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Quinoline derivatives producing *Pseudomonas aeruginosa* H6 as an efficient bioherbicide for weed management

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ABSTRACT

Regular application of weedicides has led to multiple toxic effects on human health, environment, and food safety which also facilitated an emergence of weed resistance. Development of bioherbicides using microorganisms has attained considerable attention nowadays for weed management in agricultural fields. In the present study, a rhizospheric bacteria, H6, showing a weedicide efficacy was discovered from the rhizosphere of *Momordica charantia*. By classical methods and 16S ribosomal DNA phylogenesis, H6 was identified as *Pseudomonas aeruginosa*. The weedicidal prospective of the secondary metabolites bred by H6 was investigated. Broth, supernatant culture and crude extract of H6 showed high inhibition activity in *Pennisetum purpureum*, *Oryza sativa, Pisum sativa*, and *Amaranthus spinosum.* In general, germination inhibitions of selected weeds were shown by metabolites of the strain H-6. Metabolite identified from strain H6 shows the presence of antifungal and herbicidal compounds. The herbicidal metabolites produced by *Pseudomonas aeruginosa* H6, was determined by GC- MS analysis. The results revealed that quinoline derivatives which are highly toxic to the target weeds were the distinctive metabolite found within *Pseudomonas aeruginosa* H6, making it a potential bioherbicide against weed growth. Hence, the results of the present investigation suggest that *Pseudomonas aeruginosa* H6 can be a suitable alternative to agrochemicals for weed management.

1. Introduction

The occurrence of weeds can cause severe loss in crop quality and yield. Weed control is one of the major problems faced in agricultural fields and other natural ecosystems (Banowetz et al., 2008). Vegetables being major components among horticultural crops, weed management becomes an important aspect for the successful production of these crops. Most of these crops are slow growing and have poor canopy development during the early stages. This habit makes them susceptible to competition from weeds, which adversely affect yield and quality of these crops (Bhullar et al., 2015) Weeds compete with crops for water, nutrients, space, light and oxygen resulting into a delay in maturity and low yield. Generally, these losses occur as a result of reduced yield, quality, harbouring of pests or diseases, allelopathic effects on crops etc. Thus, to get maximum returns from inputs applied to horticultural crops, there is a great need of proper weed control measures in these crops.

Usage of chemical herbicides elevates production costs and leads to the contamination of the environment with hazardous substances. Hence, a sustainable, non toxic, cost effective technologies must be acquired in order to control weed growth. With the increasing awareness about environmental protection and demand for sustainable + agriculture, development of safe, environment-friendly herbicides has become imperative (Li et al., 2004).

Environment friendly microbial herbicides can be propagated from rhizosphere soil (Halgren et al., 2013). Weed control using specific rhizosphere microorganisms is an accepted weed management strategy (El-Sayed, 2005). Rhizosphere microbiome especially includes deleterious rhizosphere bacteria (DRB). The growth of DRB is confined to the roots of weeds and concentrate their metabolic production, thus protecting other beneficial crops (Lakshmi et al., 2015). They are predominantly associated with the plant rhizosphere due to the exudation of organic acids, sugars and amino acids by the plant roots. This group of bacteria which includes *Pseudomonas, Rhizobacter, Azobacter, Rugamonas, Serpens,* and *Mesophilobacter* generally grow on simple media compared to media containing low molecular weight organic compounds (Adetunji et al., 2017). Volatile compounds arise from DRB that inhibit the weed growth. For instance, some species of *Pseudomonas* and *Chromobacterium* genus have a negative effect on plant growth due to the production of hydrogen cyanide (Park et al., 2015). Several

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rhizobacteria also produce elevated levels of indole-acetic acid (IAA) which turns out to be detrimental to the target weed.

Quinoline and its derivatives are a family of compounds intensely studied because of their wide distribution and range of activities like antitumour (Dallavalle et al., 2001), antiviral (Bernard et al., 2004), antibacterial (Kidwai et al., 2000), fungicide (Tsushima et al., 1989), and herbicide activities (Vasiliev et al., 2004). (Salmon et al., 2012) has also reported a novel synthetic herbicidal preparation comprising of quinoline derivatives which can eradicate the growth of unwanted plants. Microbes that produce secondary metabolites namely phytotoxins can be employed as an effective bioformulation for sustainable weed management. Isolation and chemical characterisation of host-specific and non-specific phytotoxins from DRB may provide templates for analog synthesis to develop bioherbicides with even more desirable characteristics than some synthetic compounds presently marketed. Phytotoxin produced by Pseudomonas *syringae* strain 3366 inhibitory to downy brome consisted of phenazine-1-carboxylic acid, 2-aminophenoxazone and 2-aminophenol (Kremer, 2014). The establishment of DRB in rhizosphere soil would be helpful to weed control management in crop fields and more economical than application of chemical herbicides (Radhakrishnan et al., 2016).

In the present study, quinoline derivatives were the active phytotoxic compounds isolated from a deleterious rhizobacterium *Pseudomonas aeruginosa* which turned out to be highly toxic towards the target weeds. Although various reports on diverse microbes that possess a broad range of phytotoxins has been published (Kremer, 2014; Radhakrishnan et al., 2016), quinoline derivatives produced by *Pseudomonas aeruginosa,* a deleterious rhizobacterium which can remarkably inhibit the seed germination and seedling growth of the target weeds has not been reported yet to the best of our knowledge. Hence, the present investigation confirms the competence of specific DRB isolate *Pseudomonas aeruginosa* which can be utilized efficiently as a suitable alternative to agrochemicals for an eco friendly and sustainable agricultural practice to suppress weed growth.

2. Materials and methods

2.1. Sampling

The soil samples were collected from the rhizosphere of different crop plants namely *Momordica charantia* (bitter gourd), *Solanum lycopersicum* (tomato), *Abelmoschus esculentus* (ladies finger) *and Solanum melongena* (brinjal)grown in agricultural fields of Athirampuzha, Kottayam (9° 35′ 0″ N; 76° 31′ 0″ E), Kerala during 2016–2017. The sampling was carried out when the ambient soil temperature was 25 °C and the samples were randomly selected. These samples were ripped out and the excess soil was separated carefully. The collected samples were transferred in sterilised polythene bags and stored at 4 °C in the refrigerator (Lakshmi et al., 2015).

