

Original Research Article

<http://dx.doi.org/10.20546/ijcmas.2016.510.034>Use of *Pseudomonas aeruginosa* as Fertilizer in *Eruca sativa*

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A B S T R A C T

P. aeruginosa is more widely known as an opportunistic pathogen for humans and animals than as soil bacteria, for this reason it was used as fertilizer in many crops as well as against root rot without harmful action. It is Pyocyanin pigment may consider as plant growth promoting, and can act as a cheap source of fertilizers to crop plants. Pyocyanin production by the selected *P. aeruginosa* isolates L. 12, L.16 and L.17 was determined in different media. The crude Pyocyanin was taken as control and other treatments were done by adding distilled water, Viz., 5%, 15% and 25%. *E. sativa* seedlings were irrigated every other day using these solutions. Measurements of leaf area, biomass Water content, photosynthesis pigments and essential and heavy elements were taken. Results revealed a pronounce increase in low Pyocyanin concentration (5%) treated plants compared to control in all measured parameter. On the other hand, high Pyocyanin concentration treated plants achieved the highest elements constituents. Pyocyanin may be further improved with the optimization and familiarization according to the current soil conditions. In future, it may replace the chemical fertilizers and help in sustainable agriculture. Further research and understanding of mechanisms of Pyocyanin is urgently needed.

Keywords

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Introduction

Eruca sativa is widely distributed all over the world and is usually consumed fresh for its special taste. It is a herb of traditional medicine and it contains many ingredients such as glucosinolates vitamin C, carotenoids, and flavonoids (Saleh *et al.*, 2009). *Pseudomonas aeruginosa* is a common Gram-negative rod-shaped bacterium that can cause disease in plants and animals, including humans.

The direct use of microorganisms to promote plant growth and to control plant

pests and diseases continue to be an area of speedily going up research. Many researchers have showed that the positive effect of *Pseudomonas aeruginosa* on the productivity of many crops in different soils and climatic conditions (Das *et al.*, 2010 and Santos *et al.*, 2013; Bashan *et al.*, (2011) concluded *Prosopis articulata*, *Parkinsonia microphylla*, *Parkinsonia florida* helped in biomass increase of seedlings. In addition, Liu *et al.*, (2013) reported that Rhizobacteria together with fertilizers helped high dry matter accumulation and nutrient uptake in the *Fraxinus americana* forest seedlings.

P. aeruginosa is more widely known as an opportunistic pathogen for humans and animals than as soil bacteria, for this reason it was used as fertilizer to many crops as well as against root rot without harmful action (Farruk *et al.*, 2007). Instead, *Pseudomonas* spp. have appeared to be an important biological mean to control plant diseases (Ultan *et al.*, 2001 and Prasada and Paramageetham, 2013).

In this study *Pseudomonas aeruginosa* species were isolated from forest litter and evaluated for its bio control activity against *Sclerotium rolfsii* Sacc. Horticulture is one of the areas where agricultural technologies are widely and intensively used. Horticultural crops may grow both in the open and closed space such as greenhouse and tunnel, and there are several practical applications such as propagation with cuttings and grafting, pruning, and soilless culture; plant growth regulators that have little or no use with other agricultural crops were used largely in horticultural crop production (Ahmet, 2011).

Research Methodology

Bacterial isolates

Pseudomonas aeruginosa isolates used in the study

Identified *Pseudomonas aeruginosa* (20 isolates) were obtained from a previous study, which was collected from contact lens storage cases, contact lenses and contact lens wearer in Saudi Arabia (Salha, 2012).

Preservation of the used organisms

Pseudomonas aeruginosa isolates were maintained on cetrimide agar at 4°C, while tested bacteria were maintained on slopes of Nutrient agar at 4°C. All the tested fungi maintained on slopes of Sabouraud medium

at 4°C. All the organisms were regenerated every six months, according to Dadgar *et al.* (2006).

Screening the ability of *P. aeroginsa* to produce pigments on Cetrimide agar

All isolates of *Pseudomonas aeruginosa* were screened for pigments production on cetrimide agar 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 the previous medium at 35°C for 3 days.

Effect of cultural conditions on the production of pyocyanin by selected isolates of *P. aeruginosa*

These experiments were performed in 250 ml Erlenmeyer flasks containing 48 ml of the production medium, inoculated with 2 ml of the overnight culture of the selected *P. aeruginosa* isolate L.12, L.16 or L.17. The flasks were incubated at 35°C for 6 days. At the end of the incubation period, the cells were collected by centrifugation at 4000 rpm for 30 min and the quantity of pyocyanin was measured as described before.

