EFFECT OF *Pseudomonas* sp. (PF-097) To SUPRESS THE ALLELOPATHIC STRESS Of *Trianthema portulacastrum* L. ON *Vigna mungo* L.

Sumaira Abdul Raouf[1], Nadia Jabeen[1*] and Sundus Akhtar[1]

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ABSTRACT

Vigna mungo L. (black gram) is a summer pulse crop endemic to Central Asia. It is one of Pakistan's top five premium food beans and a highly cost-effective crop. Allelochemicals in *Trianthema portulacastrum* L. (black pigweed) leaf extract decreased the *V. mungo* germination rate (34% to 6%), seedling length (10 cm to 3 cm), fresh biomass of seedling (2.4 g to 0.94 g) and dry biomass of seedling (1.2 g to 0.47 g). Biological agent *Pseudomonas* sp. *(*PF-097) act as bioherbicide and as plant growth promoter agent to decrease the *T. portulacastrum* allelopathic stress and to enhance the growth of *V. mungo*. Amendment of *Pseudomonas* sp. (PF-097) increase *V. mungo* percentage of germination (8-42%), seedling length (12-54%), fresh biomass of seedling (8-17%) and dry biomass of seedling (0.47 g to 1.2 g). *In vivo* pot experiment bioassay revealed that of *T. portulaacstrum* significantly suppress the shoot length (46 cm to 18 cm), shoot fresh biomass (2.6 g to 0.66 g) and shoot dry biomass of *V. mungo* (1.31 g to 0.33 g). *Pseudomonas* sp. (PF-097) PGPR incorporation in vivo experiment significantly increased the shoot length (4% to 12%), shoot fresh biomass (18% to 35%), shoot dry biomass of *V. mungo* (17% to 35%)**.** Physiological activity of Catalase and peroxidase considerably increased in negative treatments amended with only *T. portulacastrum* and decreased in positive treatments by using biological agent *Pseudomonas* sp. (PF-097). Protein content of *V. mungo* seedling was significantly increased in positive treatments as compared to negative treatments. These results showed that allelochemical stress of *T. portulacastrum* on the *V. mungo* can be countered by the herbicidal activity of the *Pseudomonas* sp. (PF-097).

Keywords: Allelopathic stress, Bioherbicide, *Pseudomonas* sp. (PF-097), *Trianthema portulacastrum, Vigna mungo,* Weed

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[1] School of Botany, Minhaj University Lahore
Corresponding author's email: drnadia.bot@mul.edu.pk

INTRODUCTION

Vigna mungo L. Hepper is a common staple crop, endemic to Central Asia that grows just once a year (Behera *et al*., 2020). It belongs to the *Fabaceae* family. It's a very economical crop of Pakistan. According to economic survey of Pakistan 2020-21 black gram was cultivated on 11.0 thousand hectares area and the production was 7.0 thousand tons that was 7.7% increase over last year (Pakistan, 2020). *V. mungo* seeds include approximately 26% protein, 60% carbohydrates, and 1.3% fat (Kumar *et al*., 2020). Nitrogen is the most prevalent component in bean plants and *V. mungo* is extremely nutritious, easily digestible, and one of the top five premium food beans in the Pakistan (Jegadeesan *et al*., 2021, Swarnalakshmi *et al*., 2020). In addition, mash bean has a high concentration of vitamins A, B, and C, as well as minerals like as potassium, phosphorus, and calcium, all of which are necessary for the human body (Kumar *et al*., 2020). *Hyptis sauveolens, Ricinus communis , Alternanthera sessilis, Ipomoea carnea, Malachra capitate, Cymbopogon citrutus* and *Trianthema portulacastrum* are the most common weeds present in black gram crop but *T. portulacastrum* weed has noxious allelopathic effect on black gram (Joshi *et al*., 2015). Allelopathy occurs when one or more biochemicals released by one organism harm the germination, growth, survival, and reproduction of other organism *(*Prabhakaran *et al*., 2015). Allelopathy is derived from the Greek terms allelo and pathy, which indicate "mutual damage" or "suffering," respectively (The effects of plants on each other) (Shankar *et al*., 2009).

Black pigweed is a significant and problematic weed found in the black gram. *T. portulacastrum* is a member of the *Aizoaceae* family (Aneja *et al*., 2012). *T. portulacastrum* inhibits the development of several crops, especially black gram (Prabhakaran *et al*., 2015). *Trianthema* species are considered noxious and dangerous weeds (Parvaiz *et al*., 2020). Herbicides have caused environmental and health problems; therefore, the development of bioherbicides is a low-cost, environmentally benign technology that is critical for agriculture's long-term viability (Khan, 2011).

The use of biofertilizers like *Pseudomonas* sp. (PF-097) can help minimize the usage of inorganic fertilizers and pollution in the environment (Suhag, 2016). *Pseudomonas* sp. promotes plant development through mechanisms such as symbiotic nitrogen fixation and phytohormone synthesis (Sivasakthi *et al*., 2014). The plant rhizosphere is a unique ecosystem with intricate plant root-soil microbial interactions (McCully, 2005). *Rhizobacteria* are a kind of soil bacteria that colonize plant roots and help them to grow by reducing disease (Chauhan *et al*., 2021). *Pseudomonas* PGPR are very helpful as their presence in plant roots (Venkatesan *et al*., 2010). This study aims to use *Pseudomonas* sp. (PF- 097) as a bioherbicidal under controlled and green house settings to inhibit the growth of black pigweed in black gram (Mehnaz, 2013).

MATERIALS AND METHODS

Collection of plant material

Fresh *T. portulacastrum* plants were collected from the fields, the bank of irrigation canals and waste land. The plants were washed 2-3 times with running tap water, and then fresh and healthy leaves were separated. The whole plant material and separate leaves were shade dried for about ten days and grounded to a fine powder using metal blender and stored in air tight bottles for the extract preparation (Rathika *et al*., 2020).

Leaf extraction

For preparing leaf extraction 50 g of powder is mixed with 100 mL of distilled water and shacked for 24h to providing uniform extraction. The aqueous extract stored at 4 ℃in dark bottles to reduce the allelochemicals degradation until further use (Akbar *et al*., 2021).

Collection of seed sample

Certified commercial seeds of black gram variety of (ADT-3) were procured from University of Agriculture, Faisalabad Pakistan. The seeds were surface sterilized by sodium hypochlorite; (NaOCl) 1% (v/v), solution for 3 min.

Subsequently seeds were washed with sterilized distill water for several times and stored at room temperature for 12 hours (Lalitha *et al.,* 2020).