2.2. Isolation of bacteria from rhizosphere

Rhizospheric bacteria were isolated from the collected soil samples using Nutrient Agar medium with the help of standard microbiological techniques. After incubation at 28 °C for 48 h, the isolated colonies were observed for its morphological characteristics (colony characteristics *viz.* form, elevation, margin, texture, pigments and opacity) to distinguish different strains (Flores Vargas and O'hara, 2006).

2.3. Surface sterilization of seeds

Seeds of *Pennisetum purpureum, Oryza sativa, Pisum sativa*, *Amaranthus spinosum* were sterilised by submerging them in 3.25% (v/v) sodium hypochlorite (NaOCl) for 1 min. Later on, they were washed with 70% (v/v) ethanol. After rinsing these seeds in sterile distilled water for five times, they were blotted on sterile filter paper (Gealy et al., 1996). Seeds were purchased from Agricultural University, Mannuthy, Thrissur.

2.4. Bacterial screening for herbicidal activity on germination

Ten bacterial strains (H1,H2,H3,H4,H5,H6,H7,H8,H9,H10) were chosen from NA agar plate and sterilised seeds were treated in advance with the culture broth and inoculated in petridish. The petridishes were carefully secured with parafilm and incubated at 25 ± 2 °C under 12 h of fluorescent light and relative humidity of 80% for 3 days. Distilled water and NB medium application were used as control. The percentage germination and seedling length and fresh weight of plant were evaluated in a completely randomized design (Park et al., 2015).

2.5. Bacterial screening for herbicidal activity on weed seedlings

Spiny amaranthus (*Amaranthus spinosus*) seedlings with uniform size were selected. The broth culture (H1, H2, H3, H4, H5, H6, H7, H8, H9, H10) of representative colonies were treated for centrifugation at 10,000 rpm for 10 min. The pellets of isolated colonies were separately dissolved in 5 ml sterile distilled water containing 500 µl Tween20 and a pinch of gum acacia. The mixture was applied to seedlings. Seedlings treated with sterilised distilled water were kept as control.

2.6. Characterisation and identification of the isolate

2.6.1. Phenotypic characteristics

Isolates which displayed the highest herbicidal activity were morphologically characterized using log phase cultures (Holt et al., 1994).

2.6.2. Identification of the potential rhizobacteria by 16S rRNA gene sequencing and phylogenetic analysis

The isolate with herbicidal activity was identified by using morphological, biochemical and 16S ribosomal DNA (rDNA) gene sequencing method. The strain was inoculated in flasks containing 250 ml Kings B broth. The flasks were incubated in a rotary shaker at 180 rpm at 37 °C for 24 h. According to the conserved bacterial 16S rDNA gene sequences, the 16S rDNA was amplified by PCR with the following primers:

8F: 5′-AGAGTTTGATCMTGG-3′
1492R: 5′-ACCTTGTTACGACTT-3′

The PCR conditions were 95 °C for 5 min, 94 °C for 30s, 55 °C for 30s, followed by 35 cycles at 72 °C for 90s, with a final step at 72 °C for 10min. The purified PCR products were then sequenced. The gene sequences obtained were subjected to BLAST sequence similarity search (http://blast.ncbi.nlm.nih.gov/BLAST) to identify the nearest taxa. Phylogenetic tree was constructed using Neighbour-joining (NJ) method using MEGA version (Yang et al., 2014).

2.6.3. Characterisation of the isolate

The different properties possessed by the isolate, due to which they exhibit herbicidal activity, were tested such as HCN production, ammonia production, indole acetic acid production, siderophore production, volatile compound production, fluorescent pigment production, antimicrobial assay and antifungal assay.

2.6.3.1. HCN production. Qualitative screening of the isolated rhizobacteria for the production of cyanide was carried out by using picrate/Na_2CO_3 saturated filter paper strips. The isolate was subcultured in Kings B Medium Base amended with 4.4 g glycine. These strips were fixed beneath the lids of the petridish, containing the lawn culture. It was then secured with parafilm prior to incubation at 28 °C. Change in color from yellow to light brown/reddish brown of the filter paper was recorded at 24, 48, 96 h as a clear sign of weak,