Effect of different media on the production pyocyanin by selected isolates of *P. aeruginosa*

Pyocyanin production by the selected *P. aeruginosa* isolates L. 12, L.16 and L.17 was determined in different media. The media used were King's medium B Base (King *et al.*, 1954), King's Medium A Base (King *et al.*, 1954), *Pseudomonas* F medium (King *et al.*, 1954), *Pseudomonas* P medium (Forbes *et al.*, 2007). After preparation of these different media in 250 ml Erlenmeyer flasks containing 48 ml of the media, they were inoculated with 2 ml of the selected bacterial isolate L.12, L.16 or L.17 and the flasks were incubated at 35°C. After 6 days of incubation period.

Applications of pyocyanin pigment

The crude pyocyanin was taken as control and other treatments were done by adding distilled water, *Viz.*, 5%, 15% and 25%. *E. sativa* seedlings were irrigated every other day using these solutions.

Measurements

One month later, many parameters have been estimated. These include leaf area, root and shoot lengths, biomass, water content, photosynthetic pigments, in addition to the amount of essential and heavy elements in shoots and roots.

Statistical Analysis

The results were analyzed statistically by using the SPSS BASE 20.0 for windows (SPSS Inc., Chicago, IL) packages. Data were tested by ANOVA and means were analyzed at $P \leq 0.05$ and $P \leq 0.01$ levels of significant, to determine the significant differences between different treatments.

Results and Discussion

Leaf is of *E. sativa* treated with different concentrations of pyocyanin

E. sativa leaf areas showed different responses when treated with different concentrations of pyocyanin pigment as was shown in Fig 1. Leaf areas were high in all treated plants compared to control. The increase followed the pattern 5% Pyocyanin (8.9 cm^2) > 15% Pyocyanin (7.8 cm^2) > 25% Pyocyanin (6.7 cm^2). The control plants recorded the lowest leaf area (3.5 cm^2).

Biomass of *E. sativa* treated with different concentrations of pyocyanin

The maximum growth in the shoots and roots lengths was observed principally in

shoots and roots of the treated plants across all treatments (Fig.2). The above and below ground lengths increase in different treatments was in the following order: 5% Pyocyanin > 15% Pyocyanin) > 25% Pyocyanin. (40cm, 23.5cm, 23cm in shoot) and (25% > 5% > 15% in roots. (10cm, 9.3cm and 8.3cm, respectively). The control plants recorded the lowest shoot and root lengths (14.4 cm and 7.3 cm, respectively).

Water Content of *E. sativa* Shoots and roots treated with different concentrations of Pyocyanin

Fig. 3 illustrates the amount of water content of shoots and roots of *E. sativa* treated with different concentrations of Pyocyanin. There was no pronounce effect in water content of shoots, nonetheless, control plants recorded the highest value (92.3%) but there is no significant difference compared to treated plants. On the other hand, treated plants showed high water content compared to control, especially 5% concentration (76.7% compared to 60.7% of the control).

Photosynthesis Pigments of *E. sativa* treated with different concentrations of Pyocyanin

In general, photosynthesis pigments showed different response to the treatments. Regardless of the treatments, all plants recorded higher amounts of carotenoides followed by chlorophyll A and less amounts of chlorophyll B (Fig. 4). Treated plants (5% and 15%) achieved the highest amount of chlorophyll A ($1.29 \mu\text{mol/g/fresh wt.}$), followed by 25% treatment ($1.02 \mu\text{mol/g/fresh wt.}$), while the control plants recorded the lowest amount of chlorophyll A ($0.26 \mu\text{mol/g/fresh wt.}$) ($P \leq 0.01$).

The same pattern was observed in the amount of chlorophyll B and the carotenoids. All treated plants recorded high

amount of chlorophyll B and carotenoids, while control plants showed the lowest amount of these pigments (Fig. 4). Worth mentioning, 5 % treatment recorded 5 times and 6 times more of chlorophyll B and carotenoids ($0.52 \mu\text{mol/g}$ /fresh wt compared to $0.099 \mu\text{mol/g}$ /fresh wt) for chlorophyll B and ($1.59 \mu\text{mol/g}$ /fresh wt compared to $0.26 \mu\text{mol/g}$ /fresh wt) for carotenoids.