Collection of PGPR sample

Pure culture of plant growth promoting rhizobacteria (PGPR) *Pseudomonas* sp. (PF-097) was utilized to study its effects on black gram under black pigweed allelopathic stress. It was collected from Fungal Culture Bank of Pakistan, University of Punjab.

Preparation of nutrient extract medium for bacteria

The nutrient medium was made by mixing 2.5 g yeast extract, 5 g dextrose, and 5 g calcium carbonate ($CaCO_3$) in 250 mL of distilled water (Ugale and Barwant 2010). It was autoclaved for 20 minutes at 121 °C at 15 psi to prevent contamination (Chauhan and Jindal, 2020). Inoculation of *Pseudomonas* sp. strain (PF-097) was done on cultured medium under laminar flow and keep in shaker incubator at 30 °C for 5 days (Blanco-Vargas *et al*., 2020).

Filtration of bacterial strain

Bacterial cultures were filtered by using distilled water to get secondary metabolites. The bacterial strains were subjected to filtration by using filter paper followed by micro filtration to get secondary metabolites (Battu and Reddy, 2009).

In vitro Bioassay

Two Sets of Petri plates, SET 1 (S I) and SET II (S II) were assembled and arranged. Five treatments (0.4, 0.8, 1.2, 1.6 and 2%) were included in S I, each with three replicates. Each sterilized Petri plate was filled with filter papers. Prior to conducting an experiment, the mash bean seeds were first steeped for 2 hours in distilled water. Each Petri dish contained 15 healthy mash seeds.

Leaf extract solutions of *T. portulacastrum* were added in in Set 1, there was no PGPR *Pseudomonas* sp. (PF-097) in Set 1. Control negative treatment was with only distilled water, and all three triplicates received nothing but distilled water and a 0% leaf extract. Five different concentration were made from (0.4, 0.8, 1.2, 1.6 and 2%). Concentration of 0.4% consist of 8.91 mL water and 0.096 mL *T. portulacastrum* leaf solution. Concentration 0.8% was made by adding 0.19 mL leaf extract solution and 8.81 mL water. Concentration 1.2% had 8.72 mL water and 0.28 mL leaf extract. Concentration1.6 was prepared by 0.38 mL leaf extract and 8.62 mL water. Last 2% concentration received 0.48 mL leaf extract and 8.52 mL water.

Experimental Set II was made by *Pseudomonas* sp. (PF- 097) and black pig weed leaf extract in varying concentrations (0.4, 0.8, 1.2, 1.6 and 2%). Control positive received *pseudomonas* sp. PGPR filtrate and distilled water. Concentration 0.4% was made by 0.012 mL leaf extract, PGPR filtrate and distilled water. Concentration 0.8% received 0.024 mL leaf extract, distilled water, and bacterial strain filtrate. Concentration of 1.2% was made by adding 0.036 mL leaf extract, bacterial filtrate and distilled water. Concentration of 1.6% was made by adding 0.48 mL leaf extract, distilled water and bacterial strain filtrate. Concentration 2% was made by adding 0.06 mL leaf extract, bacterial filtrate and distilled water.

***In vivo* bioassay**

The pot experiment was performed in greenhouse to check the effect of *T. portulacastrum* and *Pseudomonas* sp. (PF- 097) on the growth and yield of black gram. The plastic pots with capacity of 500 g sterilized soil were taken. Soil was sterilized by formalin (Abdelhafez *et al*., 2021). Soil was filled in polyethylene bag with formalin and left in sunlight for one week. After pot filling some water was added in each pot to wet the soil and left all pots for 24 hours to set soil texture properly.

Pots were arranged in two sets (SI and SII). Each set consist of treatments 0.5, 1, 1.5, 2, 2.5 and 3% and 5 replicates were taken of each treatment. In Set 1 control negative consist only soil and distilled water. In Treatment 0.5, 1, 1.5, 2, 2.5 and 3% dried biomass of whole plant material (w/w) of black pigweed added and mixed with soil and left all pots for one week for final soil texture (Ara *et al.,* 2021).

In (S II) control positive and concentrations of 0.5, 1, 1.5, 2, 2.5, and 3% were taken amended with black pigweed and *Pseudomonas* sp. (PF- 097).

In control positive, there was only *Pseudomonas* sp. (PF-097) strain. All concentrations of experiment 0.5, 1, 1.5, 2, 2.5 and 3% amended with *T. portulacastrum* powdered plant material (w/w) and 8 mL of *Pseudomonas* sp. (PF-097) strain were added in each pots. Seeds of black gram were sown after one week.

Enzyme analysis

Analysis of protein contents

To determine protein content phosphate buffer was prepared by mixing 0.1M KH2PO4 6.8 g in 500 mL distilled water and 0.1M K2HPO4 8.7 g in 500 mL distilled water. Mash bean stem was crushed by adding phosphate buffer in pestle and mortar. The reagent A was prepared by adding 2% Sodium carbonate in 0.1N Sodium hydroxide. Reagent B was prepared by adding 0.5% Copper sulphate in 1% of KNaC4H4O6.4H2O. Reagent C was made by adding one mL of reagent A and one mL of reagent B. reagent D was prepared by adding water and folin phenol in equal measurement. About 0.1 mL of centrifuged material was added in one mL of reagent C, followed by shaking it for few minutes and 0.1 mL of reagent D was added in it. Mixture was incubate at 20 to 25°C for 10 minutes. Protein contents were observed of each concentration treatment at the intensity of 6nm (Lowry, 1951).

Analysis of peroxidase (PO) activity

For peroxidase enzyme analysis plant sample (mash bean) from each concentration crushed in 5 mL phosphate buffer. Mixture was centrifuged at 3000 rpm for 10 minutes. 0.5 mL centrifuged solution and one mL of 0.05M pyrogallol and 1 mL of phosphate buffer was mixed in test tube and the sample remains incubated for few minutes at 20 °C. Absorbance was observed at the intensity of 500 nm (Chance and Machly, 1967).

Analysis of catalase activity

Catalase activity was determined by titration method. Plant sample of mash bean seedling was crushed adding phosphate buffer. Add 1 mL of 0.01 M hydrogen peroxide and incubate for 10 minutes at 25 °C. One percent of sulphuric acid was added in incubated solution. The samples were titrated against 0.05N Potassium permanganate and initial and final readings were recorded until pink color of sample solution disappears to calculate difference (Beers and Sizer, 1952).

