better diffusion of the antibiotics. A better diffusion leads to a clear zone of inhibition (Park SC, *et al*. 2004). Wells of 6 mm diameter were punctured on Muller-Hinton (MH) agar plates. The 100 µL of microbes (*E. coli, Staphylococcus aureus, lactobacillus, salmonella spp, bacillus spp* and *Serratia marces*cens) were spread uniformly on the surface of MH agar plates. Different concentrations (200 µg, 1000 µg, 2000 µg) pyocyanin pigment prepared in chloroform, were added to each well [35]. Chloroform was used as negative control. The antibacterial activity of each extract was determined in terms of the diameter of the zone of inhibition (mm) formed around each well after the incubation period. Diameter measurement scale was used to measure the zones of inhibition.

Purification by column chromatography

Pigment produced by pseudomonas aeruginosa was purified by column chromatography using silica gel (column size 20x 1.5 cm size). Silica gel as a stationary phase and a solution of methanol and chloroform at the ratio 1:1 as mobile phase. The eluted sample was further analyzed by TLC using methanol and chloroform at the ratio of 1:1 as mobile phase to check its purity and the retention factor value partially purified pigment was checked [17].

GC-MS Analysis of Bacterial Metabolites

Secondary Metabolites were analyzed by Gas chromatography coupled mass spectrometry (GC-MS) to identify the compounds present. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2µl was employed (a split ratio of 10:1). The injector temperature was maintained at 250 °C, the ion-source temperature was 200 °C, the oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase of 10 °C/min to 200°C, then 5 °C/min to 280 °C, ending with a 9 min isothermal at 280 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The total GC-MS running time was 36 min [28]. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Turbo-Mass Gold-Perkin-Elmer- mass-detector was used, and Turbo-Mass ver-5.2 software was used to handle mass spectra and chromatograms.

Preparation of MS medium

MS medium along with different concentration and various combination auxin and cytokinin was used in the media. The pH of the prepared medium was adjusted at 5.7 prior to addition of clerigel at 3.7 g/l. The medium was distributed into the culture jars (325 ml.), where each jar contained 30 ml of the medium. The culture jars were autoclaved at 121ºC at 15 lb/inch for 20 min.

Protocol for surface sterilization

Sterilization techniques are designed to kill or remove a wide range of micro-organism including protozoa, fungi, bacteria, viruses. Surface sterilization of explant is a process which involves the immersion of explants into appropriate concentration of chemical sterilant(s) or disinfectant(s) for a specified time resulting in the establishment of a contamination-free culture. The explants are washed with running tap water for half an hour followed by washing in 5ml of tween 20 solution. The surface cleaned explants were further subjected to wash in bavistin for 5 min then dip the explants into 70% ethyl alcohol and finally the surface sterilized explants were washed with 0.1% mercuric chloride ($HgCl_2$) for 4 min. Cultures were allowed to grow for 8 weeks at 25±2°C of day and night temperature. Light was provided by white fluorescent tubes giving intensity of about 1500 Lux at the explants level.

Proline assay

Proline content of the leaf sample was estimated by the method of Bates *et al*. (1973). It is necessary to study the accumulation of proline in the plant for water deficit stress activity. Fresh plant leaf samples of 0.3 g were macerated with 10ml of 3% aqueous sulphosalicyclic acid by using mortar and pestle. The leaf extract were spun at 4000 rpm for 10 minutes. Discard the pellet and then collect the supernatant solution for each samples was taken in a 2 ml of test tube and to this 2 ml of acid ninhydrin (Preparation for acid ninhydrin: 2.5 g of ninhydrin was taken and mixing to the 6 M 40 ml of orthophosphoric acid and 60 ml of glacial acetic acid with agitation until dissolved for water bath. Kept in 4 °C for reagent stored at 24 hours) were added for each samples and then 2 ml of glacial acetic acid were added for all test tubes.

The test solution were heated to kept in water bath for one hour incubation at 100°C and it was cooled under tap water for few minutes. After cooling, the test solution was transferred into a separating funnel. By adding 4ml of toluene were added in the separating funnel. The funnel was uniformly shacked during 15-30 seconds. Two different layers were separated. The upper colour layer was collected and another bottom colorless layer was discarded. The optical density was measured at 520 nm against using toluene for blank solution.