Amount of Essential and Heavy Elements of *E. sativa* Shoots treated with different concentrations of Pyocyanin

Essential elements such as Na, Mg, C, K, P, N and Cl, (Fig. 5), showed different trends as a response to treatment with different concentration of pyocyanin. For instance, 5% plants had the lowest amount of sodium while the control recorded the highest value of sodium. On the other hand, high accumulation of Ca, K and N, in the aerial parts of *E. sativa* shoots were recorded in 15% treated plants and lowest values in control. Concerning P, there were no significant differences among treatments or with the control plants. Accumulation of heavy elements in shoots of *E. sativa*, followed different trends than essential ones (Fig. 6). All plants had low amount of Pb, and Cu. 15% treated plants accumulated large amounts of B, Zn and Fe. On the other hand, 5% treated plants accumulated almost the same amount of Mn as the control plants, while 15% and 25% treated plant recorded almost the same amount of Mn.

Amount of Essential and Heavy Elements of *E. sativa* Roots treated with different concentrations of Pyocyanin

Fig. 7 and 8 illustrated the amount of essential and heavy elements accumulated in roots of treated and control *E.sativa* plant. Fig 7 indicated that 5% treated plants recorded less essential elements in terms of Na, Mg, Ca, P and N. In contrast, 15% and

25% accumulated large amount of Na, Mg, Ca, K, P and N. control plants however recorded moderate amounts of these elements in roots. Regarding heavy elements (Fig. 8), control plants recorded the highest amount of Pb, B, Cu, Mn and Fe. 5% treated plants accumulated the least amount of Pb, Zn and Mn, while 15% treated plants recorded the highest amount of Zn. 25% plants on the other hand, accumulated large amount of Pb and Mn. Worth mentioning is that, the trend of Fe was as follows, control plants> 5% treated plants> 15% treated plants> 25% treated plants.

P. aeruginosa low concentration of Pycyanin isolated in this study seemed to have positive effects on plant growth to encourage the growth and development of *E. sativa* in many ways. For Instance, leaf area increase by almost two and a half times at 5% concentration compared to the control. Leaf area growth reveals light interception and considered as an main factor in determining plant productivity (Gifford *et al.*, 1984; Koester *et al.*, 2014).

Moreover, high performance of plants frequently depends on optical methods in which leaf area growth is compared with estimates of photosynthesis for plants with altered photosynthetic rates because they are non-destructive and cost-effective (Golzarian *et al.*, 2011; Zhang *et al.*, 2012; Tessmer *et al.*, 2013 and Radhapriya *et al.*, 2015). Concerning shoot and root lengths, 5% Pyocyanin concentration pigment achieved *E.sativa* to record high values compared to control. Moreover, all treated plants showed high shoot and root lengths compared to the control.

The motivation of growth traits of *E.sativa* caused by Pyocyanin pigment was clear in the biomass of this plant in terms of shoot and root fresh and dry weights. All treated plants recorded the highest biomass in the

manner 5% > 15% > 25%. The appliance of *P. aeruginosa* strains is known to improve the shoot, root, and panicle growth as well as plant height in the case of tomato plants (Sabin and Tehmina, 2016). Miransari, (2011) reported that this increase in plant growth occurs because of the production of growth related substances. Moreover, in wheat, *P.aeruginosa* caused increased germination of seeds and plant shoot and root as was reported by Arif *et al.*, (2015).

Many researchers concluded that this increase in shoot and root growth is mainly due to the fact that, these bacteria products act directly on plant roots through the production of indole acetic acid (IAA) (Cheryl and Bermand, 2002). This phytohormone is supposed to stimulate the formation of lateral roots and increase nutrient absorption from root hairs (Sisir and Basu, 2006), and is known to stimulate the

cell elongation and cell division of plant roots (Farah *et al.*, 2004).

In addition, some authors reported that, chemical fertilization of seedlings with commercial biofertilizer resulted in a significant increase in biomass when compared with the control but was shown to be less effective than the combined application of *P. aeruginosa* RRALC3 and chemical fertilizer (Vessey, 2003).

Water contents of treated *E.sativa* shoots showed no significant effect when compared to control. However, roots of treated plants recorded high amount of water contents especially 5% treatment compared to control. Researchers concluded that plant root are potent growth signals and vital because of their influence on nutrient acquirement by plants (Travis *et al.*, 2004 and Besharat *et al.*, 2010).