RESULTS AND DISCUSSION

The results of experiment revealed that all negative concentrations (0.4, 0.8, 1.2, 1.6 and 2%) significantly suppress the germination percentage (34% - 6%), seedling length (10 cm to 3 cm), fresh biomass of seedling (2.4 g - 0.94 g), dry biomass of black gram seedling (1.2 g - 0.47 g) by increasing the allelopathic activity. The lowest germination, seedling length, fresh and dry biomass of seedling was observed at 1.6% and 2% negative leaf extract as shown in Fig. 1. *T. portulacastrum* leaf extracts had a substantial impact on black gram germination. Farooq *et al*. (2013) reported that allelochemicals from *T. portulacastrum* negatively influence the germination of black gram at low concentrations and suppress their germination at high concentrations. Moosavi *et al.* (2011) reported that as the concentration level increased the black gram germination were severely hindered (100%). *T. portulacastrum* leaf extract allelochemicals (primarily phenolic compounds) hindered cell division in plants and impacted cell elongation and seedling length (Gill, Randhawa *et al*. 1999). Giraddi *et al*. (2003) founded that seedling length of black gram was greatly reduced at higher concentrations of *T. portulacastrum* leaf extract. Allelopathic activity of *T. portulacastrum* leaf extract highly reduced the fresh and dry biomass seedling of black gram as allelopathy activity raised which is due to the presence of secondary metabolites such as alkaloids, flavonoids, tannins, and phenolic compounds, which are released into the environment via volatilization, leaching, and decomposition of other allelopathic plants (Ahmed and Slima, 2020). Aqueous solution of *A. viridis* parts inhibit the growth of poaceous crops (Muhammad *et al*., 2011). Giraddi *et al*., (2003)reported that the seedling dry weight of black grams was greatly reduced owing to the allelopathic impact of *T. portulacastrum* at higher concentrations.

The result of this experiment showed that all the positive

concentrations (0.4, 0.8, 1.2, 1.6 and 2%) significantly increase the germination percentage (8% to 42%), seedling length (12 cm – 54 cm), fresh biomass of black gram seedling (8% - 17%) and dry biomass of seedling (1.2 g - 0.47 g) amended with *Pseudomonas* sp. (PF-097) plant growth promoting rhizobacteria. The highest germination, seedling length, fresh and dry biomass of seedling was observed in control treatments as shown in Fig. 3 and 4. Bacterial plant growth promoting mechanism helps to get sustainable agriculture production under biotic and abiotic stresses (Islam *et al*., 2014). Saini and Westgate (1999) reported that amelioration of allelopathic stress by using. Zahir *et al*. (2009) reported that seeds of wheat treated with *Bacillus* sp. found significantly effective in plant growth promotion in saline soil and the use of salt tolerant PGPRs are effective for facilitating plant health in salt stress environments. Similar results were observed by Bakka and Challabathula. (2020) that *Pseudomonas* sp. (PF-097*) strain* showed promising performance under axenic conditions of salinity. *Pseudomonas* sp. significantly increased the plant height, root length and grain yield. PGPR helps in alleviation of salt stress and plant growth promotion by production of phytohormones and the enzyme ACC deaminase, (Gonzalez *et al*., 2015, Egamberdieva and Lugtenberg, 2014). Aamir *et al*. (2013) found that inoculation and co-inoculated plants with PGPR had higher relative water content and dry matter production, which may be attributed to stronger and longer roots, which resulted in greater water absorption from deeper soil. Plant dry matter improved substantially when PGPR strain *Pseudomonas* and *Rhizobium* were co-inoculated and it is also observed that sole and dual inoculation of PGPR significantly enhanced nodule quantity, nodule fresh and dry weight as compared to non- inoculated plants (Roopa *et al*., 2012, Sharma *et al*., 2019).

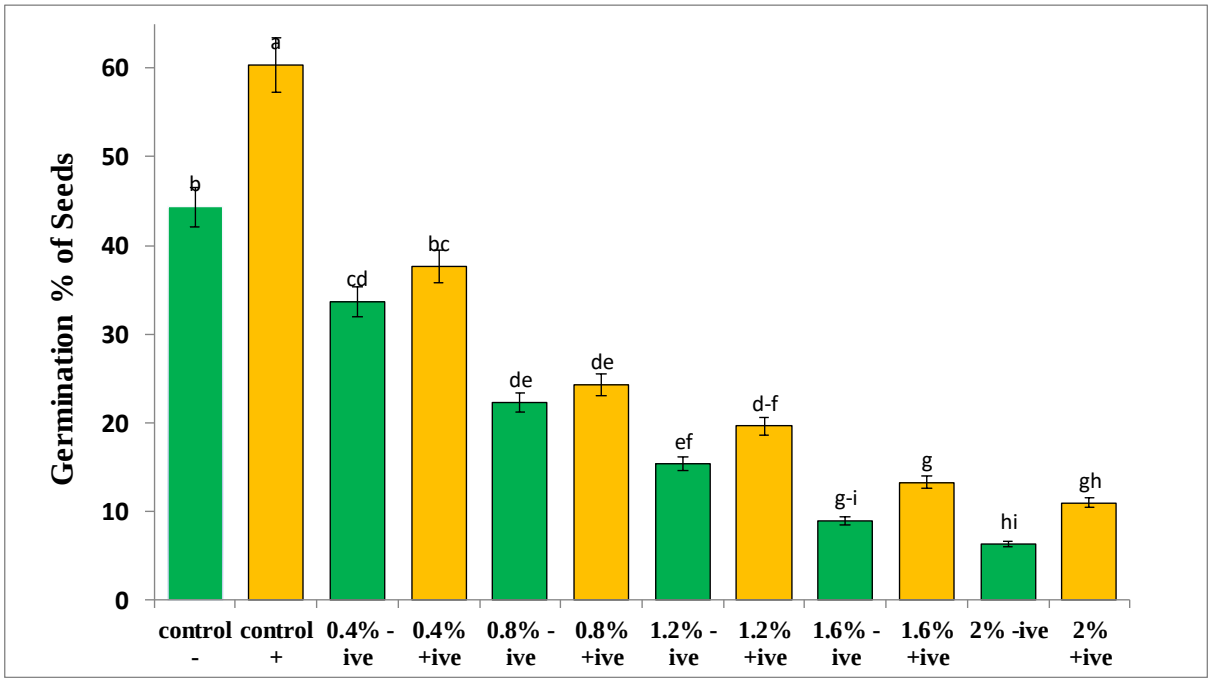


Fig. 1: Effect of *Pseudomonas* sp. (PF-097), and aqueous leave extract of *Trianthema portulacastrum* on germination percentage (%) of *Vigna mungo*. Green bars indicates the negative treatments and yellow bars shows positive treatments. Vertical bars show standard errors of means of three replicates of two separate experiments. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD Test.