Result and Discussion

Bacteria identification

The DNA of the isolate was extracted using High pure PCR template kit (Roche). The DNA is cut short and amplified using PCR primer set, 27F-5'AGAGTTTGATCMTGGCTCAG3'and 1492R-5'TACGGYTACCTTGTTACGACTT3'. This amplified product was sequenced using automated sequencer with the primer set 518F-5'CCAGCAGCCGCGGTAATACG3' and 800R-5'TACCAGGGTATCTAATCC3'. This sequence obtained is aligned and ran through BLAST to identify the organism, only sequence obtained with 1200 base pairs or above is considered to give assured result in BLAST. In gene sequencing a sequence of 1493 base pairs was obtained. And on running through BLAST, it was observed that for 100% query sequence, a 99% identity was observed for *Pseudomonas aeruginosa*. And on cross verification with the results obtained from biochemical tests, the microbe was affirmatively identified as *Pseudomonas aeruginosa*.

Table 1: Biochemical characterization of *pseudomonas aeruginosa*

S. No.	Biochemical / Morphology test	Triplicate	Result
1	Gram staining	-ve	Gram Negative
2	Capsule staining	-ve	Non- Capsulated
3	Shape	-	Rod Shaped bacteria
4	Pigment production	-	Green pigment diffusible in agar
5	Litmus milk	(-)(-)(-)	Negative
6	Lactose	(-)(-)(+)	Negative
7	Methyl red	(-)(-)(-)	Negative
8	Indole	(-)(-)(-)	Negative
9	Citrate test	(+)(+)(+)	Positive
10	Catalase test	(+)(+)(+)	Positive
11	Salt tolerance test	(+)(+)(+)	Positive
12	Urease test	(-)(-)(-)	Negative
13	Starch Hydrolysis	(+)(+)(+)	Positive
14	Gelatin Hydrolysis	(+)(+)(+)	Negative
15	Casein Hydrolysis	(+)(+)(+)	Positive
16	Voges Proskauer	(-)(-)(-)	Negative
17	Oxidase	(+)(+)(+)	Positive

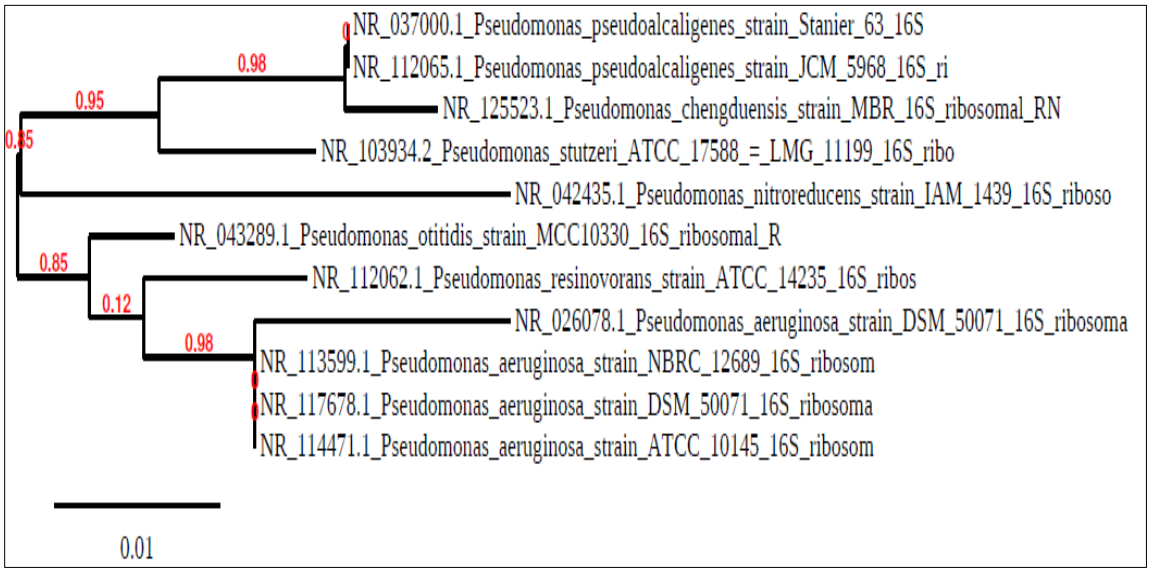

Fig 1: Phylogenetic tree

Extraction of pigments

The pseudomonas aeruginosa culture broth were centrifuged to obtain cell free supernatant. to the 5ml supernatant 2.5ml of chloroform was added. The pigment dissolves in chloroform making it blue. To the 2.5ml of chloroform 0.5ml of 0.2N HCL was added and vortexes. The HCL layer turns pink. To 200µl of this pink solution 2.3ml of 0.2N HCL was added and absorbance was measured at 520nm.