Fig.1 Leaf area (Cm²) of *E. sativa* and shoot and Root lengths treated with different concentration of *P. aeruginosa* pigments

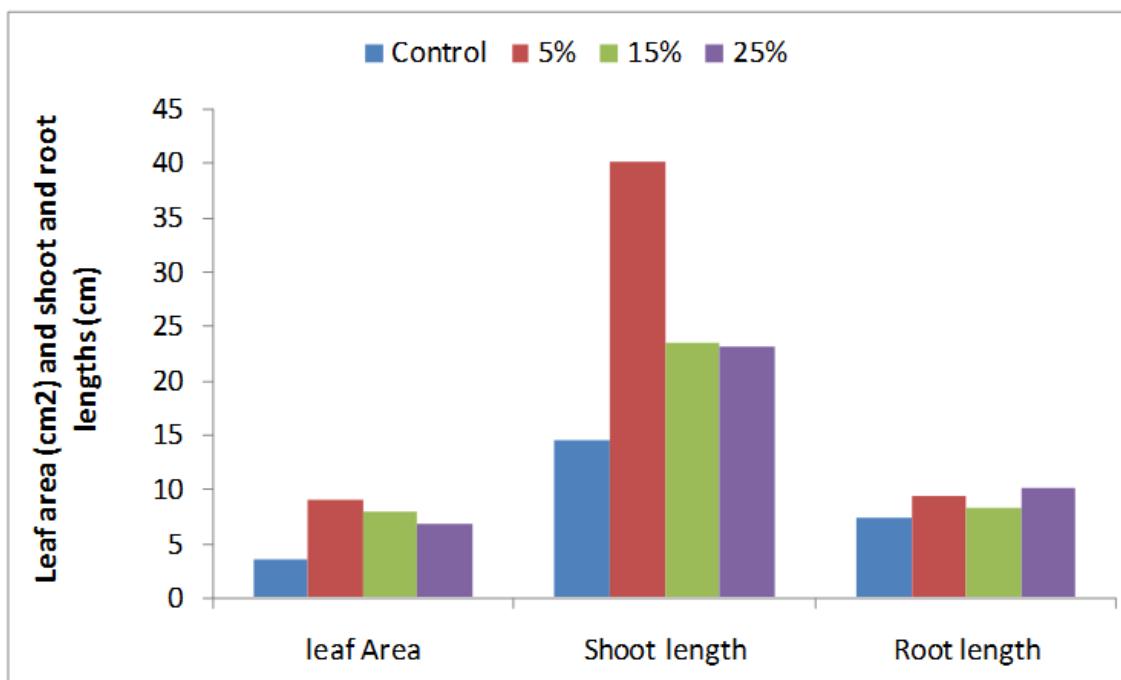


Fig.2 Biomass (g) of *E. sativa* treated with different concentration of *P. aeruginosa* pigments.