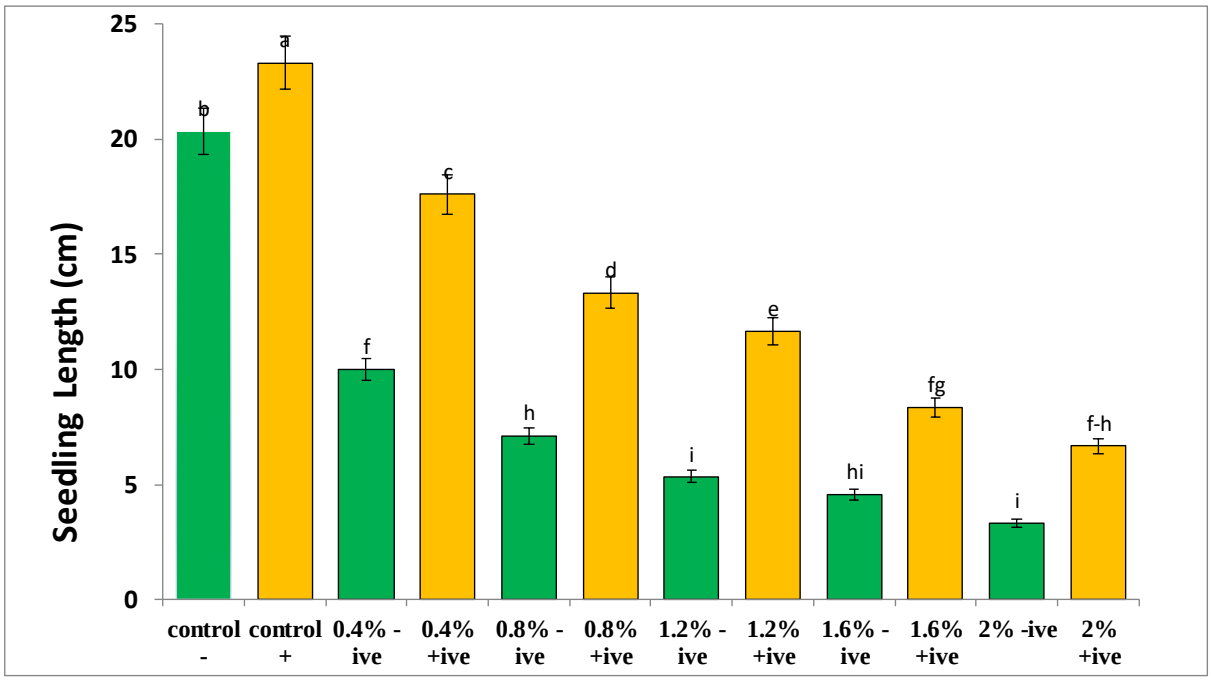


Fig. 2: Effect of *Pseudomonas* sp. (PF-097), and aqueous leave extract of *Trianthema portulacastrum* on length of seedlings of *Vigna mungo*. Green bars indicates the negative treatments and yellow bars shows positive treatments. Vertical bars show standard errors

of means of three replicates of two separate experiments. Values with different letters at their top show significant difference (P ≤ 0.05) as determined by LSD Test.

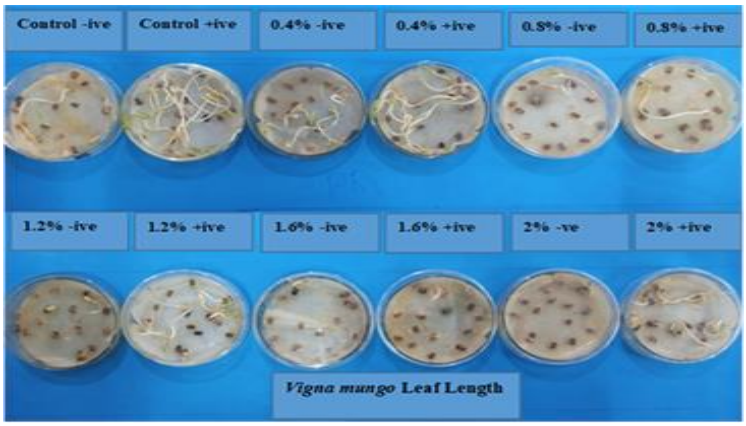
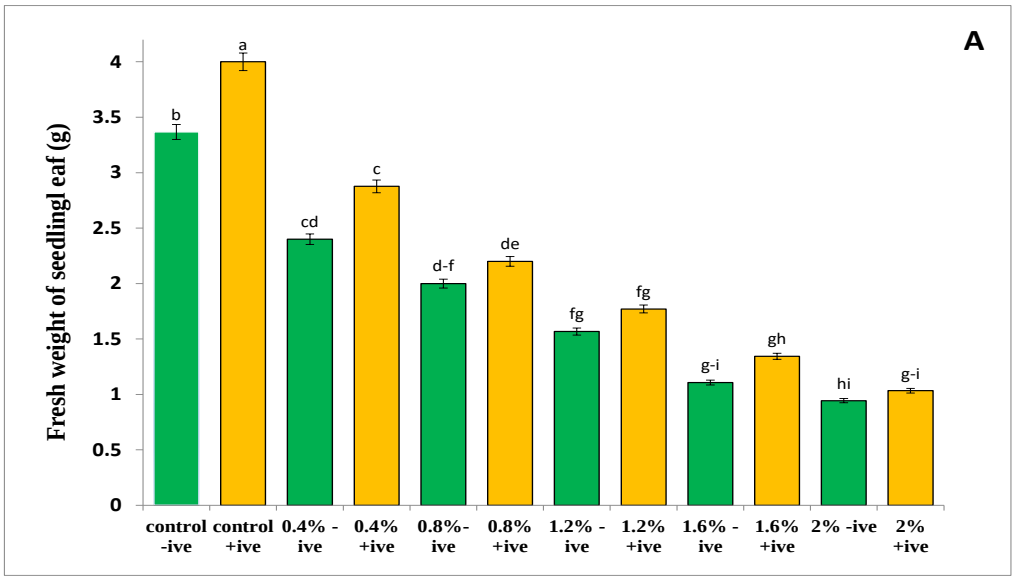


Fig. 3: Effect of *Pseudomonas* sp. (PF-097) and aqueous leave extract of *Trianthema portulacastrum* on length of seedlings of *Vigna mungo*



