Effects of PGPR on growth and photosynthetic pigment of *Trigonella foenum-graceum* and *Brassica juncea* in PAH-contaminated soil

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Abstract

This study aimed to evaluate the impact of plant growth-promoting rhizobacteria (PGPR) on growth and photosynthetic pigment of two plant species, *Trigonella foenum-graceum* (methi) and *Brassica juncea* (mustard), grown in PAH-contaminated saline soil. Pot experiments were conducted for 30 days with the selected plant species in saline soil artificially supplemented with phenanthrene (5, 50, 200 and 400 mg/kg) and naphthalene (5, 50, 200 and 400 mg/kg). After 30 days of growth, both plants were evaluated for morphological changes including root and shoot length and biomass of mucilaginous material using three-way ANOVA and Duncan's new multiple range test ($P \leq 0.05$). Experimental results clearly indicated that PGPR had a positive impact on overall growth as compared to the non-PGPR-treated plant species.

Keywords Winter crops · PAH compounds · Chlorophyll content · Growth parameters

1 Introduction

Petroleum is an integral part of industrialized society. Polycyclic aromatic hydrocarbons (PAH) are the by-product of incomplete combustion of petroleum products [10]. The Department of Environment (1987) and Environment Agency (2002) have formulated guidelines regarding toxic levels of PAHs based on land use [9, 14]. There are various sources from which PAHs accumulate in the soil, such as combustion of petroleum products, burning of coal for domestic purposes and leakage from gas pipelines. The effect of PAHs on plants was recently documented [9], showing that PAHs lead to decreased photosynthetic activity of enzymes which are antioxidant in nature. PAHs also affect lipid peroxidation, damage DNA and reduce plant growth [2]. The removal of PAH compounds from the soil is difficult due to their resistant nature, with the exception of naphthalene. PAH compounds having a structure of 3–4 benzene rings are insoluble in water and less volatile in nature; thus they show less degradation in soil [5, 18].

PAH contamination of soil leads to changes in the various chemical and physical properties of the soil and microorganisms [12]. Measurable changes in soil microbes affect biochemical processes in the soil, such as the mobility of elements that exist in trace amounts [21]. A change in the bioavailability of trace elements leads to inhibited plant growth and reduced chlorophyll content [22]. Addressing problems related to PAH compounds in our environment has become a global challenge [30]. Phytoremediation is a developing technology which is used to clean oil-contaminated sites, water bodies and soil with the help of flora [8]. Various plant species have shown promise for phytoremediation of PAH pollutants [11]. There are two major techniques involved in phytoremediation of contaminated soils. The first involves dissipation of pollutants from the soil from inside the plant, which is known as phytoextraction. The second is rhizoremediation, in which plants degrade pollutants in the soil with the help of exudates secreted by the plants and the microbial community present in the rhizospheric zone [3, 12]. The ultimate goal of

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the use of vegetation is to remove PAH compounds from the contaminated soil. This can be done through the use of plant–microbe interactions in PAH-contaminated soil, which improve the chemical properties and increase the microbial population in the rhizospheric zone [5]. This technology is cost-effective and environmentally friendly, and is generally socially acceptable [6]. It has many benefits, including reduced soil erosion and water run-off, fortification of plant roots and enhanced aesthetic value [8]. To achieve a reduction in PAH compounds in soil, a stable vegetation cover native to the area is needed. The plants should have maximum root area, should be tolerant of stress conditions and should have good seed germination in contaminated soil [5, 27]. The plant species that are used are endemic and tolerant of the soil conditions. In the present study, we have taken *Trigonella foenum-graceum* and *Brassica juncea* plant species, which are stress-tolerant and produce a large amount of exudates that are helpful in increasing the microbial load in the rhizospheric zone and seed germination in contaminated soil [4]. To enhance degradation of PAH compounds in the soil, we inoculated plant growth-promoting rhizobacteria (PGPR), consisting of *Azotobacter* and *Pseudomonas*, with two PAH compounds, naphthalene and phenanthrene, because these compounds are present in the environment and easily accumulate in the soil. The aim of the study is to evaluate the effect of PGPR on the growth parameters and chlorophyll content of *Trigonella foenum-graceum* and *Brassica juncea*.