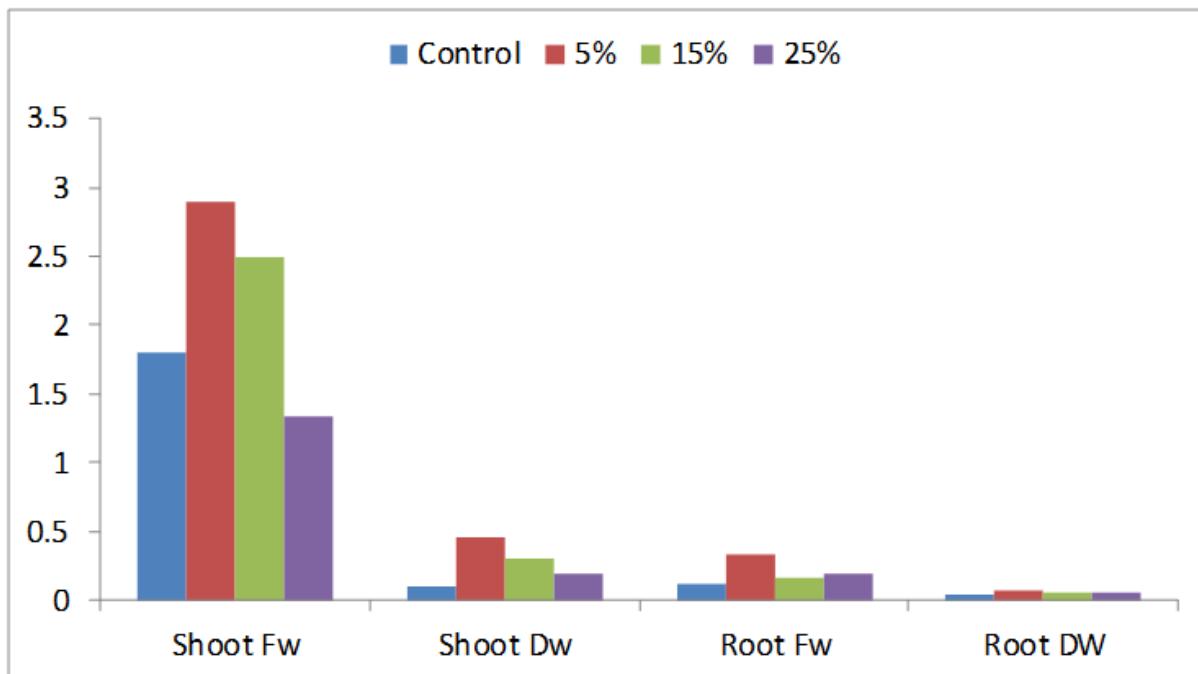


Fig.3 water Content (%) of *E. sativa* treated with different concentration of *P. aeruginosa* pigments

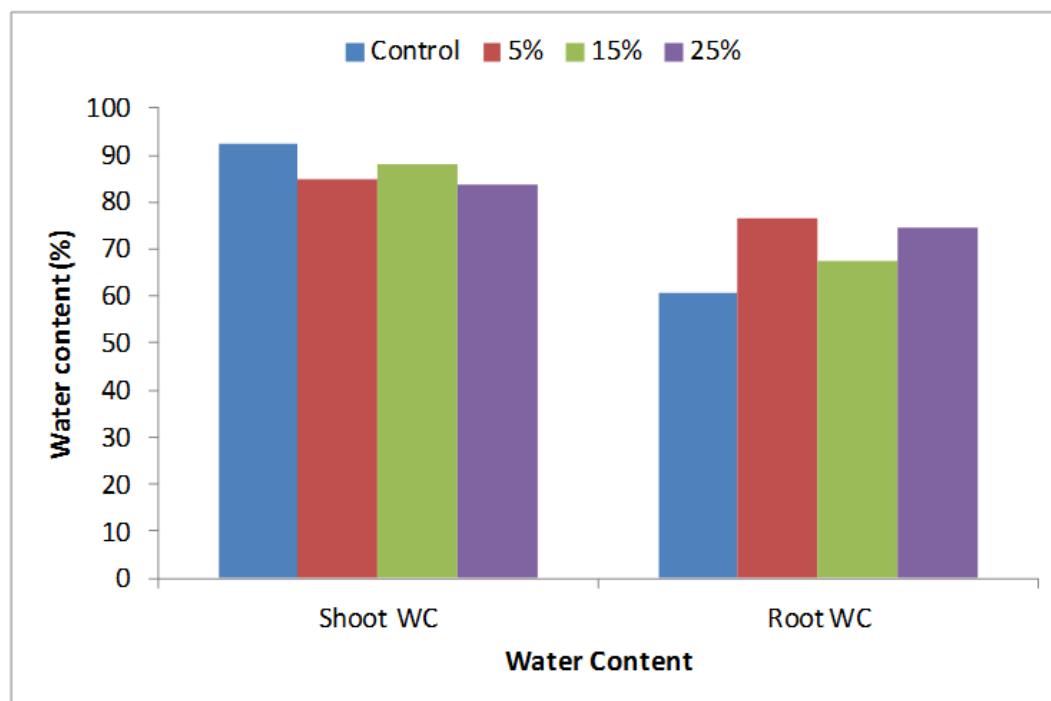


Fig.4 Photosynthetic Pigments ($\mu\text{mol/g/fresh wt.}$) of *E. sativa* treated with different concentration of *P. aeruginosa* pigments