Concentration of pyocyanin pigment (µg/ml) = OD@520nm x dilution factor x 17.072

Presumption test for extracted pigments

The pseudomonas aeruginosa culture broth were centrifuged to obtain cell free supernatant and pigment was extracted with chloroform. Addition of 2-3 mL chloroform in extracted pigment, bluish color was developed and after addition of 0.2N HCl, it gave pinkish red color which is confirmation test for Pyocyanin pigment. This result was assured the positive presumptive test for pyocyanin.

Factors affecting on pyocyanin production

From each and every growth media the pigment was extracted using chloroform and is separated using acidified HCL and is quantified at 520nm. The media that showed highest absorbance. Nutrient broth with glycerol (2%) is showed to have high pyocyanin content. table 4.2 shows the absorbance of pigment at 520nm for respective media.

Table 2: Pigment production media optimization

S. No	Media	Absorbance @ 520nm	Pigment concentration (µg/ml)
1	Nutrient broth	0.242	51.6428
2	Nutrient broth + glycerol (2%)	0.246	52.4964
3	Mineral salt media + lactose	0.013	2.7742
4	Mineral salt media + glycerol	0.027	5.7611
5	Kings B medium	0.132	28.1655

Effect of Different pH and Temperature

Nutrient broth supplemented with glycerol is prepared for 6, 7, 8, and 9 pH. After inoculation the culture broths were incubated at 35ºC and 110 rpm. Figure-2 shows the pigment yield for the cultures grown at the respective pH. From the observation of the effect of pH it is evident that pH of range 7-8 shows high pyocyanin production. Thus pH 8 is set as the optimum pH for pyocyanin production for further studies. Similarly, nutrient broth supplemented with glycerol is prepared at pH 8 and is incubated for a day at different

temperatures 28, 35, 40, and 50ºC. Since the incubation period is only two days, 1 ml inoculum is taken for 100 ml media. Figure-6 shows the effect of different temperature in pyocyanin production.

On observing the effect of temperature, it is evident that the isolated *Pseudomonas* was able to produce pigment in high temperature such as 40ºC, but highest yield is recorded at 35ºC. Hence for further production of pigment, Nutrient broth supplemented with glycerol at pH 8 is used and the culture broths are incubated at 35ºC for a period of 3 days. Almost similar parameters and pyocyanin production was seen in work done by *Pseudomonas aeruginosa.*

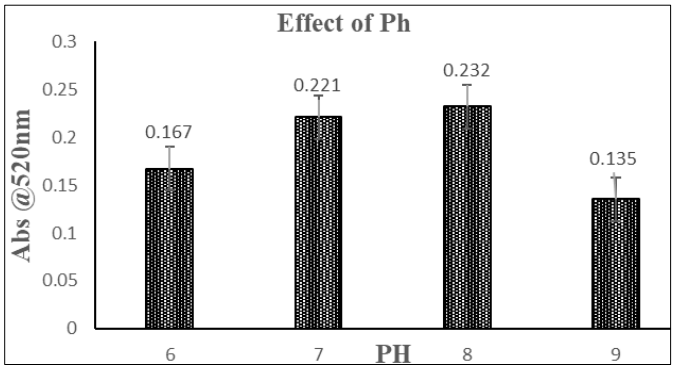

Fig 2: Effect of pyocyanin in Different pH

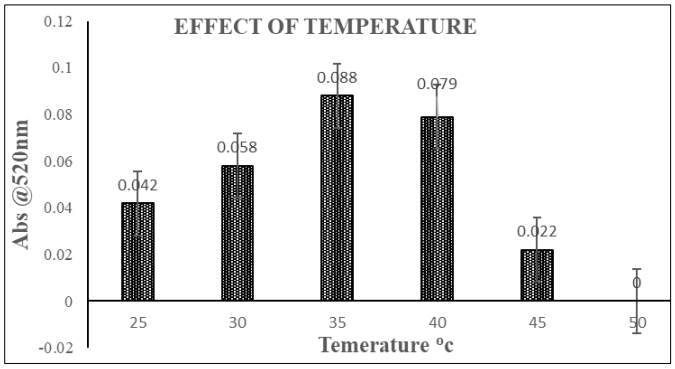

Fig 3: Effect of pyocyanin in Different Temperature

Antimicrobial activity

The Agar well diffusion method was the best plating procedure for detecting the antibacterial activity. The zone of inhibition observed in Muller Hinton agar plates for pyocyanin against *Escherichia coli, Staphylococcus aureus, lactobacillus spp, salmonella spp, bacillus subtilis* and *Serratia marcescens*. Results of the inhibition zone values for pyocyanin showed high antibacterial activity against gram negative *E.coli* and *serratia marcescens*. Pyocyanin had better antibacterial activity against gram positive Staphylococcus aureus and bacillus spp.