2 Material and methodology

In this experiment, *Trigonella foenum-graceum* (Pusa Early Bunching, PEB) and *Brassica juncea* (Raya RH-749) of the Fabaceae family were used, and plant seeds of this variety were procured from the CCS Haryana Agriculture University, Hisar (Haryana).

2.1 Plant and soil preparation

The seed surfaces were sterilized with mercuric chloride for 1 min and then rinsed with tap water three times before they were used for sowing. Seeds were sown in pots containing sandy loamy soil which was contaminated with naphthalene and phenanthrene at different concentrations:

1. Naphthalene: 5, 50, 200 and 400 mg/kg.
2. Phenanthrene: 5, 50, 200 and 400 mg/kg.

In the experiment, we used 5-kg pots and soil taken from Guru Jambheshwar University (29°9'12.6072"N and 75°42'42.0732"E), Hisar (Haryana, India). The experiment

was performed in triplicate for each treatment (96 pots) of phenanthrene and naphthalene, and a control without any treatment was also maintained. PGPR (*Azotobacter* and *Pseudomonas*) were also added to 48 of the pots with phenanthrene and naphthalene. The PGPR was collected from the Microbiological Lab of Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, 125001. In each pot, 25 ml of PGPR was added along with 0.5 g of seeds for each plant species (*Trigonella foenum-graceum* and *Brassica juncea*); tap water was added to maintain 60% moisture content.

2.2 Analysis of root length and shoot height

After 1 month of plant growth, plants were removed from the different pots and weighed to determine their “fresh weight”. Then the height of shoots and length of roots and primary roots were measured with a measuring tape. All plants were then dried in an oven at 94 °C for 52 h, after which they were allowed to cool at room temperature, and were subsequently weighed to determine their “dry weight”. The water content and standing biomass were then calculated as shown below:

Water content = Fresh weight–Dry weight

Standing Biomass = Dry weight/plot area.

2.3 Photosynthetic pigment analysis

Fresh leaves (0.5 g) were put in 70% acetone and homogenized in 50 ml of acetone. The homogenate was centrifuged at $12,180 \times g$ (RCF) in a REMI PR-24 centrifuge for 10 min and then filtered. The supernatant was removed and placed in graduated cylinders. Leaf extract was transferred to a 1-cm cuvette, and absorbance readings were taken using a UV–Vis spectrophotometer (Cary 300) at 663-nm and 645-nm wavelengths to determine the concentration of photosynthetic pigment.

$\text{Chl - a} = 12.7\text{DX}_{663} - 2.69\text{DX}_{645} \times V/1000W$ (ug g⁻¹F Wt)

$\text{Chl - b} = 22.9\text{DX}_{645} - 4.68\text{DX}_{663} \times V/1000W$ (ug g⁻¹F Wt)

$\text{Carotenoids} = \frac{(A_{480} + 0.114 \times A_{663} - 0.638 \times A_{645}) \times AV \times 1000}{112.5 \times \text{weight of sample}}$

DX = the absorbance of the extract at the x nm; V = total volume of the chlorophyll solution (ml); W = weight of the tissue extract (g)

2.4 Measurement of microbial load

One gram of soil was dissolved in 100 ml of distilled water, and the solution was mixed thoroughly by vortexing. In the next step, 1 ml of the diluted bacterial sample

was taken from the stock solution. The sample was then poured into a test tube and diluted to a volume of 10 ml. The procedure was repeated until dilutions of 10^{-7} were obtained. Next, nutrient agar plates were prepared, and 0.3 ml of suspension solution from each dilution was spread over the plates. The plates were then incubated at 37 °C for 24 h, after which bacterial colonies were counted.

Counting colony forming unit

$$= \frac{\text{Number of CFU}}{\text{Volume plated(ml)} \times \text{Total dilution used}}$$

2.5 Statistical analyses

The effects of treatments were evaluated by ANOVA, and means were compared using Duncan's test ($P < 0.05$).