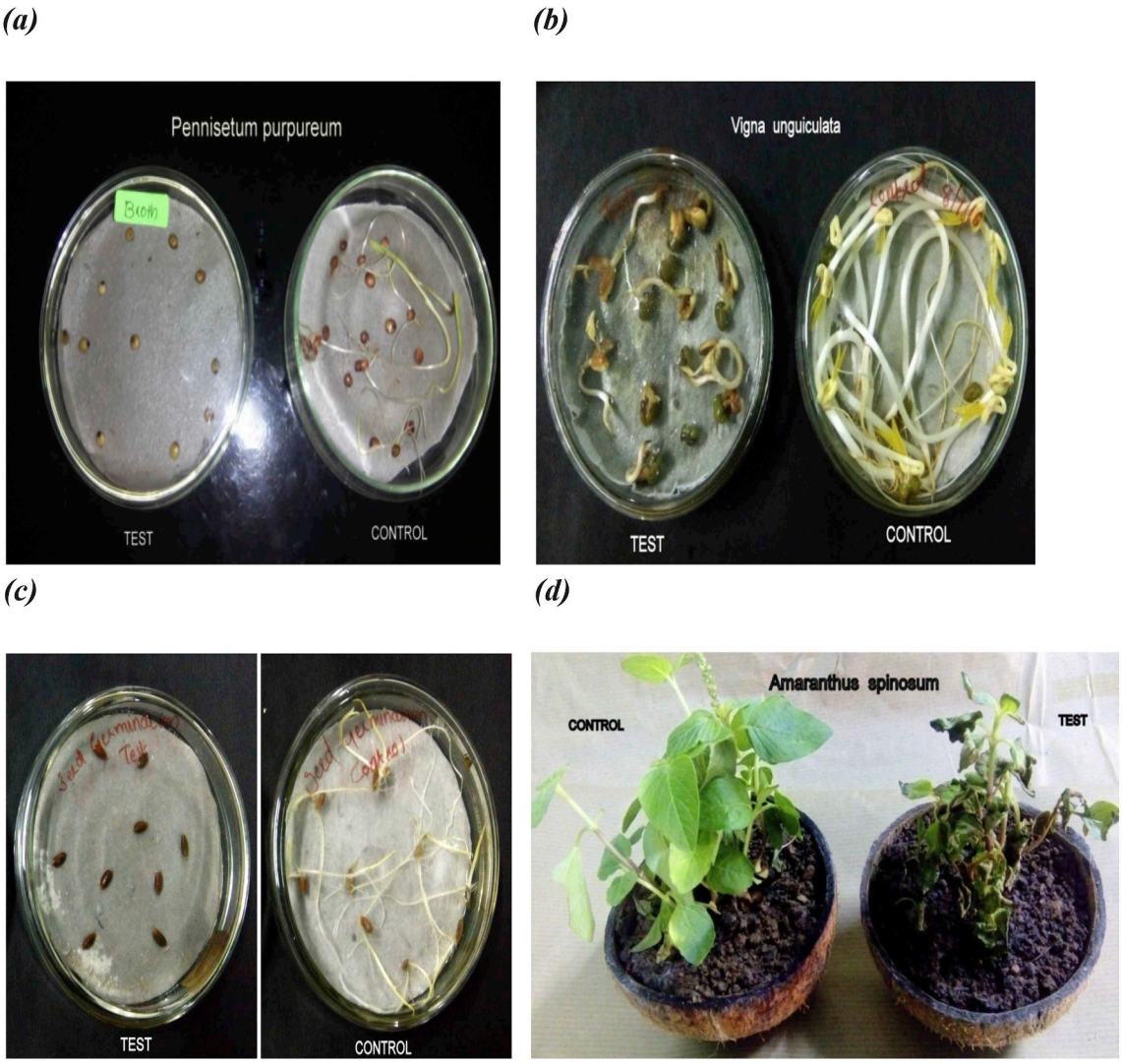

Fig. 1. Rhizobacterial screening of the isolate for herbicidal activity on weed germination
(a) Pennisetum purpureum
(b) Pisum sativa
(c) Oryza sativa
(d) Amaranthus spinosum.

moderate or strong cyanogenic potential respectively. The results obtained were visually compared with the uninoculated control plates (Bakker and Schippers, 1987).

2.6.3.2. Ammonia production. Ammonia production from the isolate was determined using peptone water. Log phase cultures were inoculated in 10 ml peptone water and incubated at 28 ± 2 °C for 48–72 h. Nesslers reagent (0.5 ml) was added to each tube and the tube which showed a color change from yellow to brown was considered as positive test for ammonia production (Cappuccino and Sherman, 1992).

2.6.3.3. Siderophore production. The isolates were inoculated on Kings B broth and incubated for 30 °C for 24–48 h. To 1 ml of incubated culture, 1 ml of 2%Aqueous Ferric Chloride solution was added. Orange or red color indicates the presence of siderophore.

2.6.3.4. Indole acetic acid (IAA) production. IAA production was detected by a modified method. The concentration of tryptophan used is (50 µg/ml). 72hr old fully grown culture was subjected to centrifugation at 10,000 rpm for 30min.The supernatant (2 ml) was mixed with two drops of orthophosphoric acid and 4 ml of the Salkowski reagent (50 ml, 35% of perchloric acid, 1 ml 0.5 M $FeCl_3$ solution). Development of pink color upon addition of the reagents was considered to be a positive test (Bric et al., 1991).

2.7. Anti fungal assay

The following plant pathogens were used to determine antifungal activity of the isolates: *Colletotrichum gleoeosporioides, Phythium myriotylum, Fusarium oxysporum, Rhizoctonia solani, Phythophora infestans.* Fungal cultures were cultured using potato dextrose agar (PDA) at 25 °C for 13 days. The antifungal bioassay was carried out in vitro by growth inhibition of phytopathogenic fungus on NA:PDA media. The bacterial inoculum was streaked on two sides of the petridish aseptically. Fungal inoculum consisted of an agar disc (1 cm diameter) punched out with sterilised gel puncture from the growing margin of colonies were placed at the centre of each inoculated plates and to the control medium. The plates with phytopathogens alone served as control. These plates were incubated at 28 °C for five days. The diameter of the zones of inhibition were measured and the experiment was repeated thrice (Altindag et al., 2006).