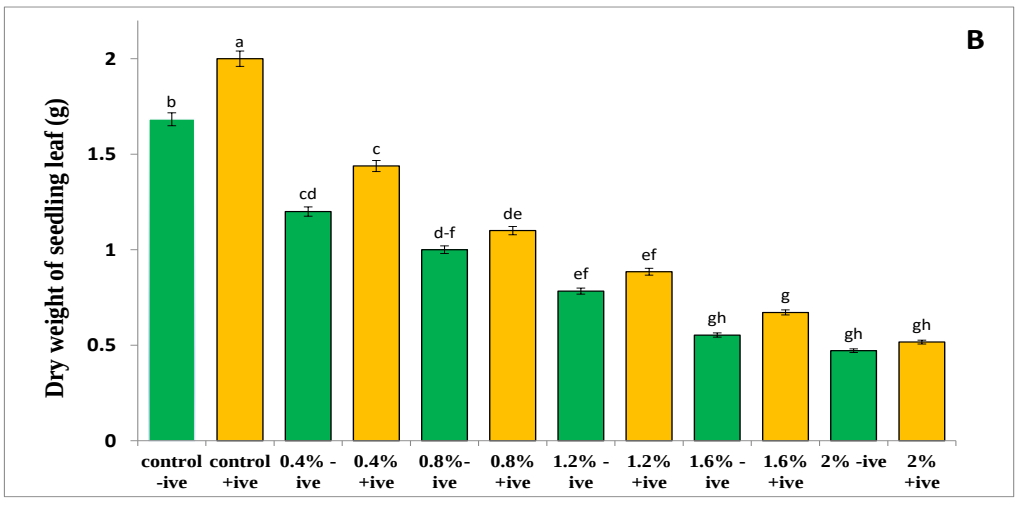


Fig. 4: Effect of *Pseudomonas* sp. (PF-097) and aqueous leave extract of *Trianthema portulacastrum* on fresh and dry biomass of *Vigna mungo*. Green bars indicates the positive treatments and yellow bars shows negative treatments. Vertical bars show standard errors of means of three replicates of two separate experiments. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD Test.

In vivo* shoot length, fresh and dry biomass of *Vigna mungo* under the allelopathic stress of *Trianthema portulacastrum* and *Pseudomonas* sp. *(PF-097)

The result of study revealed that all negative concentrations from (0.5, 1, 1.5, 2, 2.5 and 3%) significantly suppress the shoot length (46 cm to 18 cm), shoot fresh biomass (2.6 g - 0.66 g) and shoot dry biomass (1.31 g - 0.33 g) of *V. mungo* by the allelopathic stress of *T. portulaacstrum*. The lowest, seedling length, fresh and dry biomass of seedling was observed at 2.5% and 3% negative whole plant extract as shown in Fig. 5 and 6. Allelopathic stress of black pigweed was also observed when the quantities of black pigweed were greater it significantly decreased the shoot length and shoot fresh and dry biomass of black gram (Khan and Ahmad (2011). The entire plant extract of *T. portulacastrum* exhibited a considerably greater allelopathic impact than other sections of the black pigweed (Kadioglue *et al*). It was observed that phenolic chemicals negatively influence cell membrane permeability by making it non-specifically permeable, causing ion fluctuations and hydraulic conductivity in roots to be disrupted. *T. portulacastrum* whole-plant showed more phenolics and inhibited the germination and seedling length. Seed protease activity was reduced when water absorption was disrupted, which was important for protein degradation during germination and, to a great degree, was linked to seed imbibition and water uptake (Ahmed, 2020).

The result of study revealed that all positive concentrations from (0.5, 1, 1.5, 2, 2.5 and 3%) significantly increased the shoot length (4% to 12%), shoot fresh biomass (18% to 35%), shoot dry biomass (17% - 35%) amended with this *Pseudomonas* sp. (PF-097) PGPR. The highest, seedling length, fresh and dry biomass of seedling was observed control treatments. All treatments were significantly different from each other. Ross *et al*. (2000) founded that *Pseudomonas* sp. as PGPR improves soil physical conditions, increasing the soil's water holding capacity and reducing stress intensity, leading in improved growth and development of black gram. In a pot experiment co-inoculation of *Bacillus* and *Pseudomonas* sp. enhanced nutrient content in grain and various regions of the plant (Qureshi *et al*. 2011). Munns *et al.* (2018) reported that single and combined inoculation of PGPR reduced Na content in grain and enhanced K/Na ratio under allelopathic stress. Jaleel *et al*. (2007) used *Pseudomonas fluorescens* as biofertilizer to enhance the plant growth and yield in drought-stricken stress conditions. *Pseudomonas* sp. increase number of tillers, shoot length, spike length, and seed weight during drought stress. Activity of PGPR stimulated the plant growth by regulating hormonal and nutritional balance, solubilize nutrients and induce resistance mechanism (Ali *et al*., 2009, Nadeem *et al*., 2014) . PGPR *Bacillus pumilus* and *Bacillus licheniformis* have a strong growth-promoting ability *(Gutiérrez-Mañero et al*., 2001, Ali *et al*., 2010) . They produce high amount of physiologically active gibberellins and enhance stem elongation. PGPR also enhance the endogenous indole-3-acetic acid content and development of *Vigna radiata* bacterial strains of *Micrococcus, Bacillus, Escherichia, Pseudomonas* and *Staphylococcus* improve indole-3-acetic acid (IAA) content and growth of wheat (Velazhahan *et al*., 2020). PGPR increased the number of tillers, shoot length, spike length, and seed weight. Drought tolerant rhizobacteria *Pseudomonas* and *Bacillus* also reduce the water stress of wheat (Raheem *et al*., 2018, Ali *et al*., 2009).