3 Results and discussion

Phenanthrene and naphthalene were used in this experiment. Phenanthrene had adverse effects on both *Trigonella foenum-graceum* and *Brassica juncea* as compared to naphthalene. The experiment was conducted over a period of 1 month, during which maturity was achieved by both plant species. Sampling was conducted in December 2016, and growth and chlorophyll parameters were analysed using Duncan's new multiple range test method at

the 5% level. Values of $P \leq 0.05$ were considered statistically significant. In order to understand the tolerance effect of both plant species and the effect of PGPR in the contaminated soil mixed with PAHs at different concentrations, physiological changes including growth parameters and chlorophyll content were taken into consideration.

3.1 Growth parameters

Figures 1, 2 and 3 display the total root length, shoot length and plant biomass. Phenanthrene and naphthalene affected plant growth in different ways and at different concentrations of the pollutants in the soil. The addition of phenanthrene at different concentrations in soil (5, 50, 200 and 400 mg/kg) caused a measurable change (decrease) in shoot, root and total biomass in the two plant species, as compared to those of control plants (no treatment). Root length and biomass were affected to some extent (i.e. 13.53 cm, 0.01186 g/cm² at 50 mg/kg; 12.13 cm, 0.01176 g/cm² at 200 mg/kg; 11.50 cm, 0.0114 g/cm² at 400 mg/kg) for *Trigonella foenum-graceum*, and to a greater extent (32.50 cm, 0.0133 g/cm² at 50 mg/kg; 31.73 cm, 0.0129 g/cm² at 200 mg/kg; 30.40 cm, 0.0126 g/cm² at 400 mg/kg) for *Brassica juncea* (excluding at 5 mg/kg, which showed exclusive reduction i.e. 0.0087 g/cm², 9.53 cm for *Trigonella foenum-graceum* and 29.50 cm, 0.0087 g/cm² for *Brassica juncea*). Shoot length decreased with doses of 50 mg/kg (58.43 cm), 200 mg/kg (54.63 cm) and 400 mg/kg (52.63 cm) in *Brassica juncea*, whereas in *Trigonella foenum-graceum*,

Fig. 1 **a** Effects of naphthalene (NHE) and phenanthrene (PHE) on root length of *Trigonella foenum-graceum* with or without PGPR. **b** Effects of naphthalene (NHE) and phenanthrene (PHE) on biomass

of *Trigonella foenum-graceum* with or without PGPR. Data for each parameter were analysed separately using three-way ANOVA followed by Duncan's new multiple range test

Fig. 2 **a** Effects of naphthalene (NHE) and phenanthrene (PHE) on root length of *Brassica juncea* with or without PGPR. **b** Effects of naphthalene (NHE) and phenanthrene (PHE) on biomass of *Brassica*

juncea with or without PGPR. Data for each parameter were analysed separately using three-way ANOVA followed by Duncan's new multiple range test

Fig. 3 **a** Effects of naphthalene (NHE) and phenanthrene (PHE) on shoot length of *Trigonella foenum-graceum* with or without PGPR. **b** Effects of naphthalene (NHE) and phenanthrene (PHE) on shoot

length of *Brassica juncea* with or without PGPR. Data for each parameter were analysed separately using three-way ANOVA followed by Duncan's new multiple range test

the values were 18.63 cm, 17.40 cm and 16.90 cm for doses of 50, 200 and 400 mg/kg, respectively. Toxicity symptoms such as chlorosis and stunted growth were observed at the 5-mg/kg dose 10 days after applying phenanthrene, whereas in the case of naphthalene, visually evident toxic effects and reduced growth were less pronounced at different concentrations for both *Trigonella foenum-graceum* and *Brassica juncea*. The addition of naphthalene had a marked effect on the growth of *Brassica juncea* but not *Trigonella*

foenum-graceum; nevertheless, the decreased growth was less pronounced than that for treatment with phenanthrene. The study revealed that the effects on the growth of both species were markedly affected by phenanthrene as compared to naphthalene, whereas *Trigonella foenum-graceum* showed resistivity towards both compounds. Degradation of PAHs in *Triticum aestivum* has been reported; the observed results, based on the degradation ratios of PAHs in different soil (i.e. planted and unplanted), confirm