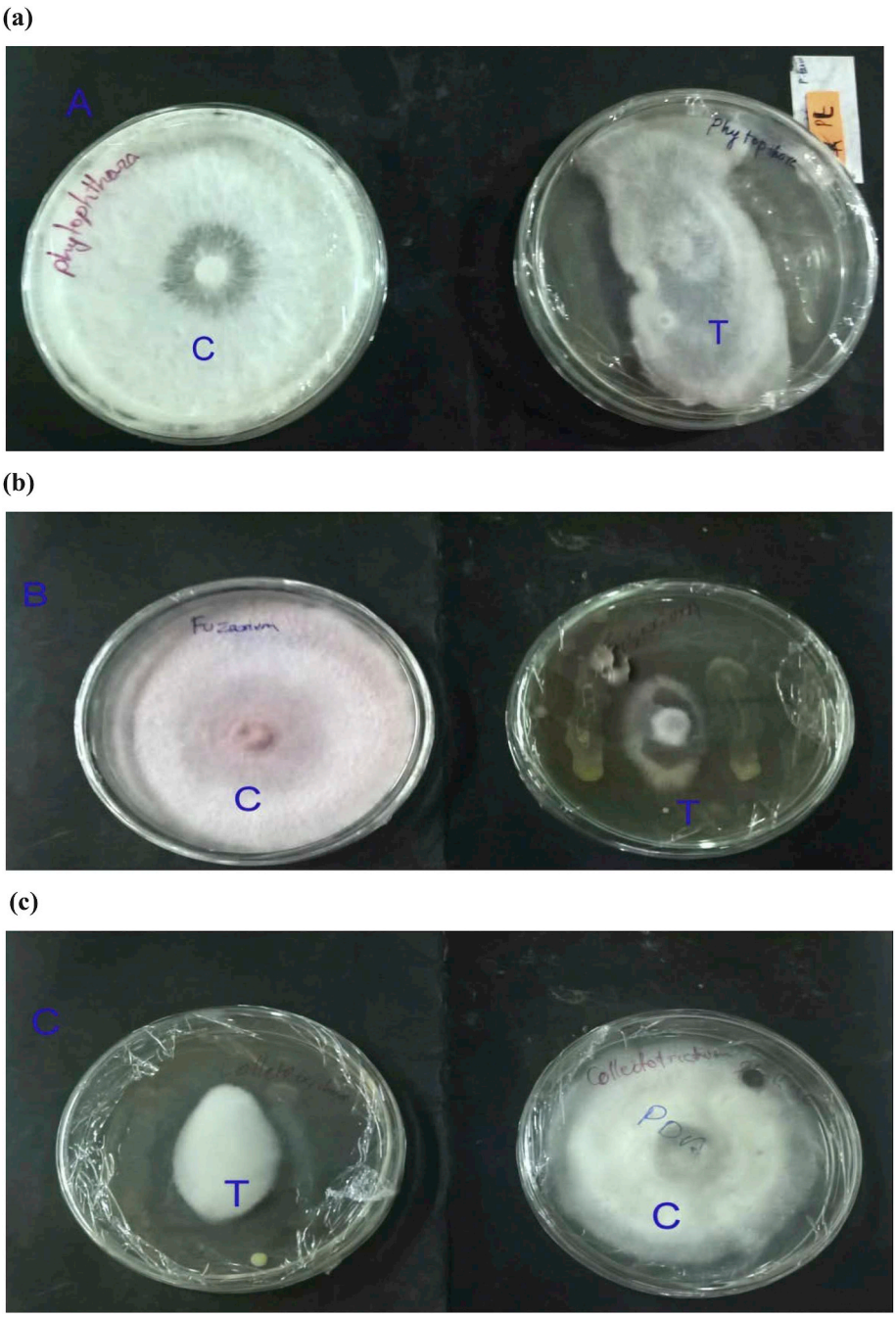

Fig. 2. Antifungal activity of H6 (T) against phytopathogens
A) *Phythophora infestans*
B) *Fusarium oxysporum*
C) *Colletotrichum gleoeosporioides* along with the control (C).

2.8. Volatile compound assay

Seedling bioassay was carried out in order to check the response of volatile metabolites of the isolate on root development. Pregerminated surface-sterilised seeds (*Pennisetum purpureum, Oryza sativa, Pisum sativa*, *Amaranthus spinosum*) were arranged in equal distance apart on sterilised Whatmann No:1 filter paper. Quarter-strength Kings B agar was used to culture the isolate of interest and incubated for 24hr. A paired-plate assembly was used wherein each inoculated Kings B agar plate was paired with a plate containing the pre-germinated seeds. These plates were sealed with parafilm and incubated at 27 °C in the dark. This experiment was repeated four times, and the root lengths were measured after 48 h. Non-inoculated Kings B agar were served as controls (Alstrom and Burns, 1989).

2.9. Production of herbicidal metabolites from rhizosphere isolate using fermentation technology

Various media were used for the optimum production of effective metabolites from rhizosphere isolate using fermentation technology.

2.9.1. Preparation of the inoculum

The selected media Kings B was prepared individually. The effective strain was cultured in 250 ml of Kings B media in 500 ml conical flask

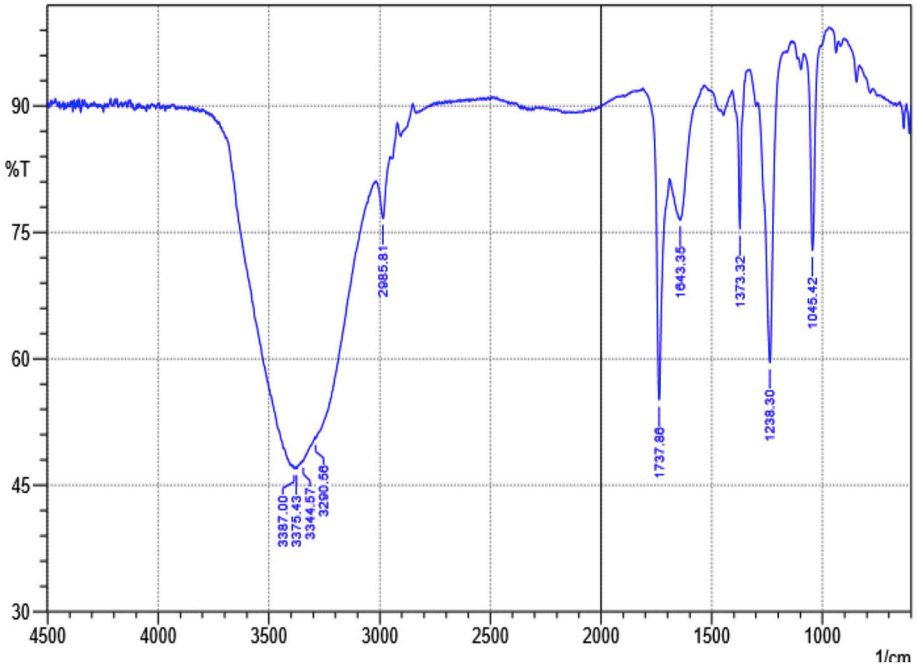

Fig. 3. FTIR analysis of ethyl acetate fraction of the isolate H6.

incubated in an orbital shaker at 28 °C, 120 rpm for 5 days. The culture media was later subjected to centrifugation at 10000 rpm for 15min to separate the cell free filtrate. This filtrate was used to monitor the herbicidal activity of the strain against target weeds and fungal pathogens.

2.9.2. Extraction of crude metabolites using selected organic solvents

Extraction of crude metabolites from the isolate of interest was carried out using Kings B medium by partitioning with double volume of organic solvents like Hexane, Petroleum ether, Chloroform, Ethyl acetate (in the order of polarity) the four solvents being tried for the extraction of secondary metabolites. Extraction was carried out in the order of polarity Hexane > Petroleum ether > Chloroform > Ethyl acetate in a separating funnel. This was continuously shaken for some time and placed in a stand for few minutes and the process was repeated for 4–5 times. The organic phase were collected and concentrated by using Rotary evaporator at 60 °C. The extracted metabolites were tested for their efficacy against weeds and fungal pathogens (Reddy et al., 2009).

