

Article

# Alleviation of Cadmium Adverse Effects by Improving Nutrients Uptake in Bitter Gourd through Cadmium Tolerant Rhizobacteria

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**Abstract:** Cadmium is acute toxicity inducing heavy metal that significantly decreases the yield of crops. Due to high water solubility, it reaches the plant tissue and disturbs the uptake of macronutrients. Low uptake of nutrients in the presence of cadmium is a well-documented fact due to its antagonistic relationship with those nutrients, i.e., potassium. Furthermore, cadmium stressed plant produced a higher amount of endogenous stress ethylene, which induced negative effects on yield. However, inoculation of 1-amino cyclopropane-1-carboxylate deaminase (ACCD), producing plant growth promoting rhizobacteria (PGPR), can catabolize this stress ethylene and immobilized heavy metals to mitigate cadmium adverse effects. We conducted a study to examine the influence of ACCD PGPR on nutrients uptake and yield of bitter gourd under cadmium toxicity. Cadmium tolerant PGPRs, i.e., *Stenotrophomonas maltophilia* and *Agrobacterium fabrum* were inoculated solely and in combination with recommended nitrogen, phosphorus, and potassium fertilizers (RNPKE) applied under different concentration of soil cadmium (2 and 5 mg kg<sup>-1</sup> soil). Results showed that *A. fabrum* with RNPKE showed significant positive response towards an increase in the number of bitter gourds per plant (34% and 68%), fruit length (19% and 29%), bitter gourd yield (26.5% and 21.1%), N (48% and 56%), and K (72% and 55%) concentration from the control at different concentrations of soil cadmium (2 and 5 mg kg<sup>-1</sup> soil), respectively. In conclusion, we suggest that *A. fabrum* with RNPKE can more efficaciously enhance N, K, and yield of bitter gourd under cadmium toxicity.

**Keywords:** ACC deaminase; heavy metal stress; PGPR; fertilizers; nutrients; yield

## 1. Introduction

High use of pesticides, inorganic fertilizers, and untreated sewage water has significantly contributed to the buildup of heavy metals in agricultural soils [1,2]. These heavy metals become part of soil at the exchange site and remain readily available for plants. Rapid industrialization and anthropogenic activities are also allied factors responsible for the accumulation of toxic metals beyond their threshold limit in cultivatable lands [3–6]. Among different heavy metals, cadmium (Cd) is an acute toxin due to its high resistance time, i.e., >1000 years and water solubility [7]. Presence of cadmium below  $0.5 \text{ mg kg}^{-1}$  soil is considered a safe limit, but depending upon parent material, it can be accumulated up to  $3.0 \text{ mg kg}^{-1}$  soil [8]. Being a part of phosphate fertilizers (up to  $4.4 \text{ mg kg}^{-1}$ ), it is easily taken up by crops as Cd-supplement [9,10].

Cadmium causes cardiovascular, respiratory, cancer, and renal, skeletal system in humans when taken up beyond the threshold limit [11,12]. The high concentration of Cd in plant tissues disturbs nutrient uptake and creates water imbalance that results in poor photosynthesis [13]. It also causes lipid membrane instability, alteration in membranes permeability, and chlorosis in plants [14,15]. Due to its divalent nature, it competes with divalent essential nutrients, i.e., P, Ca, Mg, and decreases their uptake in plants [16–18]. Bioavailability of K is also affected when Cd is present in higher concentration in soil [19]. Heavy metal toxicity and physiochemical properties soil depend on the land use [20–22]. Different crops, the biological adsorption factor ( $\text{mg Cd/kg plant ash}$ ,  $\text{mg Cd/kg soil}$ ) based on Cd content in plant ash, is different, i.e., winter wheat grains (5.97) and straw (2.50), barley grains (4.06) and straw (2.50), sugar beet roots (4.63) and tops (1.41), pea beans (3.22) and straw (0.88), corn grains (8.75) and straw (2.53), soya beans (4.31) and straw (4.63), sunflower seeds (10.8) and stem (4.28) [23]. Moreover, biosynthesis of endogenous stress ethylene under Cd toxicity plays a notorious role that aids in poor root growth [24–26].