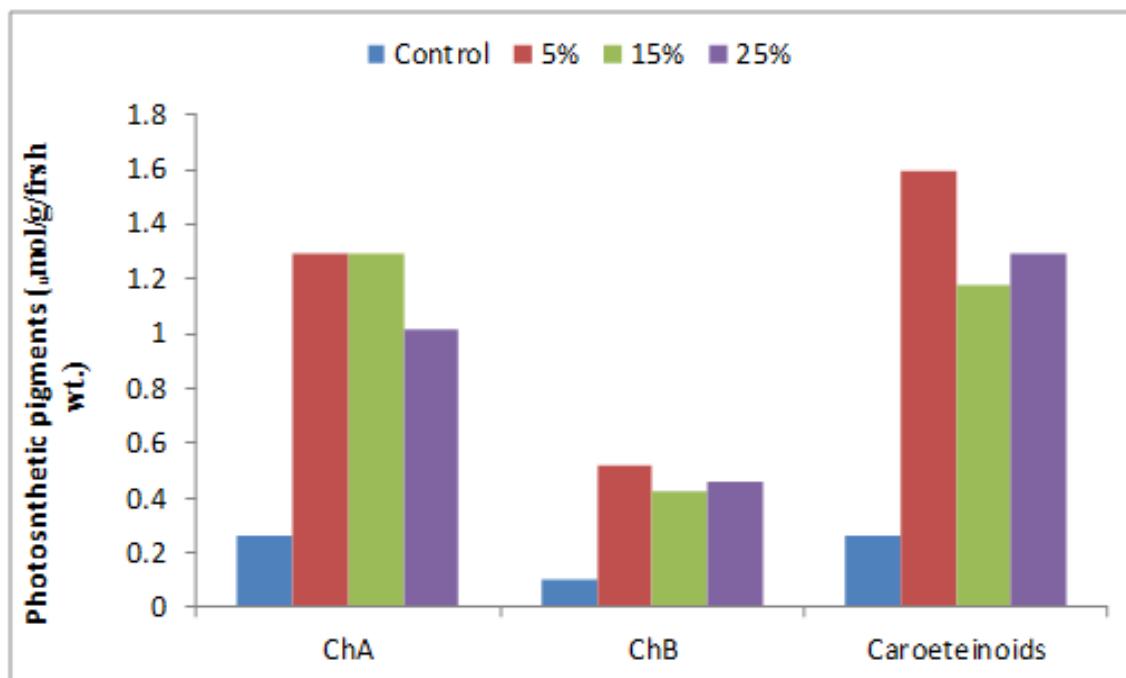


Fig.5 Amount of Essential Elements (%) of *E. sativa* Shoots treated with different concentration of *P. aeruginosa* pigments

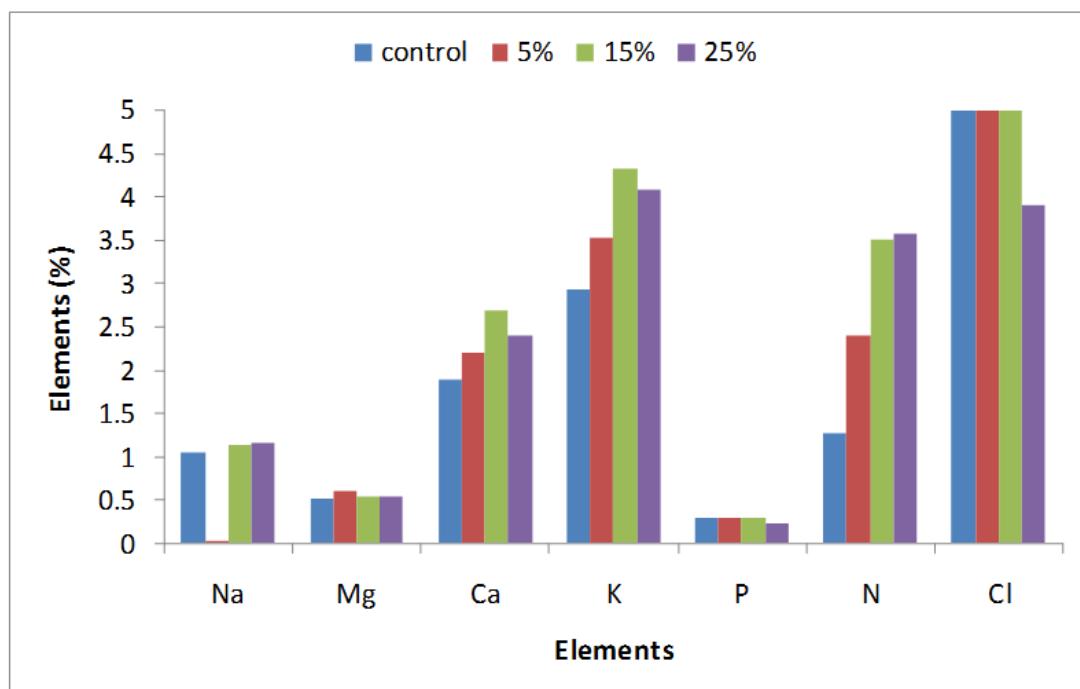


Fig.6 Amount of Heavy Elements (ppm) of *E. sativa* Shoots treated with different concentration of *P. aeruginosa* pigments