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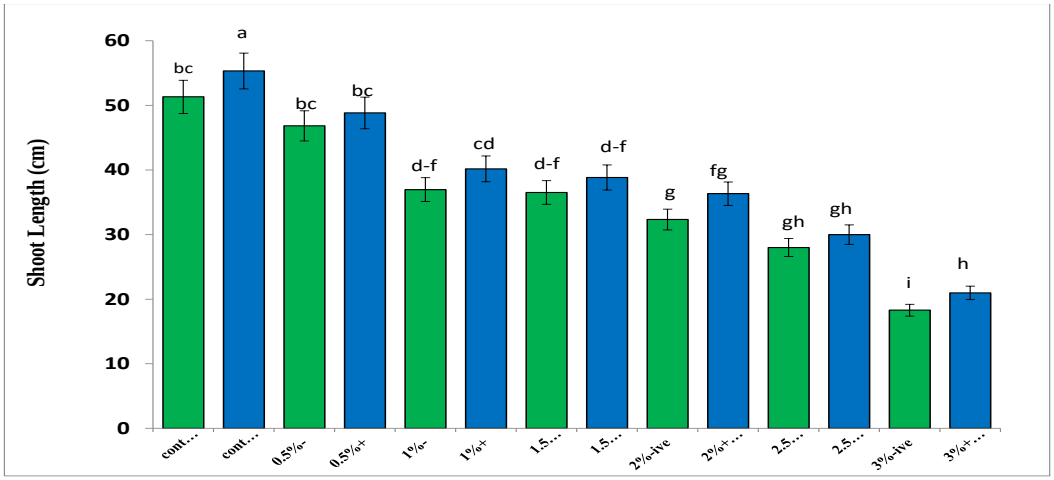

Fig. 5: Effect of *Pseudomonas* sp. (PF-097) and *Trianthema portulacastrum* dried plant powder on length of shoot of *Vigna mungo*. Green bars indicates negative treatments and blue bars shows positive treatments. Vertical bars show standard errors of means of three replicates of two separate experiments. Values with different letters at their top show significant difference (P ≤ 0.05) as determined by LSD Test.

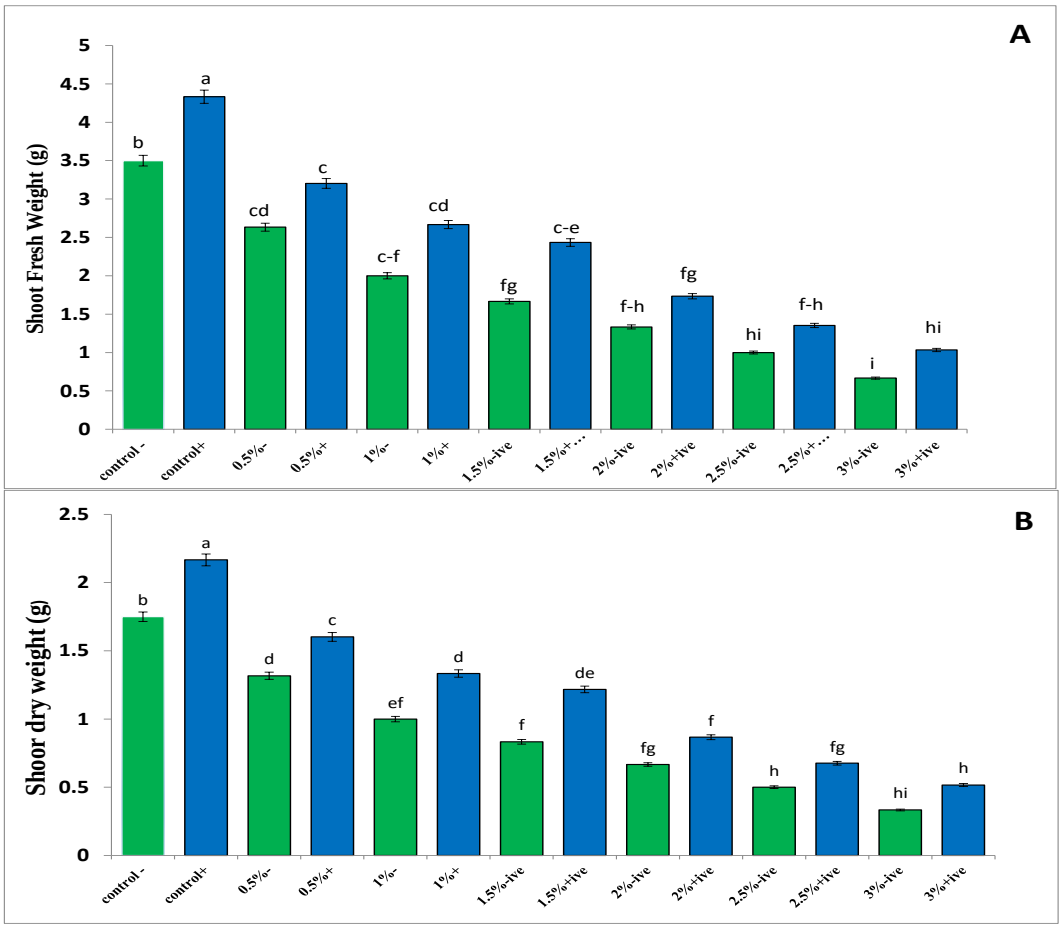

Fig. 6: Effect of *Pseudomonas* sp. (PF-097) and *Trianthema portulacastrum* dried plant powder on fresh and dry biomass of shoot of *Vigna mungo*. Green bars indicates the negative treatments and blue bars shows positive treatments. Vertical bars show standard errors of means of three replicates of two separate experiments. Values with different letters at their top show significant difference (P ≤ 0.05) as determined by LSD Test.

Estimation of physiological parameters in *Vigna mungo* plant

Peroxidase activity of black gram considerably increased in control negative treatments and decreased in control positive treatments as represent in Fig. 7 by the amendment of *Pseudomonas* sp. (PF- 097). Peroxidase activity in control positive treatment was reduced to -24% than control negative treatment. Peroxidase activity decreased (-54 - -21%) in control positive concentrations. Peroxidase activity increased (-35 - -319%) in control negative concentrations. There was significant difference in peroxidase activity of all negative and positive treatments. PGPR produced several active enzymes under drought, heavy metal and salt stress. During salinity stress enhanced peroxidation was observed, but PGPR inoculation decreased the peroxidase and catalase activity and protect the cell from destruction (Jha and Subramanian, 2014). *Pseudomonas* sp. PGPR also reduced the caspase like endopeptidase enzyme activity and decreased the peroxidative activity and also regulate the antioxidant enzymatic activity (Jha and Subramanian, 2014). El-Shora *et al*. (2014) found that allelopathic stress of *T. portulacastrum* increased the activity of stress-related enzymes (peroxidase and catalase) under negative treatments. Weeds enhanced the activity of enzymes such as peroxidase, superoxide dismutase, and catalase, as well as decrease chlorophyll content, resulting in allelopathic stress (Motmainna *et al*., 2021, Patel and Saraf, 2013). PGPR produced several active enzymes under drought, heavy metal and salt stress. (Kang *et al*., 2014, Kohler *et al*., 2009) reported that PGPR mitigate oxidative stress by reducing the activities of peroxidase, catalase and polyphenol oxidase. PGPR alleviate the peroxidase under severe drought condition they can used as inoculant to alleviate the oxidant damage by elicited by drought stress (Sen and Chandrasekhar, 2014).