In addition, ethylene ( $\text{C}_2\text{H}_4$ ) is a plant-signaling molecule. It is involved in seed germination flower senescence, root elongation, fruit ripening, and leaf abscission. Mostly ethylene is synthesized in a two-step process, i.e., (1) enzymatic conversion of S-adenosyl methionine (SAM) to 1-amino cyclopropane-1-carboxylic acid (ACC); (2) conversion of ACC to ethylene, which is catalyzed by ACC-oxidase [27]. However, synthesis of endogenous ethylene level is significantly enhanced upon exposure of plants to abiotic stresses, including low soil fertility [28,29]. This endogenous stress ethylene negatively affects gas exchange attributes, nutrients and water uptake, and yield of different crops under any stress conditions [30,31].

To overcome these problems, inoculation of ACC deaminase producing plant growth promoting rhizobacteria (PGPR), could be an efficacious and nature friendly technique [32–36]. Certain PGPRs can improve growth attributes of crops under heavy metals toxic conditions by secretions of ACC deaminase, siderophores, indole acetic acid, gibberellins, and better availability of water and nutrients [37–40]. Enzyme ACC deaminase cleaves stress ethylene into intermediate compounds; thus, decreases the stress generating factors in plants [41,42].

Among different crop plants, bitter melon is a rich source of vitamins, carbohydrates, and proteins [43,44]. As compared to cucumber and tomato, one cup of bitter melon juice (94 g) has 93% reference daily intake (RDI) of vitamin C [45]. It is cultivated in Pakistan (6107 hectares), with an annual production of 57,190 tons [46]. However, the yield of bitter melon is negatively affected when cultivated in Cd pollution. As improvement in N, P, and K can mitigate the stress of Cd toxicity in plants [3], which is why the current study was conducted to explore the efficacy of ACC deaminase producing PGPR with recommended NPK fertilizers (RNPKE) on bitter melon nutrients uptake and yield under Cd toxicity.

The present study aimed to explore (1) effectiveness of rhizobacteria in the improvement of nutrients uptake; (2) effect of nutrients on bitter gourd yield under cadmium-induced stress; (3) correlation of inorganic fertilizer with rhizobacteria on yield and nutrients attributes of bitter gourd under Cd stress. We hypothesized that ACC deaminase-producing PGPRs could improve nutrient uptake and alleviate adverse effects of Cd in bitter gourd for yield improvement.

## 2. Materials and Methods

### 2.1. Experimental Site and Treatments

A pot experiment was conducted in the Department of Soil Science research area, Bahauddin Zakariya University, Multan, Pakistan. The soil was characterized as dark brown and saline with JAKHAR soil series [42]. Six treatments were applied in four replication by following two factorial completely randomized designs (CRDs). The treatments were control (without NPK or bacterial strains), recommended NPK fertilizers (RNPKF), *Stenotrophomonas maltophilia*, *Agrobacterium fabrum*, RNPKF + *S. maltophilia*, and RNPKF + *A. fabrum*. All treatments were added in the soil at 2 and 5 mg Cd kg<sup>-1</sup> soil. Artificial toxicity of Cd was developed by using analytical grade salt of CdCl<sub>2</sub> [25]. As per treatment plan, two levels of Cd were maintained, i.e., 2 and 5 ppm (mg kg<sup>-1</sup> soil), keeping in mind the Cd concentration of pre-experimental soil. Rhizobacteria were inoculated at the time of sowing. However, required fertilizers were applied at the time of pot preparation.

### 2.2. Collection of Bacterial Strains and Broth

ACC deaminase producing PGPRs *S. maltophilia* (ACC deaminase activity = 71.78 μmol α-ketobutyrate mg<sup>-1</sup> protein h<sup>-1</sup>) and *A. fabrum* (ACC deaminase activity = 432.6 μmol α-ketobutyrate mg<sup>-1</sup> protein h<sup>-1</sup>) were taken from the Laboratory of Soil Microbiology, Department of Soil Science. Both PGPRs were documented Cd tolerant previously, i.e., survive over 5.0 mg Cd kg<sup>-1</sup> soil toxicity [25]. For seeds inoculation, Dworkin and Foster (DF) media without agar was used for inoculum preparation [47]. Loop of each rhizobacteria was taken in the sterilized flask and incubated at 25 ± 3 °C and 100 rpm for 48 h. After that, broth optical density (OD) was measured by spectrophotometer (540 nm wavelength). Finally, dilution was made with sterilized distilled water to achieve 0.45 nm OD, to achieve a uniform population of 10<sup>7</sup>–10<sup>8</sup> colony forming units (CFU) mL<sup>-1</sup>.