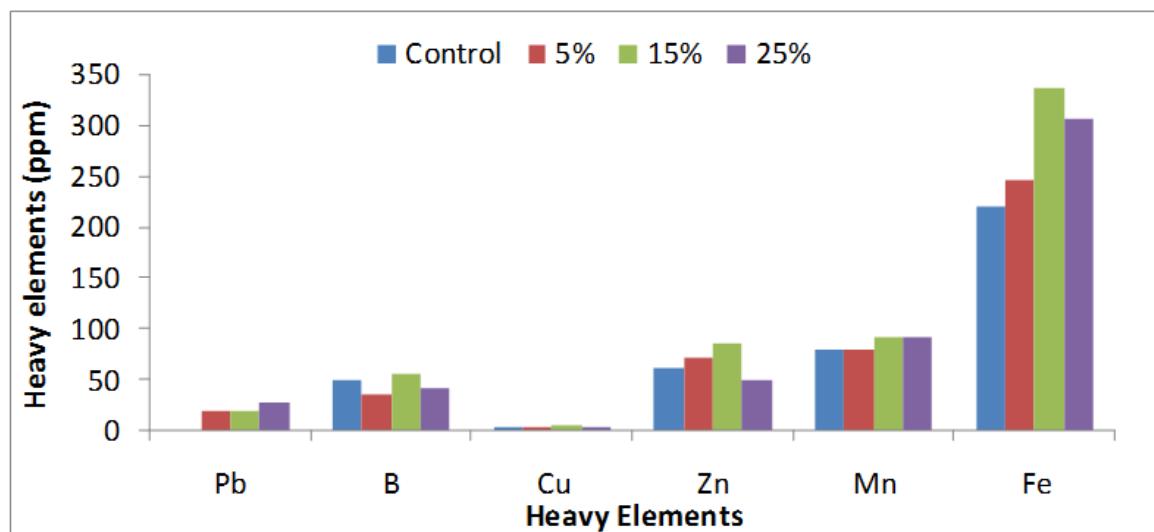


Fig.7 Amount of Essential Elements (%) of *E. sativa* Roots treated with different concentration of *P. aeruginosa* pigments

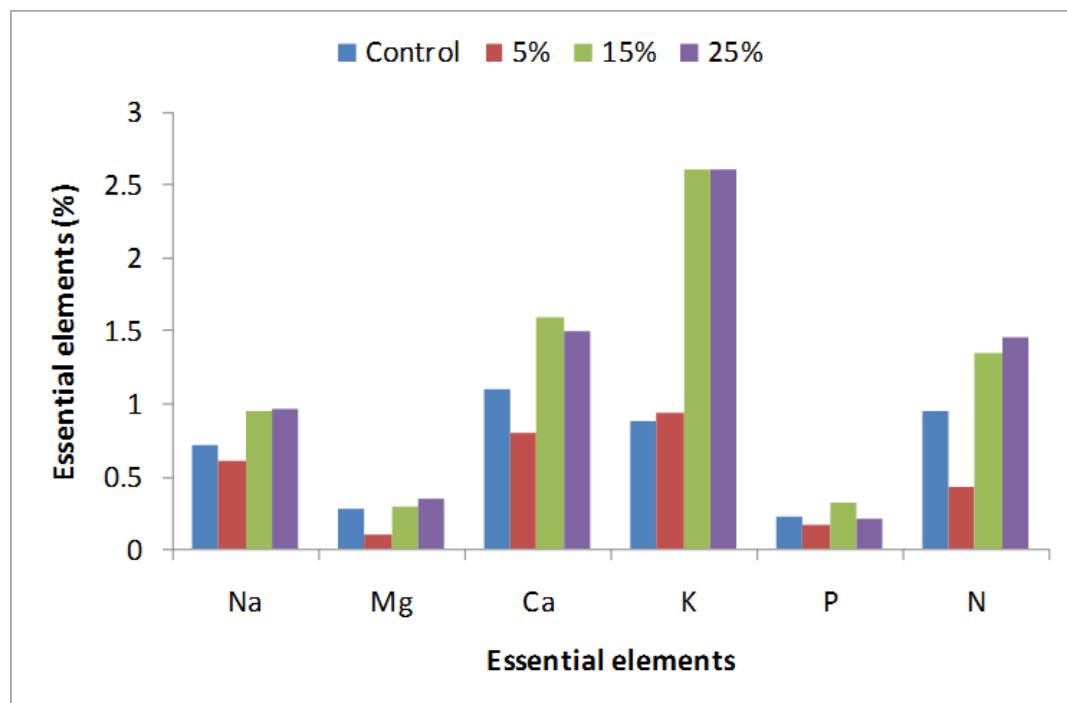
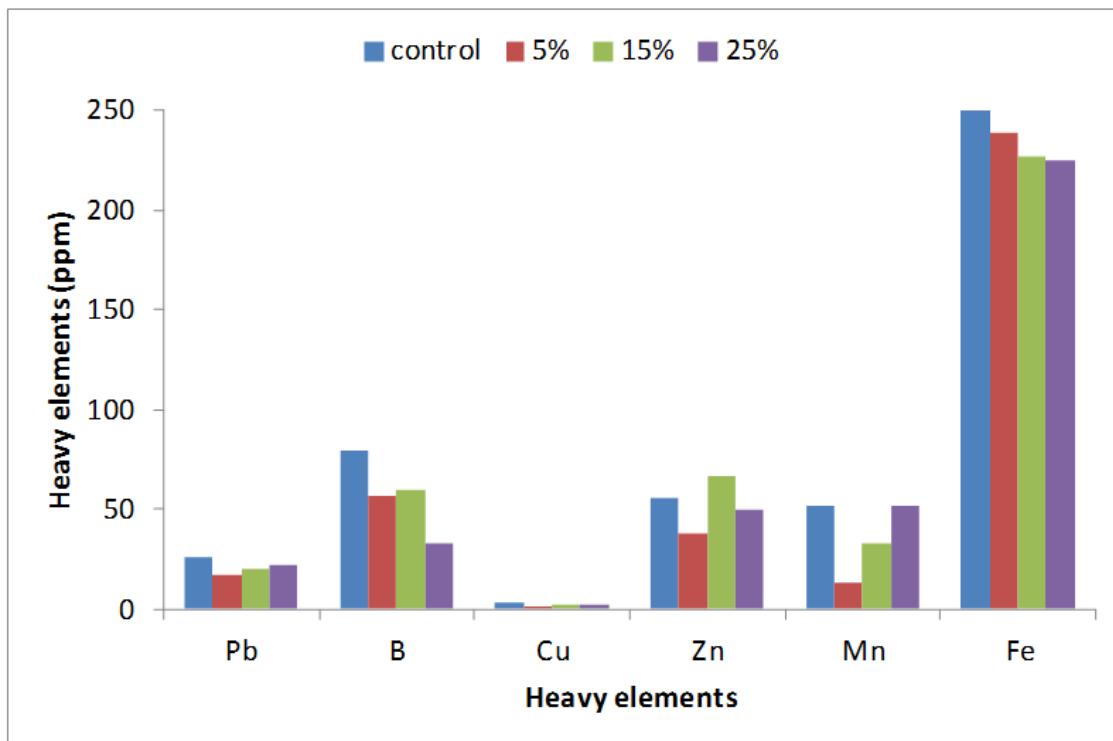