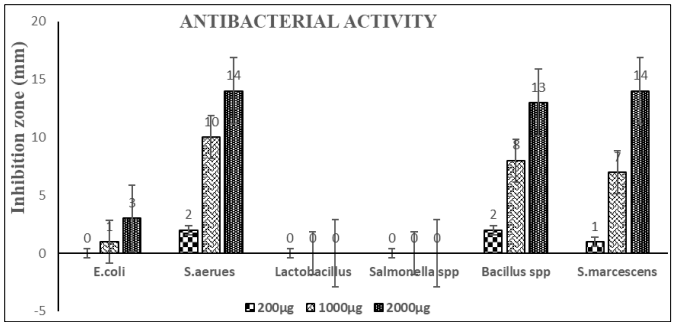

Fig 4: Antibacterial activity of pyocyanin

Purification of pyocyanin in column chromatography

Purification of extracted pigment was done by column chromatography (column size 20x 1.5 cm) using silica gel as a stationary phase and a solution of methanol and chloroform at the ratio 1:1 as mobile phase. The eluted sample was further analyzed by TLC using methanol and chloroform at the ratio of 1:1 as mobile phase to check its purity and the retention factor value partially purified pigment was checked.

Chromatographic analysis of the pigments

The pigment extracted using chloroform was run in TLC sheet using chloroform and methanol (1:1). From analysis, it is verified that the extracted pigment was pyocyanin as the retention factor was 0.74 (solvent front = 5 cm and solute front = 3.7 cm).

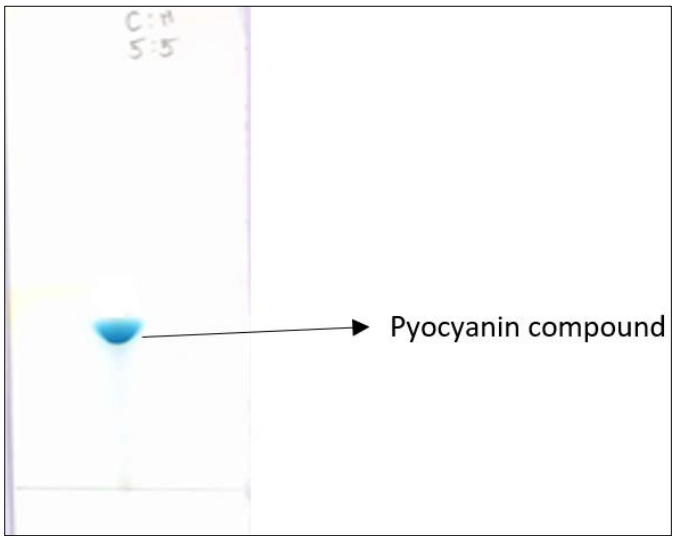

Fig 5: TLC analysis

Gas chromatography-Mass spectrometry of pyocyanin in the present study, showed the presence of 1-Hydroxy phenazine compound. The result was showed the related pyocyanin pigment extracted from Pseudomonas aeruginosa. From the GCMS result of the extract was found that the structure, 1-Hydroxy phenazine.

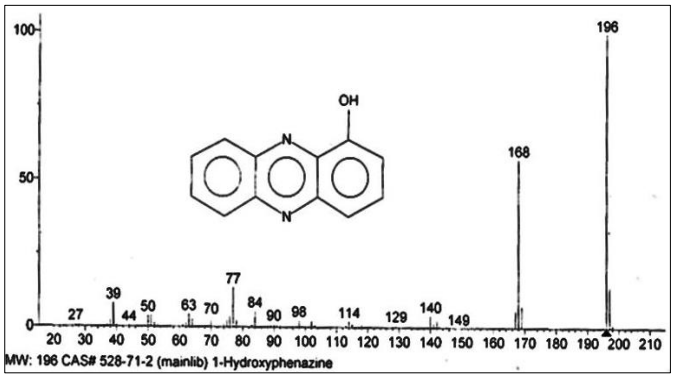

Fig 6: GCMS analysis of pyocyanin

Mass Multiplication of *Dendrocalamus asper*

The plants which are inoculated on the MS medium supplement with BAP (1- 3mg/l) was initiated after three week of inoculation in the culture room. After three weeks duration the initiated culture were sub cultured in the same medium for multiplication. The importance of the application of high BAP concentration to initiate bud formation from explants. A reduction in the number as well as length of root

that occurred with exposure to high levels of BAP alone (5mg/l) in bamboo plant. Synergistic effects of plant growth regulators have influenced the cultural response in bamboo shoot proliferation and elongation. The media was optimized with BAP 1 mg/l is produced multiplication of shoot and mass culture.