### 2.3. Seeds Sterilization and Sowing

HgCl<sub>2</sub> (0.1%) solution was used for sterilization of seeds. All seeds were placed for 5 min in the solution followed by, three times, washing with sterilized deionized water [48]. Moreover, 1mL respective PGPR inoculum was used for seeds inoculation along with sugar (30% sucrose), peat, and clay (1:1) in 1:2:6 ratio for 100 g seeds. Four inoculated seeds were sown in each pot. Sowing of bitter gourd seeds was done by hand. After 20 days of sowing, only three healthy seedlings were maintained in each pot by thinning.

### 2.4. Irrigation and Fertilizer Application

In pots, 65% field capacity was maintained on a weight basis during the experiment. To fulfil the requirement of crop nutrients (187N, 75P, and 225K kg ha<sup>-1</sup>) urea, K<sub>2</sub>HPO<sub>4</sub> and K<sub>2</sub>SO<sub>4</sub> were applied.

### 2.5. Harvesting and Samples Analyses

Harvesting was done at the time of fruit maturity. Samples were digested for the determination of biochemical attributes. The number of bitter gourds was counted manually. For fruit length, standard measuring tape was used. For determination of yield per plant, fruits were collected and weighed on the analytical balance. With the help of diacid mixture nitric acid and perchloric acid (2:1 ratio), the tissues of the plant were digested for P and K analyses [49]. Phosphorus in the samples was determined by using ammonium molybdate and ammonium metavanadate yellow color method [50].

However, for analyses of K in samples, the digested solution was run on flame photometer [51]. For determination of nitrogen, samples were digested in concentrated  $H_2SO_4$ , and digestion mixture ( $K_2SO_4$  (100): $CuSO_4 \cdot 5H_2O$  (10): $FeSO_4$  (1)). Distillation was performed in Kjeldahl distillation apparatus, using boric acid as a collector [52].

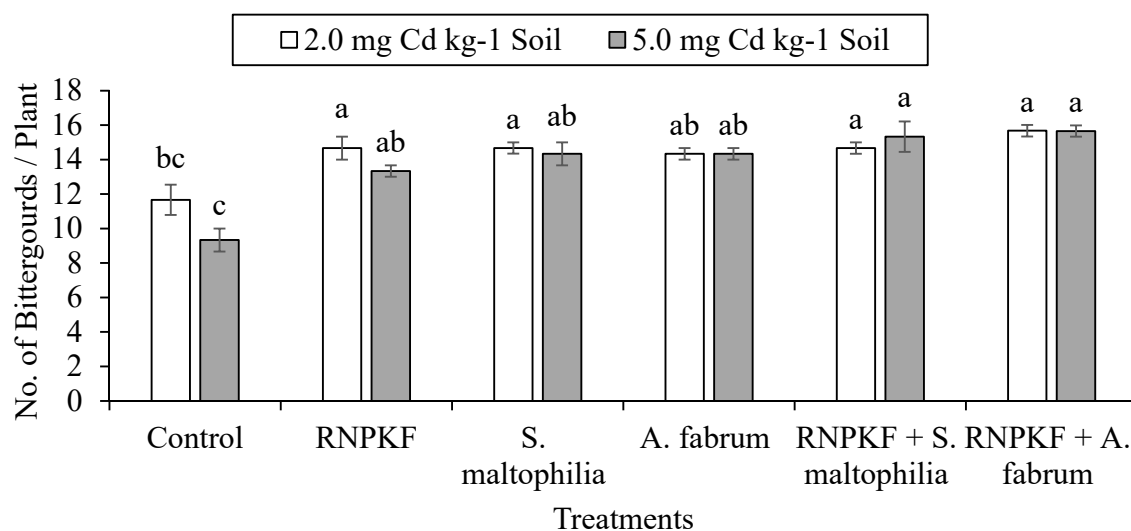
## 2.6. Statistical Analyses

One-way ANOVA was used to assess the effects of treatments. Two factorial ANOVA was conducted separately to compare PGPRs and RNPKE interaction under different levels of Cd. Treatment comparison was computed at  $p \leq 0.05$  by Tukey's Test.