the impact of *Triticum aestivum*. PAH content was lower in the soil in which different species were planted. The loss of the PAH is due to biotransformation, biodegradation and uptake by plants from the soil [19, 20, 23, 28]. Many research studies have reported that the accumulation of PAHs in plants is negligible [16, 32]. Loss of phenanthrene is due predominantly to plant-promoted biodegradation. The enhanced dissipation of phenanthrene in the planted soil is due to the microorganisms present in the rhizospheric zone. Soil containing plant litter and root exudates is more favourable for transformation of the microbes, providing substrates for co-metabolic dissipation and enhancing the bioavailability of the contaminants [16]. To enhance the growth rate of the plants, PGPR was added to the soil, which led to 75% enhancement of biomass in *Trigonella foenum-graceum*, while shoot and root length were increased by 68%. Similarly, in the case of *Brassica juncea*, biomass increased by 65%, whereas the shoot length and root length were increased by 80%.

3.2 Effect of PGPR

PGPR were used to enhance plant growth under conditions of stress and contamination. PGPR showed a

tremendous effect on both growth and chlorophyll content of plants. The growth parameters of PGPR-treated plants differed significantly ($P \leq 0.05$) from those of non-PGPR-treated plants, as assessed by three-way ANOVA and Duncan's new multiple range test. In *Trigonella foenum-graceum*, 75% increased biomass was observed as compared to non-PGPR plants, whereas shoot and root length were increased by 68%. Similarly, in the case of *Brassica juncea*, biomass increased by 65%, while shoot and root length increased by approximately 80%. To estimate the effect of PGPR on microbial growth, the colony counting method was used. The bacterial colonies were observed to increase with increased concentrations of phenanthrene and naphthalene, as shown in Table 1. Among the four concentrations, maximum growth of bacterial colonies (9.89×10^{10}) was observed at 400 mg/kg.

The study showed that PGPR counterbalanced the deleterious effect of phenanthrene and naphthalene by enhancing microbial growth in the soil, leading to enhanced stress tolerance in these plants as compared to the non-PGPR plants.

Table 1 CFU values obtained by ANOVA (Duncan's new multiple range test) for *Trigonella foenum-graceum* and *Brassica juncea*

Treatment (mg)	Bacterial colonies in 1 g of soil planted with <i>Trigonella foenum-graceum</i>	
	Without PGPR	PGPR
Phenanthrene		
5	3.56×10^{10}	4.47×10^{10}
50	4.98×10^{10}	6.31×10^{10}
200	7.24×10^{10}	9.76×10^{10}
400	8.76×10^{10}	9.89×10^{10}
Naphthalene		
5	2.84×10^{10}	3.31×10^{10}
50	3.86×10^{10}	4.17×10^{10}
200	5.20×10^{10}	5.43×10^{10}
400	6.01×10^{10}	6.75×10^{10}
Treatment (mg)	Bacterial colonies in 1 g of soil planted with <i>Brassica juncea</i>	
	Without PGPR	PGPR
Phenanthrene		
5	2.36×10^{10}	3.27×10^{10}
50	3.62×10^{10}	4.11×10^{10}
200	5.24×10^{10}	6.26×10^{10}
400	7.46×10^{10}	8.19×10^{10}
Naphthalene		
5	1.34×10^{10}	2.01×10^{10}
50	2.16×10^{10}	3.27×10^{10}
200	3.40×10^{10}	4.13×10^{10}
400	5.51×10^{10}	5.93×10^{10}