Catalase activity was considerable increased in negative treatments amended with only *T. portulacastrum* and decreased in positive treatments amended with biological agent *Pseudomoans* sp. (PF-097). By using biological agent *Pseudomonas* sp (PF-097) catalase activity decrease (-53 -- 3%) in positive concentrations. Catalase activity increased (-6 - -622%) in negative concentrations as shown in Fig. 8. Farhoudi and Lee (2013) found that by the use of black pigweed extract on *Avena ludoviciana* and *Hordeum spontoneum* the activity of antioxidant enzymes increase and sugar content and photosynthesis rate decrease. Raheem *et al*. (2018) reported that drought tolerant rhizobacteria *Pseudomonas* and *Bacillus* sp. reduce the allelopathic drought stress in black gram. It was found that allelochemicals increased catalase and peroxidase and decrease total protein content of plant (El-Shora *et al*., 2014). PGPR alleviate the catalase under severe drought condition they can used as inoculant to alleviate the oxidant damage by drought stress (Sen and Chandrasekhar, 2014). Activity of antioxidant enzymes GR, CAT, APX in wheat leaves significantly reduced when treated with PGPR strains (Upadhyay *et al*., 2012). According to the study of Maurya (2020) the reactive oxygen species (ROS) such as superoxide radical (O^{2-}), hydrogen peroxide (H_2O_2), hydroxyl radical (OH), singlet oxygen ($^1O_2$), peroxy radical (ROO), and alkoxyl radicals (RO) are produced at low temperature within a threshold concentration in the plant cell under ambient environmental conditions. However, the extreme environmental conditions trigger excessive production of ROS. Moreover, ROS damage molecular and cellular components due to the oxidation of biomolecules (lipid, carbohydrates, proteins, enzymes, DNA) and cause plant death (Bhuyan *et al.,* 2020). To avert the damages, plants tightly regulate ROS production via the recruitment of enzymatic and non-enzymatic antioxidants. The enzymatic antioxidant system comprising superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), peroxidase (POX), etc. and non-enzymatic antioxidants such as vitamins, flavonoids, stilbenes, and carotenoids quench the excess ROS, thereby providing a shield against oxidative stress (Hasanuzzaman

et al., 2020; Wongshaya *et al.*, 2020). Unfettered propagation of oxygen (O_2) derived reactive species is detrimental to the plant health. However, a controlled ROS production participates in redox signaling, plant growth and development during stress (Florez-Sarasa *et al*., 2020). That's why in the present findings, the defensive mechanism activated in the tested plant when treated with *T. portulacastrum* that ultimately increased the catalase activity.

By the amendment of *Pseudomonas* sp. (PF- 097) protein content of black gram seedling was significantly increased in positive treatments as compared to negative treatments. Control positive treatment characterized with higher protein content (89%). Protein activity increased (14 - -57%) in positive concentrations and decreased (-6 - -622) in negative concentrations as shown in Fig. 9. A substantial increase in protein content was found in positive treatments modified with *pseudomonas* sp. (PF-097). Khan *et al*. (2018) found that inoculation with *pseudomonas, Bacillus subtilis, B.s thuringiensis,* and *B. megaterium*, as well as plant growth regulators (Salicylic acid and Putrescine), increased chlorophyll, protein, and sugar content in chickpeas. Mesa-Marín *et al*. (2018) reported that PGPRs reduce the activity of antioxidant enzymes in plant roots (Mesa-Marín et al., 2018). Gutiérrez-Mañero *et al*. (2001) reported that PGPR *B. pumilus* and *B. licheniformis* have a strong growth-promoting ability. They produce high amount of physiologically active gibberellins and enhance stem elongation.

It could be speculated that when black gram plant was under the stress of *T. portulacastrum* only, overall changes in plant growth, physiology and antioxidant enzymes could be due to destabilization of membrane function that may consequences in loss of plant intrinsic balance. As there is threshold of enzyme activity, and it seems that increase in activity of stress related enzyme was not sufficient to protect plant from oxidative stress that's why the growth of black gram decreased in the stress and due to the incorporation of mended with biological agent *Pseudomoans* sp. (PF-097) reduced the stress in the vicinity of plant roots and triggered the better growth in tested plant (Akhtar *et al*., 2016).

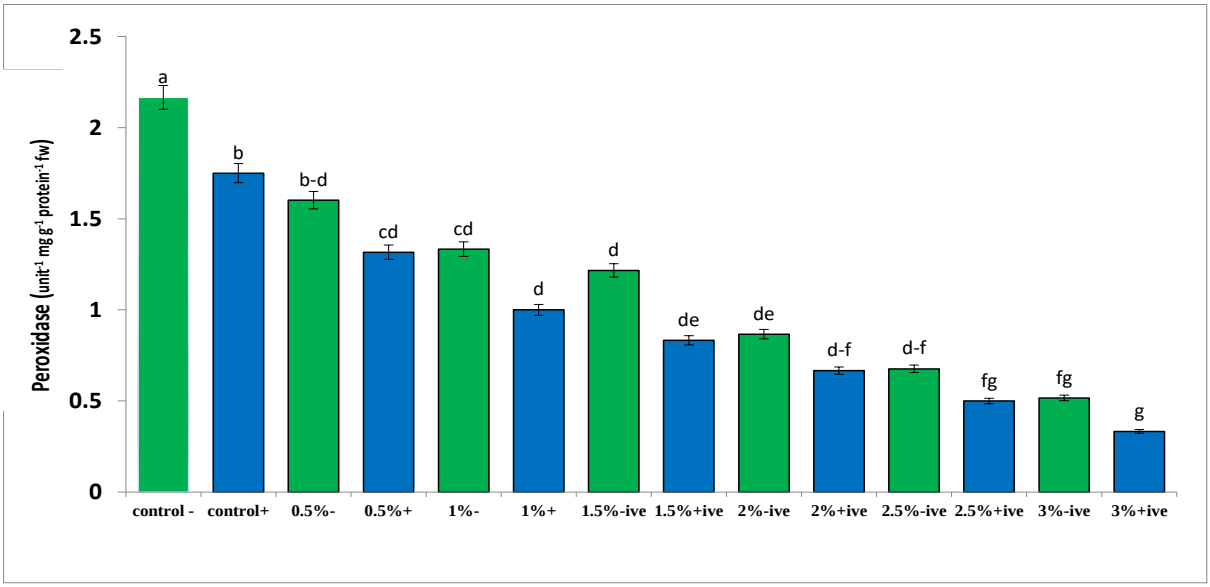

Fig. 7: Effect of *Pseudomonas* sp. (PF-097) and *Trianthema portulacastrum* dried plant powder on peroxidatase activity of *Vigna mungo*. Green bars indicates the positive treatments and yellow bars shows negative treatments Vertical bars show standard errors of means of three replicates of two separate experiments. Values with different letters at their top show significant difference (P ≤ 0.05) as determined by LSD Test.