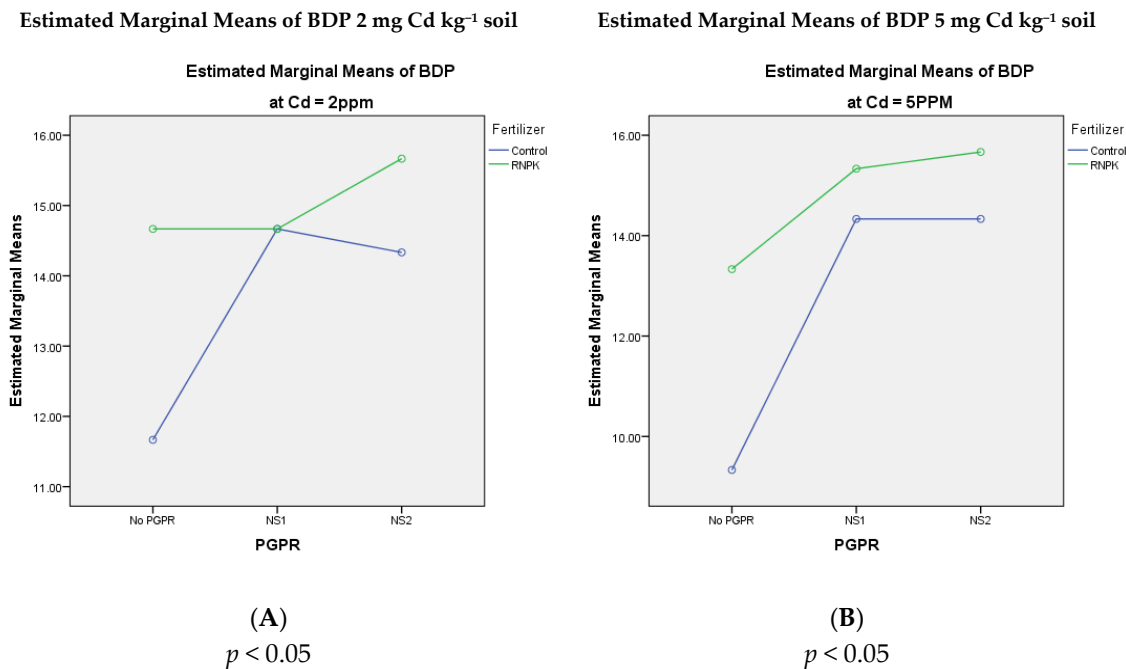
## 3. Results

### 3.1. Number of Bitter Gourds per Plant

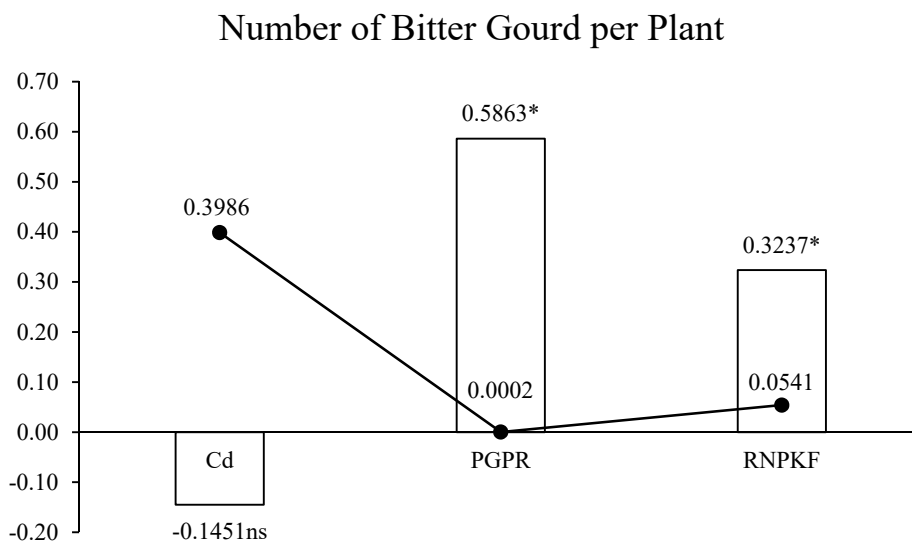
Effects of PGPRs and RNPKE under different levels of Cd were significant ( $p \leq 0.05$ ) on the number of bitter gourds per plants (BDP). Inoculation of PGPRs and RNPKE have significant main and interactive effects on BDP at 2 and 5 mg  $kg^{-1}$  soil. Application of RNPKE + *S. maltophilia*, RNPKE + *A. fabrum*, RNPKE, *S. maltophilia* and *A. fabrum* showed significant positive effect over control at 2 and 5 mg  $Cd\ kg^{-1}$  soil for BDP (Figure 1). Interaction between PGPRs and RNPKE at 2 mg  $Cd\ kg^{-1}$  soil (Figure 2A) and 5 mg  $Cd\ kg^{-1}$  soil (Figure 2B) were significant ordinal for BDP (Figure 2B). It was noted that Cd showed non-significant negative correlation ( $-0.1451$ ;  $p = 0.3986$ ) with BDP. However, PGPR ( $0.5863$ ;  $p = 0.0002$ ) and RNPKE ( $0.3237$ ;  $p = 0.0541$ ) showed positive significant correlation with BDP (Figure 3). The maximum increase of 34% and 68% in BDP was observed from control where RNPKE + *A. fabrum* was applied at 2 and 5 mg  $Cd\ kg^{-1}$  soil, respectively.