2.9.3. Screening for herbicidal and antifungal activity with the crude extract of the isolate

Seeds of *Pennisetum purpureum, Oryza sativa, Pisum sativa, Amaranthus spinosum* were sterilised, soaked in a solution of the extract in hexane, chloroform, petroleum ether and ethyl acetate and transferred to petriplates containing sterile moistened Whatmann filter paper. The plates were sealed and incubated at 20 °C in the dark. Germination and root length were recorded after 7 days. Seeds treated with different solvents were served as control to determine the percentage of inhibition. Each bioassay was conducted in triplicates containing five seeds per plate (Pedras et al., 2003).

Antifungal assay was carried out using the phytopathogen *Colletotrichum gleoeosporioides.* Assay was carried out using different crude solvent extract of hexane, petroleum ether, chloroform, ethyl acetate. Fungal cultures were cultured at 25 °C for 13 days using potato dextrose agar. The antifungal assay was performed in vitro using NA:PDA media. The agar block of *Colletotrichum gleoeosporioides* were spot inoculated on to the centre of petriplate by gel puncture. Four wells were punctured in the agar plate and filled with 1 ml crude extracts of different solvents and control plates were filled with 1 ml of different solvents. The petriplates were incubated at 28 °C for five days. The zone of inhibition was measured and the experiment was carried out in triplicates.

2.10. Characterisation of antifungal and herbicide metabolites by HPLC, NMR, IR and mass spectroscopy

2.10.1. HPLC

HPLC analysis were carried out with a HPLC equipped with quaternary pump, automatic injector and diode array detector (wavelength range 190–600 nm), degasser and a Hypersil ODS column (5 mm particle size silica,4.6 i. d × 200 mm), equipped with an in-line filter. Mobile phase system A:0.1%TFA in H_2O-0.1% TFA in CH_3CN(40:60) to 100% CH3CN containing 0.1% of TFA, for 30 min, linear gradient,1.0 ml/min; system B:0.1%TFA in H_2O −0.1%TFA in CH_3CN (20:80) to 100% CH_3CN containing 0.1% of TFA, for 25 min, linear gradient,1.0 ml/min. For the semi preparative separation an Econosphere 1 ODS column (10 mm particle size silica, 10.0 i. d.250 mm) equipped with an in-line filter was used with an isocratic elution (0.1%TFA in H_20−CH_3CN, 35:65)for 25 min, flow rate 5.0 ml/min (Pedras et al., 2003).

2.10.2. GC-MS

GC analysis was carried out with a Hewlett-Packard 5890 gas chromatograph equipped with a chiral capillary column (Chirasil-Vall column, Alltech 25 m × 0.32 mm id, film thickness 0.2 mm), with Helium as carrier gas (flow 30 cm/s, measured at 70 °C); detector at 240 °C; injector at 220 °C; oven temperature at 70–200, at 4 °C min 1; samples were injected in split model (ca 1:50) with CH_2Cl_2 as solvent. Mass spectra (MS) high resolution(HR), electron impact (EI), chemical ionization(CI) or fast atom bombardment (FAB) were obtained on a VG-70VSE magnetic spectra mass spectrometer using double focusing EB geometry operating at an accelerated voltage of 8 kV; EL/CI source operating with a electron energy of 70eV and a source temperature of 200 °C; in FAB the primary Cs energy was 28 kV and the sample was introduced in a matrix containing either nitrobenzyl alcohol or glycerol on the tip of a FAB insertion probe. Specific rotation[a] D were determined at ambient on a Perkin Elmer-141 polarimeter using a 1 ml,