3.3 Chlorophyll and carotenoid content

The effect of phenanthrene and naphthalene on the chlorophyll parameters is given in Table 2. From the results, it is evident that phenanthrene and naphthalene had different effects on the two plant species *Trigonella foenum-graceum* and *Brassica juncea*. Although both treatments had a negative effect on all the measured chlorophyll parameters, the effects of phenanthrene were greater than those of naphthalene. The highest inhibition with 5-mg/kg treatment (1.365 ± 0.0015 mg/m⁻², 3.31 ± 0.002 mg/m⁻² and 0.298 ± 0.001 mg/m⁻² for Chl-a, Chl-b and carotenoids, respectively) was documented in phenanthrene-supplemented soil as compared to control. The increased chlorophyll values with naphthalene treatment, however, were not significantly different ($p < 0.05$) from the control. In contrast, for *Brassica juncea*, the negative effects of phenanthrene and naphthalene were significantly different from *Trigonella foenum-graceum*. The measured deleterious effects on chlorophyll content (i.e. Chl-a, Chl-b and carotenoids) of *Brassica juncea* in phenanthrene-contaminated soil at 5 mg/kg were 0.500 ± 0.001 , 0.884 ± 0.001 and 0.081 ± 0.002 , respectively. On further addition of phenanthrene and naphthalene up to 400 mg/kg, the chlorophyll parameters did not differ significantly from controls. The addition of PGPR to contaminated soil had a considerable mitigating effect on phenanthrene: the chlorophyll content increased to 3.131 ± 0.0001 , 5.26 ± 0.001 and 0.72 ± 0.001 for Chl-a, Chl-b and carotenoids, respectively, with 5 mg/kg treatment of *Trigonella foenum-graceum*. For *Brassica juncea*, the chlorophyll content increased to 1.087 ± 0.002 , 1.97 ± 0.002 and 0.208 ± 0.0002 for Chl-a, Chl-b and carotenoids, respectively, with 5-mg/kg treatment. In contrast, the addition of PGPR to the phenanthrene and naphthalene treatment in soil planted with *Brassica juncea* showed no significant effect as compared to *Trigonella foenum-graceum*, as shown in Table 2.

The above results reveal that the microbial load increased with increased concentrations of phenanthrene and naphthalene. This is because microorganisms in the rhizosphere use pollutants as a carbon source for their energy; thus the microbial load was greatest with 400 mg/kg for both phenanthrene and naphthalene. However, if we compare phenanthrene and naphthalene, the microbial load was lower for naphthalene, as it is more volatile in nature than phenanthrene. In addition, we found that resistance to phenanthrene and naphthalene in both plant species was associated with shoot length, chlorophyll content and biomass of the plant. Plant biomass is a key factor in the phytoremediation process. Comparing selected treatments under the various levels of pollutants

in soil, the plants and the PGPR both produce an increase in shoot height and dry weight, and there is no effect on the content of chlorophyll, as analysed by three-way ANOVA and Duncan's new multiple range test ($p \leq 0.05$). Even under poor conditions, PGPR shows a good impact on the growth of the plants. In comparing the results with previous studies, Janoušková and Vosátka [15] revealed that inoculation of carrot with arbuscular mycorrhizal fungi (AMF) caused growth inhibition resulting from toxic cadmium (Cd) compared with the non-inoculated control. Hong et al. [13] revealed that PGPR enhanced the shoot length and biomass of maize grown in soil contaminated with diesel oil. The synergistic effect of PGPR is due to the release of organic acids, siderophores and phytochelators, which further help to reduce soil pH. The excellent growth parameters of plants grown in PAH-contaminated soil provide an important indication that these plants have potential value for phytoremediation. The removal of PAHs is achieved by several techniques including biodegradation [1, 26], photo-oxidation, leaching and bioaccumulation [24]. Many recent studies have reported the effects of PAHs on plants [31]; in yellow lupine plants, 80% reduction in the upper portion of the plant and 40% reduction in root length were observed. The plant species of rye, red clover and white mustard showed that engine oil mixed with soil enhanced the toxic effects of xenobiotics [23]. This occurs because xenobiotics in soil disturb essential nutrient uptake, which results in stunted plant growth [25]. In this study, phenanthrene and naphthalene were used as two pollutants and were mixed in the soil prior to growing seeds. The results revealed that phenanthrene exerted a greater deleterious effect than naphthalene on both *Brassica juncea* and *Trigonella foenum-graceum*. The degradative effects of these chemicals were dependent mainly on the molecular weight and structure of the compounds. Phenanthrene was more stable in the soil because of its three angular rings and lower desorption rate [7, 29] as compared to naphthalene, with its two-ring straight structure and easy solubility in aqueous phase. Both plants species showed high tolerance and resistivity towards these PAH compounds. Significant effects ($p \leq 0.05$) were shown only with a low concentration of PAH compounds (i.e. 5 mg/kg), because at lower PAH concentrations, the plants take up these compounds through the roots, showing the effect on the morphology and photosystem, and the remaining pollutants which were unavailable to the plants were degraded by microorganisms present in the soil. With a high concentration, however, the phenanthrene and naphthalene microbial degradation increased because of the availability of carbon sources for the microbes. Naphthalene was easily degraded by microbes