Fig.8 Amount of Heavy Elements (ppm) of *E. sativa* Roots treated with different concentration of *P. aeruginosa* pigments



Photosynthesis pigments, in terms of chlorophyll A and carotenoids showed pronounce increase in treated plants compared to control. Increased photosynthesis pigment with *Pseudomonas* treatment was reported by Islam *et al.*, (2014) in wheat. In general, the beneficial effect of high photosynthesis pigments is well known fact (Lawlor, 2002 and Chaves *et al.*, 2009).

Element accumulation in shoots revealed that, 5% treatment recorded low Na amount while the control plants recorded the highest value. In general low concentrations recorded the fewer amounts in case of essential and heavy elements, while the high concentrations accumulated more amounts of these elements.

Additionally, in the present study, it was found that high concentration treatment of

Pyocyanin (15% and 25%) increased the Na, Mg, K, Ca and Cl content of *E. sativa* seedlings roots. This might be credited to the production of organic acids by the plants themselves as well as by various bacteria in the rhizosphere because production of organic acids is influential in decreasing the soil pH and rousing the accessibility of micronutrients as was reported by Berg (2009). Regarding heavy elements, low concentration (5%) treated plants accumulated less Pb, Zn and Mn, while the other treatments recorded high amount of these elements. It was noticeable that Fe was abundant in shoots and roots of treated plants. This considered a good factor for the growth of *E. sativa*. Iron is a vital nutrient for almost all forms of life. All microorganisms essentially require iron (Neilands, 1995). In the aerobic environment, iron come up primarily as Fe^{3+} and is likely to form insoluble

hydroxides and oxyhydroxides, thus making it commonly unreachable to both plants and microorganisms (Rajkumar *et al.*, 2010).

In conclusion, plant growth promoting Pyocyanin, may act as a cheap source of fertilizers to crop plants. The productive competence may be further improved with the optimization and familiarization according to the current soil conditions. In future, they are expected to replace the chemical fertilizers and help in sustainable agriculture. Further research and understanding of mechanisms of Pyocyanin is urgently needed.

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