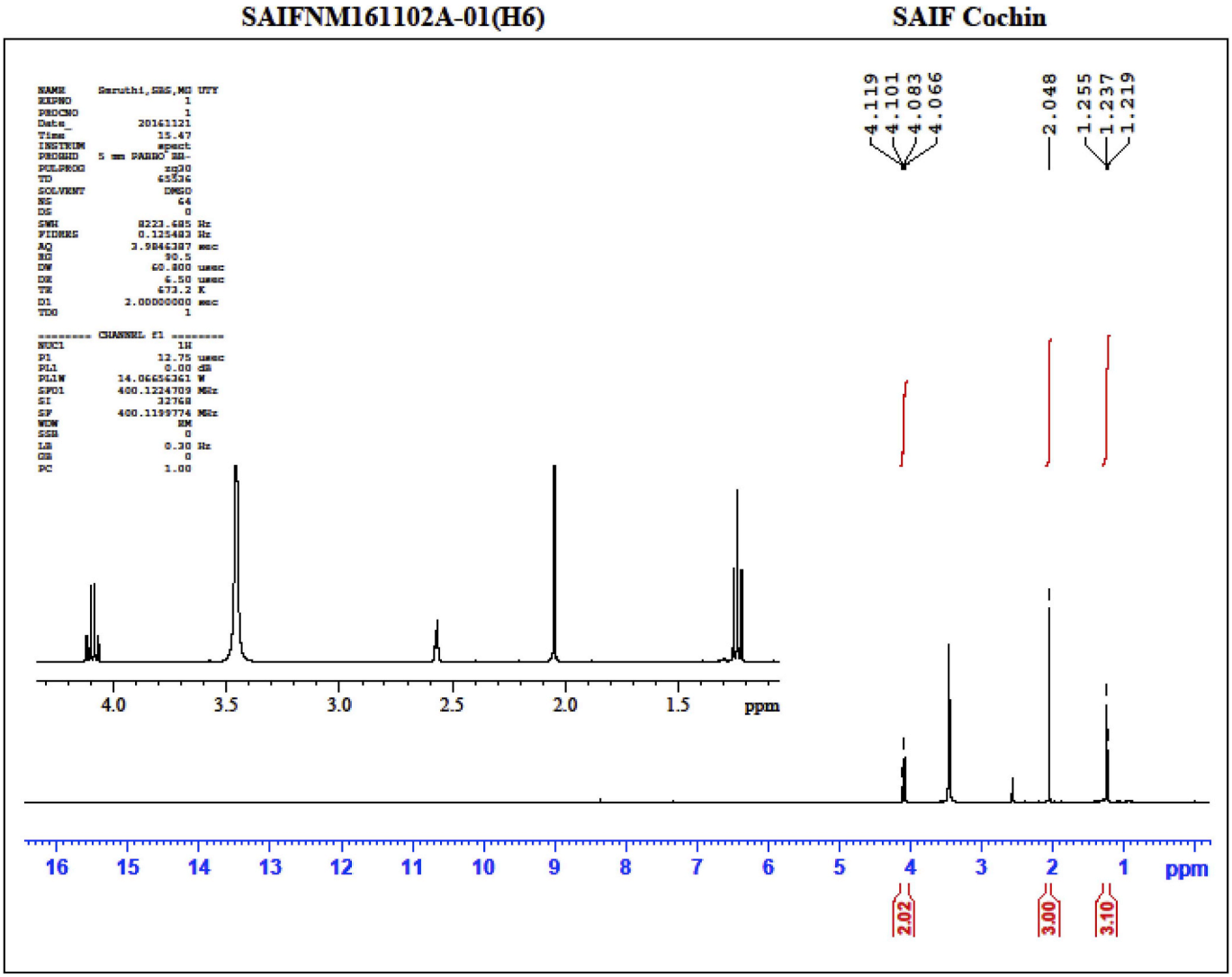

Fig. 4. NMR analysis of ^{1}H ethyl acetate extract.

10 cm path length cell; the units are 10-1 deg cm2 g-1 and concentrations(c) were reported in g/100 ml (Pedras et al., 2003).

2.10.3. FTIR

Fourier Transform infrared (FTIR) spectra were obtained on a Bio-Rad FTS-40 spectrometer using a diffuse reflectance cell (Pedras et al., 2003).

2.10.4. NMR

NMR spectra were recorded on a Bruker AMX 300, Bruker AMX 500 or Bruker Avance 500 spectrometers; for 1H (300 or 500 MHz), d values were referenced to CD_3OD (CD_2HOD 3.31 ppm), $CD_3S(O)CD_3$ ($CD_2HS(O)CD_3$, 2.50 ppm) or $CD_3(O)CD_3$ ($CD_2HC(O)CD_3$ 2.05 ppm) and for 13C(75.5 or 125.8 MHz) referenced to CD_3OD (49.15 ppm), $CD_3S(O)CD_3$ (39.51 ppm) or $CD_3C(O)CD_3$ (206.68, 29.92 ppm) (Pedras et al., 2003).

3. Results

3.1. Isolation and screening of rhizospheric bacteria for germination inhibition

Ten bacterial isolates were isolated from the rhizosphere of different crop plants (as mentioned above) grown in agricultural fields of Athirampuzha, Kottayam. They were screened for germination inhibition of weed seeds of *Pennisetum purpureum, Oryza sativa, Pisum sativa* and *Amaranthus spinosum*. The result of the preliminary screening showed that only the isolate H6 from the rhizosphere of *Momordica charantia* (bitter guard) plant presented the highest herbicidal activity on the target weeds compared to the control seeds grown on agar alone (Fig. 1a,b,c) Supernatant of the culture H6 also arrested the germination of weed seeds. The NB without culture was taken as control.

3.2. Rhizobacterial screening of the isolate for herbicidal activity on weed seedlings

Only strain H6 inhibited Spiny *amaranthus*. They induced clear and identifiable symptoms, such as discoloration and distortion of roots, depletion of root hair development and necrotic lesions (Fig. 1d).

3.3. Phenotypic and genotypic characterisation

Based on the morphological and biochemical characteristics, the isolate was tentatively identified as *Pseudomonas*. The bacterial isolate of 1–2.5 mm diameter mucoid colonies, appeared yellowish white with smooth, regular margins and fruity odour on Kings B agar plates at 28 °C. It also showed the presence of fluorescent green pigment on King's B medium under UV light. Phenotypic characterizations revealed

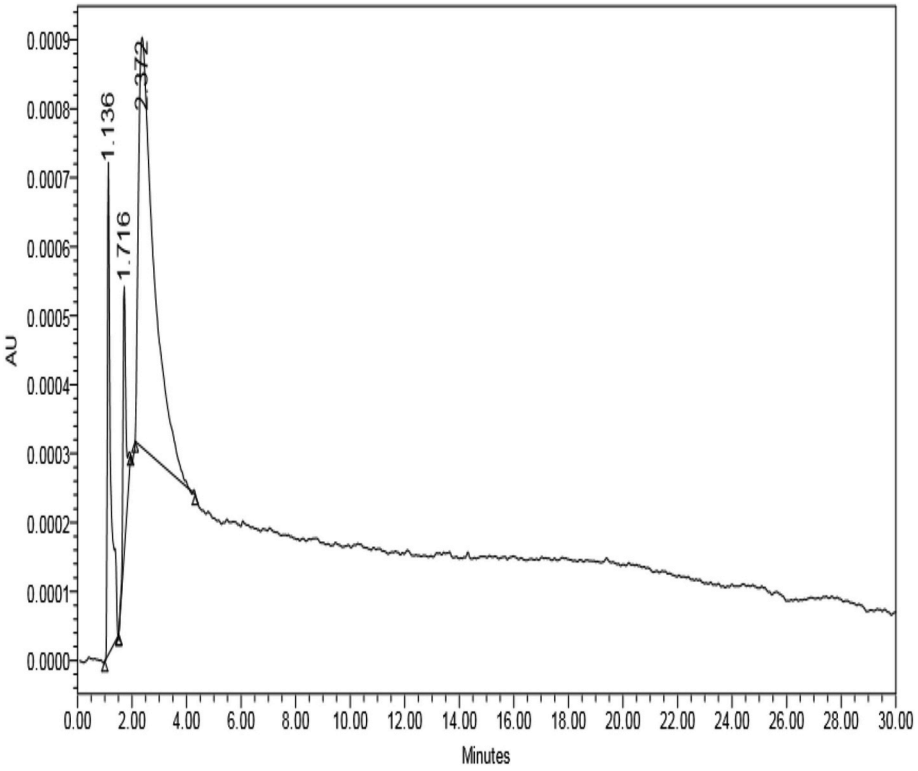

Fig. 5. HPLC analysis of ethyl acetate fraction of the isolate H6.