Table 2 Effect of naphthalene (NHE) and phenanthrene (PHE) on chlorophyll content of *Trigonella foenum-graceum* and *Brassica juncea* with and without PGPR

Treatment	Plant species	Chl-a (mg/ml)	Chl-b (mg/ml)	Carotenoid (mg/ml)
Control without PHE	<i>Trigonella foenum-graceum</i>	3.768 ± 0.0015	6.28 ± 0.002	1.23 ± 0.001
	<i>Brassica juncea</i>	1.348 ± 0.0015	3.260 ± 0.0015	0.487 ± 0.001
Control + PGPR + PHE	<i>Trigonella foenum-graceum</i>	5.833 ± 0.0015	9.48 ± 0.001	2.039 ± 0.002
	<i>Brassica juncea</i>	2.148 ± 0.001	4.72 ± 0.002	0.622 ± 0.0002
5 mg/kg PHE	<i>Trigonella foenum-graceum</i>	1.365 ± 0.0020	3.31 ± 0.002	0.298 ± 0.001
	<i>Brassica juncea</i>	0.500 ± 0.001	0.884 ± 0.001	0.081 ± 0.002
5 mg/kg PHE + PGPR	<i>Trigonella foenum-graceum</i>	3.131 ± 0.0001	5.26 ± 0.001	0.72 ± 0.001
	<i>Brassica juncea</i>	1.087 ± 0.002	1.97 ± 0.002	0.208 ± 0.0002
50 mg/kg PHE	<i>Trigonella foenum-graceum</i>	2.283 ± 0.0001	3.69 ± 0.002	0.427 ± 0.002
	<i>Brassica juncea</i>	1.106 ± 0.001	1.88 ± 0.001	0.211 ± 0.001
50 mg/kg PHE + PGPR	<i>Trigonella foenum-graceum</i>	4.986 ± 0.0001	8.63 ± 0.002	1.72 ± 0.002
	<i>Brassica juncea</i>	2.135 ± 0.001	3.83 ± 0.002	0.523 ± 0.001
200 mg/kg PHE	<i>Trigonella foenum-graceum</i>	2.185 ± 0.0002	3.48 ± 0.001	0.357 ± 0.002
	<i>Brassica juncea</i>	0.88 ± 0.001	1.504 ± 0.001	0.161 ± 0.001
200 mg/kg PHE + PGPR	<i>Trigonella foenum-graceum</i>	4.336 ± 0.0002	7.47 ± 0.002	1.45 ± 0.001
	<i>Brassica juncea</i>	1.821 ± 0.001	3.27 ± 0.001	0.411 ± 0.001
400 mg/kg PHE	<i>Trigonella foenum-graceum</i>	2.094 ± 0.0002	3.05 ± 0.001	0.286 ± 0.003
	<i>Brassica juncea</i>	0.814 ± 0.002	1.363 ± 0.001	0.145 ± 0.002
400 mg/kg PHE + PGPR	<i>Trigonella foenum-graceum</i>	3.864 ± 0.0002	6.56 ± 0.001	1.25 ± 0.001
	<i>Brassica juncea</i>	1.721 ± 0.001	3.09 ± 0.001	0.354 ± 0.001
Control without NHE	<i>Trigonella foenum-graceum</i>	3.768 ± 0.0015	6.286 ± 0.002	1.236 ± 0.001
	<i>Brassica juncea</i>	1.348 ± 0.0015	3.260 ± 0.001	0.487 ± 0.001
Control + PGPR + NHE	<i>Trigonella foenum-graceum</i>	5.833 ± 0.0015	9.483 ± 0.001	2.0397 ± 0.001
	<i>Brassica juncea</i>	2.481 ± 0.001	4.724 ± 0.002	0.622 ± 0.002
5 mg/kg NHE	<i>Trigonella foenum-graceum</i>	1.994 ± 0.0015	3.402 ± 0.001	1.529 ± 0.001
	<i>Brassica juncea</i>	0.923 ± 0.0015	1.647 ± 0.001	0.196 ± 0.001
5 mg/kg NHE + PGPR	<i>Trigonella foenum-graceum</i>	3.211 ± 0.001	5.382 ± 0.001	0.972 ± 0.001
	<i>Brassica juncea</i>	1.087 ± 0.002	1.97 ± 0.002	0.246 ± 0.002
50 mg/kg NHE	<i>Trigonella foenum-graceum</i>	3.285 ± 0.002	5.546 ± 0.001	2.626 ± 0.001
	<i>Brassica juncea</i>	1.254 ± 0.002	2.202 ± 0.002	0.310 ± 0.001
50 mg/kg NHE + PGPR	<i>Trigonella foenum-graceum</i>	5.452 ± 0.001	9.250 ± 0.001	1.954 ± 0.001
	<i>Brassica juncea</i>	2.406 ± 0.001	4.345 ± 0.001	0.599 ± 0.002
200 mg/kg NHE	<i>Trigonella foenum-graceum</i>	3.242 ± 0.0002	5.475 ± 0.002	2.591 ± 0.001
	<i>Brassica juncea</i>	1.216 ± 0.001	2.139 ± 0.002	0.310 ± 0.001
200 mg/kg NHE + PGPR	<i>Trigonella foenum-graceum</i>	5.090 ± 0.001	8.852 ± 0.001	1.843 ± 0.001
	<i>Brassica juncea</i>	2.083 ± 0.001	3.780 ± 0.001	0.516 ± 0.001
400 mg/kg NHE	<i>Trigonella foenum-graceum</i>	3.238 ± 0.0001	5.46 ± 0.001	2.586 ± 0.002
	<i>Brassica juncea</i>	0.863 ± 0.002	0.993 ± 0.001	0.213 ± 0.002
400 mg/kg NHE + PGPR	<i>Trigonella foenum-graceum</i>	4.864 ± 0.001	8.469 ± 0.001	1.767 ± 0.001
	<i>Brassica juncea</i>	0.960 ± 0.001	1.753 ± 0.001	0.354 ± 0.0010