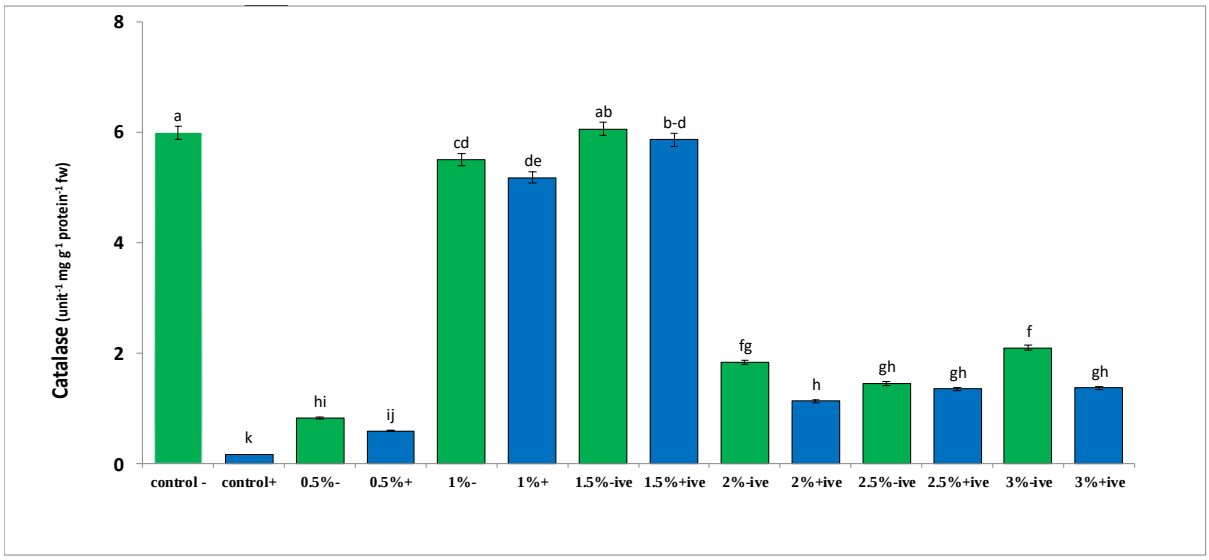

Fig. 8: Effect of *Pseudomonas* sp. (PF-097) and *Trianthema portulacastrum* dried plant powder on catalase activity of *Vigna mungo*. Green bars indicates the positive treatments and blue bars shows positive treatments Vertical bars show standard errors of means of three replicates of two separate experiments. Values with different letters at their top show significant difference (P ≤ 0.05) as determined by LSD Test.

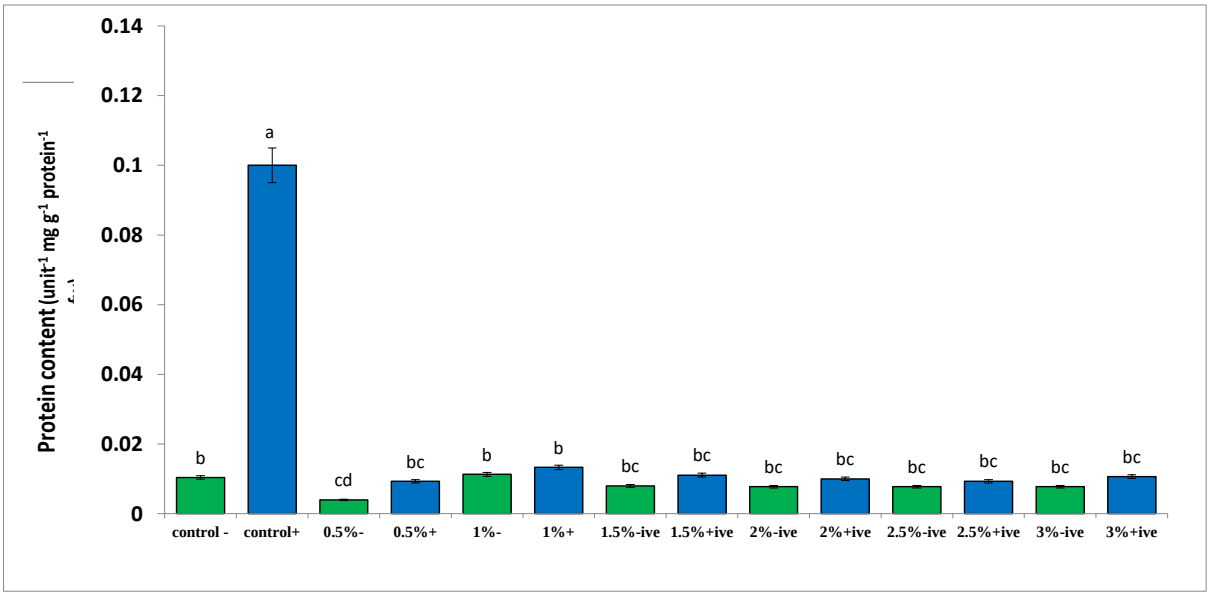

Fig. 9: Effect of *Pseudomonas* sp. (PF-097), and *Trianthema portulacastrum* dried plant powder on protein content of *Vigna mungo*. Green bars indicates the negative treatments and blue bars shows positive treatments. Vertical bars show standard errors of means of three replicates of two separate experiments. Values with different letters at their top show significant difference (P ≤ 0.05) as determined by LSD Test.

Conclusion

The leaf extract of *T. portulacastrum* decreased the *V. mungo* leaf germination, seedling length, fresh biomass of seedling, dry biomass of black gram seedling. Amendment with the *Pseudomonas* sp. (PF-097) PGPR amendment increased the said parameters in *V. mungo*. The high concentration of phenolic acids in *T. portulacastrum* weed indicates that it must be controlled early in its life cycle to minimize the crop damage. Thus, the amendment *Pseudomonas* sp. (PF-097) in the soil could suppress the allopathic affect caused by *T. portulacastrum* and could enhance the growth of black gram. So far, further field trials are required so that the application by the farmers in fields could be make affective.

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