Table 1
Herbicidal compounds identified through GC-MS analysis.

Sr. No.	Compound	Function	References
1	2,5-Cyclohexadiene-1,4-dione, 2-(1,1-dim	Inhibition of Hydroxyphenylpyruvate dioxygenase	Giovanni Meazza et al., 2002
2	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1	Inhibition of Hydroxyphenylpyruvate dioxygenase	Giovanni Meazza et al.,2002
3	Quinoline, 1,2-dihydro-2,2,4-trimethyl	Preventing plant growth	David T. Mowry and Arthur H.1953
4	4-Cyano-2-quinolinecaboxamide	Preventing plant growth	David T. Mowry and Arthur H.1953
5	6,7-Methylenedioxy-thieno(2,3-b)quinolin	Preventing plant growth	David T. Mowry and Arthur H.1953
6	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahy	antimicrobial	Samantha Lee 2016
7	4-Methoxybenzamide, N-(4-methylbenzyl)	Inhibitor of Cell wall synthesis(cellulose)	Weed Science Society Of America.

that the isolate was gram negative, short rods in appearance (1.9 μm × 0.5 μm) and motile. Its growth temperature ranged from 4 °C to 42 °C but optimum growth was recorded at 30 °C.

The isolate showed positive reactions for citrate utilization, catalase activity, oxidase activity, gelatine hydrolysis and lysine decarboxylase enzyme. It was also able to utilise glucose, galactose, fructose, mannitol, trehalose and glycerol as sole carbon sources.

The isolated rhizobacteria was identified as *Pseudomonas aeruginosa* with an Accession No.**KU598682.1** by 16S rRNA gene sequencing showing 100% similarity with the type strain of *P. aeruginosa* available in the NCBI GenBank.

3.4. Characterisation of the isolate

3.4.1. HCN production

The isolate H6 showed positive results in terms of HCN production by indicating a color change in the impregnated filter paper (treated with picric acid and sodium carbonate) from deep yellow to orange at 24hr of incubation, followed by a reddish brown coloration after 48hr of incubation. HCN being one of the essential constituent of volatile metabolites produced by cyanogenic DRB, it may be responsible for arresting seed germination. If roots are exposed to higher levels of HCN produced by DRB, it gets rapidly dissipated and further degrades the root surfaces hampering root development.

3.4.2. Siderophore production

Upon the addition of 1 ml of 2% Aqueous Ferric Chloride solution to the incubated broth, the color changed from yellow to brown which indicates siderophore production.

3.4.3. Ammonia production

Another volatile product of H6 is ammonia which plays a vital role in biocontrol activity. Production of ammonia was detected by a color change from deep yellow to brown after the addition of Nessler's reagent to the inoculated peptone water.

3.4.4. Volatile compound assay

Paired-plate assembly was carried out to detect the production of inhibitory volatile compounds by the isolate. Seeds in the test plate did not germinate compared to the control plate which contained germinated seeds in the absence of the isolate. These results confirmed that the cyanogenic isolate was responsible for the production of volatile metabolites during their growth which inhibited the germination of weed seeds.

3.4.5. Indole acetic acid production

Production of IAA was examined using Salkowski reagent. A color change was observed initially at the highest IAA concentration within minutes and after a duration of 30 min, the color intensity continued to

increase. The ability of the isolate to produce IAA in pure culture in the presence and absence of the precursor L-tryptophan was checked. In the absence of L-tryptophan, low amount of IAA was produced by all the isolates as compared to that produced in the presence of tryptophan.

3.5. Antifungal assay

The antifungal assay was carried out with the isolate against following plant pathogens: *Colletotrichum gleoeosporioides, Phythium myriotylum, Fusarium oxysporum, Rhizoctonia solani, Phythophora infestans*. The zone of inhibition displayed by the isolate varied in diameter from 1 cm to 5 cm. The isolate showed good antifungal activity in the order of *Fusarium oxysporum* > *Colletotrichum gleoeosporioides* > *Phythium myriotylum* > *Rhizoctonia solani* > *Phythophora infestans*. *Fusarium oxysporum* was most inhibited by the isolate with the percentage of inhibition (I) as 77% (Fig. 2).

3.6. Production of metabolites from the isolate using fermentation technology

It was observed that King's B medium was the most appropriate medium for effective production of metabolites through fermentation technology using the parameters 120 rpm, pH 7.0 and temperature 28 °C. The metabolites were extracted from *P. aeruginosa* culture filtrate (2:1 ratio) using various organic solvents like Ethyl acetate, petroleum ether, chloroform, and hexane. The bioefficacy of every single solvent extract was experimented on weed seeds for herbicidal activity.

3.7. Herbicidal and antifungal assay using solvent extract

From the metabolites tested on the weed seeds, the one extracted with ethyl acetate significantly inhibited (90–98%) weeds and phytopathogens. The compound responsible for germination arrest factor may be dissolved in ethyl acetate. The other solvents retarded the growth of seeds as compared to the control. Chloroform extracts showed the reduction of root length. Petroleum ether and hexane showed similar results as compared to the control. The root lengths were comparatively same as control in case of hexane and petroleum ether. The antifungal activity was remarkably significant in ethyl acetate fraction. The other solvent extracts inhibited the fungal growth compared to the control fungal plates except in the case of petroleum ether.

3.8. Identification of active compound responsible for herbicidal activity

FTIR spectrum of H6 exhibited absorbance peaks at 1045.42cm-1, 1238.30cm-1, 1373.32cm-1, 1643.35cm-1, 1737.86cm-1, 3290.53cm-1, 3344.57cm-1, 3375.43cm-1, 3387cm-1(Fig. 3). The peaks exhibit alkanes, alkenes, ethers, amines, ketones, nitriles, nitrites, amines, alcohol functional group (Pedras et al., 2003). 1H NMR spectrum showed signals between 4 and 1 in DMSO solvent. Detailed analyses indicated the existence of amino acids like leucine, isoleucine and the compound C10 HO-acid (Fig. 4). The retention time of the compound as per HPLC analysis of the ethyl acetate fraction is 1.136, 1.716, and 2.372. Peak at 2.37 indicates the presence of cyclic peptides (Fig. 5).