Data for each parameter were analysed separately using three-way ANOVA followed by Duncan's new multiple range test. Values designated for chlorophyll content which we have taken (Chl-a, Chl-b and carotenoids) differ significantly ($p < 0.05$). Means and SD are shown ($n = 5$)

because of its two rings and more volatile nature, as it can be easily evaporated as compared to phenanthrene, with its angular structure and lower degradability and volatility [17]. This shows that phenanthrene has more deleterious effects on plant species than naphthalene.

4 Conclusion

The plant species tested in this study germinated and grew in soil contaminated with PAH (phenanthrene and naphthalene). The negative impact of phenanthrene and

naphthalene was observed in the growth parameters and chlorophyll content in both *Brassica juncea* and *Trigonella foenum-graceum*. PGPR plays an important role in mitigating stress conditions by enhancing the degradation of PAH compounds. This property can be exploited in the future to ensure food security. The plants treated with PGPR showed excellent tolerance and resistivity towards PAH compounds even at high concentrations in soil. Further, the experimental plants showed that the adverse effects of phenanthrene were greater than those of naphthalene in both plant species. The experiment proved that the benefits of phytoremediation increased exponentially at higher PAH concentrations for both plant species, which was due to the influence of PAH soil concentration on rates of biodegradation. Under the experimental conditions, the microbes tended to utilize the PAH compounds as nutrients. As the concentration increased, the degradation rate also increased, with optimal conditions at 400 mg/kg of phenanthrene and naphthalene. Based on the results of this study, further biodegradation studies are in progress to evaluate the extent of degradation of PAH compounds in soil by these plant species monitored using gas chromatography techniques.

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Compliance with ethical standards

Conflict of interest On behalf of all the authors, the corresponding author declares that there is no conflict of interest in this research article.

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