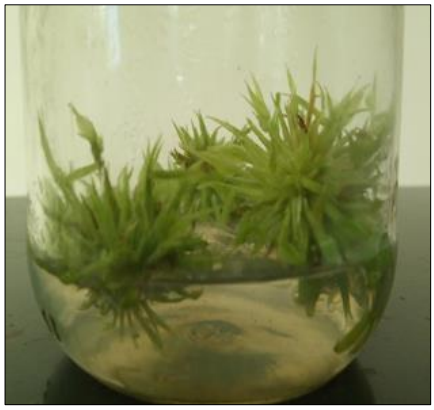

Fig 7: BAP 1mg/l

Fig 8: BAP 2mg/l

Fig 9: BAP 3mg/l

Stress Tolerance plant production

P. aeruginosa species to impact root physiology through production of plant hormones such as auxin or cytokinins, by stimulating root growth, or by altering root system architecture. Moreover, *P. aeruginosa* species provide protection against pathogens, tolerance to abiotic stress, and resistance to insect or herbivore attack. Bacteria communicate with plants through secreted signaling factors. *P. aeruginosa* strains also triggered an oxidative burst and phytoalexin accumulation in *Dendrocalamus asper* cells and primed leaves for accelerated phytoalexin production. Redox-active pyocyanin (PCN) secreted by *P. aeruginosa* triggers systemic resistance. The *Dendrocalamus asper* plants which are inoculated on the MS medium supplement with pyocyanin (0.2, 0.4, 0.6 and 0.8mg/l). Cultures were allowed to grow for three weeks at 25±2°C of day and night temperature. Light was provided by white fluorescent tubes giving intensity of about 1500 Lux at the explants level. These results suggest that *Pseudomonas*-derived metabolites can induce reactive oxygen species (ROS).

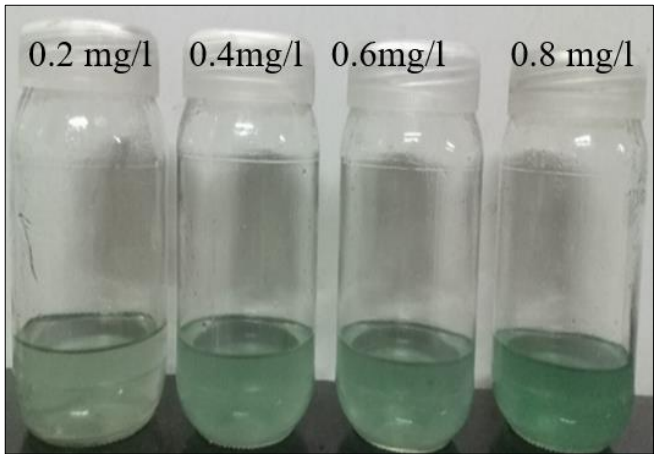

Fig 10: MS Medium prepared with pyocyanin

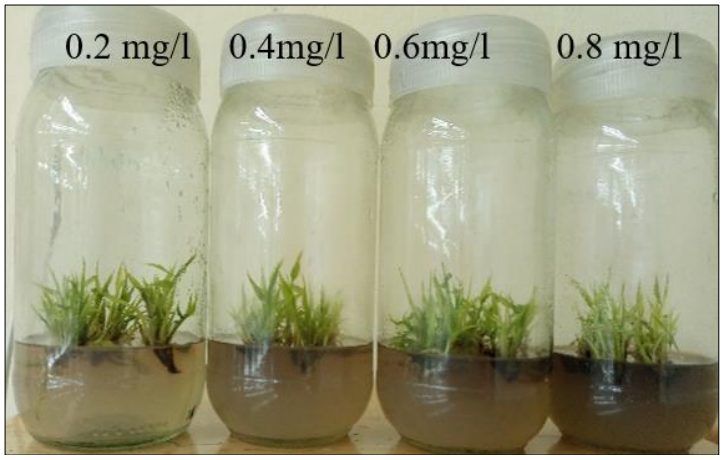

Fig 11: Plant growth with pyocyanin

Proline content of *Dendroclamus asper*

Proline contents increases significantly under pyocyanin stress (fig 4.12). Proline is responsible for protecting plant tissues from stress injury. It is responsible for imparting abiotic tolerance to plant tissue, but proline accumulation seems to be a symptom of cells injury rather than to an indicator for abiotic resistance. In our work it was observed that pyocyanin stress are acting as critical phenomena for bamboo micro propagation efficiency.