3.9. GC-MS analysis

From the GCMS analysis, 47 compounds have been identified. Literature studies shows that quinoline compounds and its derivatives can be used as herbicidal compounds. The compound identified is confirmed by the FTIR, by the identification of functional groups. One of the compound identified, 1, 2-dihydroquinolines, is patented to be used as herbicide. Another report states that 2,2,4′,8-tetramethyl compounds of quinoline are preferred because they are particularly effective. Four of the compounds identified through GCMS were 1,2-dihydro-2,2,4trimethyl Quinoline, Quinazoline, 4-methyl,4-Cyano-2-quinolinecaboxamide and 6,7-Methylenedioxy-thieno(2,3-b) quinoline (Table 1).

4. Discussion

Among the different crop plants chosen, *Momordica charantia* being a medicinally important cosmopolitan vegetable crop, it is known to produce various bioactive secondary metabolites which have favourable or unfavourable effects on growth and development of plants or microorganisms. These bioactive secondary metabolites are released in the environment as root exudates, plant residues decomposition, leaf leachates and microbial metabolic activity (Singh and Sunaina, 2014). These root exudates attract and promote the growth of microorganisms in the rhizosphere and microorganisms and their products in turn interact with the target weeds displaying significant herbicidal activity (Singh et al., 2017). Hence, *Pseudomonas aeruginosa* isolated from the rhizosphere of *Momordica charantia* presented the highest herbicidal activity on the target weeds compared to the control seeds grown on agar alone.

Growth inhibition of numerous weeds using DRB are reported in many research studies (Bakker and Schippers, 1987; (Kremer and Souissi, 2001; Kremer and Kennedy, 1996). The present study focussed only on fast-growing bacteria rather than on fungi or actinomycetes because of their rapid growth. Strain H6 displayed significant detrimental effects on target weeds by inhibiting their root growth of *Pennisetum purpureum*, and other weeds. They also induced fine and distinct symptoms mainly retarded root hair development, browning and discoloration of roots, distortion of leaves and roots and necrotic lesions on the target weeds. The results obtained were in agreement with the previous reports and has explored the competence of various rhizospheric bacteria to interfere with the growth of weed seedlings (Gealy et al., 1996; Adam and Zdor, 2001; Begonia and Kremer, 1994).

Morphological characteristics of the isolate was carefully examined by Gram staining. Biochemical characteristics corresponded to *P. aeruginosa*, but the isolate identity was confirmed on the basis on 16S rDNA sequencing and alignment at NCBI database.

Our results revealed the ability of H6 to produce HCN as a secondary metabolite. HCN, being a volatile compound, it plays a major role in photosynthesis by disrupting the oxygen reduction in the cytochrome respiratory chain and electron transport. This mechanism leads to the significant growth retardation in weed seeds (Bakker and Schippers, 1987). According to (Albert and Anderson, 1987), cyanogenic rhizobacteria engage in the depletion of plant growth without evident plant cell damage, an effect accredited to the metabolites produced by rhizobacteria which are being absorbed by the roots (Kremer and Kennedy, 1996).

Plant growth retardation is a prominent property of cyanide producing rhizobacteria. A systemic bioassay driven approach was followed in order to understand the role of cyanogenesis in plant infection model system. The cyanogenic effect of strain H6 was monitored on weeds such as *Pennisetum purpureum,* Spiny *amaranthus* etc. An observable depletion in root and shoot length was found while treating with *Pseudomonas* produced metabolites, which was later on linked to HCN production. The results of the paired plate assembly exhibited the presence of diffusible volatile metabolites further contributing to the enhanced germination arrest of weed seeds.

The antifungal activity against the five tested fungi was broadly exhibited by the isolate. The isolate showed good antifungal activity in the order of *Fusarium oxysporum* > *Colletotrichum gleoeosporioides* > *Phythium myriotylum* > *Rhizoctonia solani* > *Phythophora infestans.* The antifungal activity of the test isolate displayed a synergistic interaction between HCN production and siderophore or with other metabolites.

By fermentation technology for the production of metabolites, it revealed that ethyl acetate fraction showed herbicidal action against the weeds and phytopathogenic fungi. The compound responsible for

herbicidal activity was identified through FTIR, GCMS, HPLC, NMR etc. *Pseudomonas aeruginosa* has been known to produce compounds that irreversibly arrest the germination and inhibit the growth of weed seeds (Charles and Julius, 2013). Through this analysis it was concluded that quinoline derivatives were responsible for herbicidal activity of *P. aeruginosa*. According to the literatures, cyclic peptide is a component of herbicide. FTIR analysis confirmed this result by the presence of functional groups such as amide, amine. NMR confirmed the presence of amino acids such as leucine, isoleucine, and a compound C10 HO-acid. It shows resonance at 4.08, 1.25 (Pedras et al., 2003).

Weed control strategies using DRB helps to regulate the growth of weeds before or in coincident with the emergence of the crop plants. Therefore, DRB do not essentially eliminate the problem of weeds, but considerably deplete the early growth of weeds and enable the beneficial crops to effectively compete with the weakened weed seedlings (Kremer and Kennedy, 1996; Schroth and Hancock, 1982).

5. Conclusion

The rising problem of weed competition in agriculture has disrupted the consistency of the crop yield. An eco-friendly solution to this would be the use of herbicidal DRB to control the weed growth. The isolated rhizobacteria *P. aeruginosa* H6 produced certain metabolites like HCN which was capable of acting against the weeds. They also possess certain enzymes such as cellulase, pectinase, chitinase, and protease and also displayed antibiotic and antifungal activity. Another volatile compound present in the isolate is ammonia. The presence of compounds responsible for herbicidal activity was confirmed by GCMS, NMR, HPLC and FTIR analysis. The compound analysis showed the presence of quinoline and its derivatives which are proven to be herbicidal compounds. Likewise, this competent strain *P. aeruginosa* H6 could also demonstrate an inherent capability of inhibiting the growth of weed seedlings in vitro. Thus, this novel isolate can be efficiently used for the control of weeds in agriculture.

Compliance with ethical standards

Conflict of interest

The authors hereby declare that there is no conflict of interest.

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