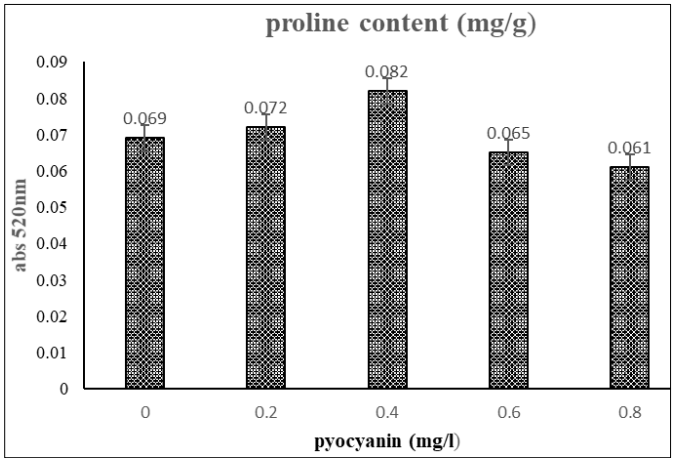

Fig 12: proline content of pyocyanin treated plant

Primary hardening and secondary hardening of *Dendrocalamus asper*

Primary hardening is an integral and vital activity of the whole process of tissue culture technology. In the present study cocopeat showed to be far superior to other potting media in terms of percentage survival of plantlets (95.00%), plantlet height (5.58 cm), number of leaves (3.20), plantlet diameter (4.59 mm), number of primary roots per plantlet (5.20), length of primary roots (5.18 cm) and number of secondary roots per plantlet (25.50 cm). Some of the findings of earlier workers indicated a survival percentage ranging from 80-100 when bamboo rooted plantlets were transferred to *ex vitro* hardening media under Green House or Shade House conditions. The potting mixture consisting of red soil: sand: cocopeat (1:1:1) recorded the best results in terms of plantlet height (20.50), plantlet diameter (11.60 cm) length of leaves (15.43 cm), width of leaves (6.47 cm), number of primary roots per plantlet (12.30) and number of secondary roots per plantlet (150.20). Probably cocopeat might have helped in improving physical and chemical properties of the growing media, consequently resulted in better growth of bamboo plantlets.

Conclusion

Abiotic stress signaling is an important area with respect to increase in plant productivity. Some Microbial metabolites are improved the plant immunity, altered the signaling pathway and produce stress tolerance plant. *Pseudomonas aeruginosa* was isolated from soil and identified using 16S rRNA gene sequencing**.** In gene sequencing a sequence of 1493 base pairs was obtained. And on running through BLAST, it was observed that for 100% query sequence, a 99% identity was observed for *Pseudomonas aeruginosa*. The pyocyanin production medium were optimized and successfully extracted their extracellular metabolites. These compounds are purified using column chromatography and identified using GCMS. The GCMS result of the extracted pigment was found that the structure, 1-Hydroxy phenazine. It is electron losing yellow color pyocyanin compound. The pyocyanin have good antimicrobial activity against *Escherichia coli, Staphylococcus aureus, lactobacillus, salmonella spp, bacillus subtilis* and *Serratia marcescens*. Pyocyanin are altered root formation and improved plant immunity. *Dendrocalamus asper* plant was developed under stress condition by giving different concentration of bacterial metabolites. Dendrocalamus asper growth media was optimized with BAP 1mg/l is produced multiplication of shoot and mass culture. The plants which are supplemented with pyocyanin (0.2, 0.4, 0.6 and 0.8mg/l) under *in vitro* condition. The Chlorophyll pigment present in the plant was decreased due to stress condition. Pyocyanin induced an accumulation of phytohormones in the leaves under abiotic stress. In pyocyanin treated plants are significantly increase the proline content of the bamboo. Pyocyanin was found to be effective against abiotic stress with the help of Morphological and Physiological test.

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