**Figure 1.** Number of bitter gourds per plant (BDP) treated with plant growth promoting rhizobacteria (PGPRs), recommended NPK fertilizers (RNPKE), and their combination under 2 and 5 mg  $Cd\ kg^{-1}$  soil. Different small letters express significant differences ( $p \leq 0.05$ ).



**Figure 2.** Interaction graph of *S. maltophilia* (NS1), *A. fabrum* (NS2), and RNPkF at 2 (A) and 5 mg Cd kg<sup>-1</sup> soil (B) for number of bitter gourd per plant (BDP).

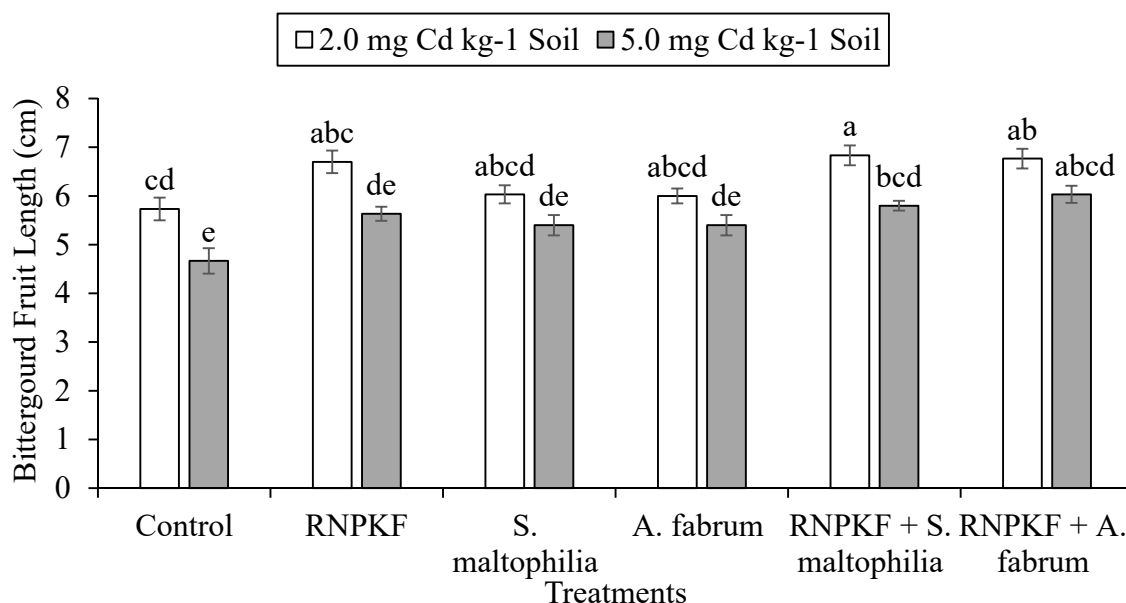


**Figure 3.** Pearson correlation of Cd, PGPRs, and RNPkF for number of bitter gourd per plant (BDP). \* = significant ( $p \leq 0.05$ ); ns = non-significant.

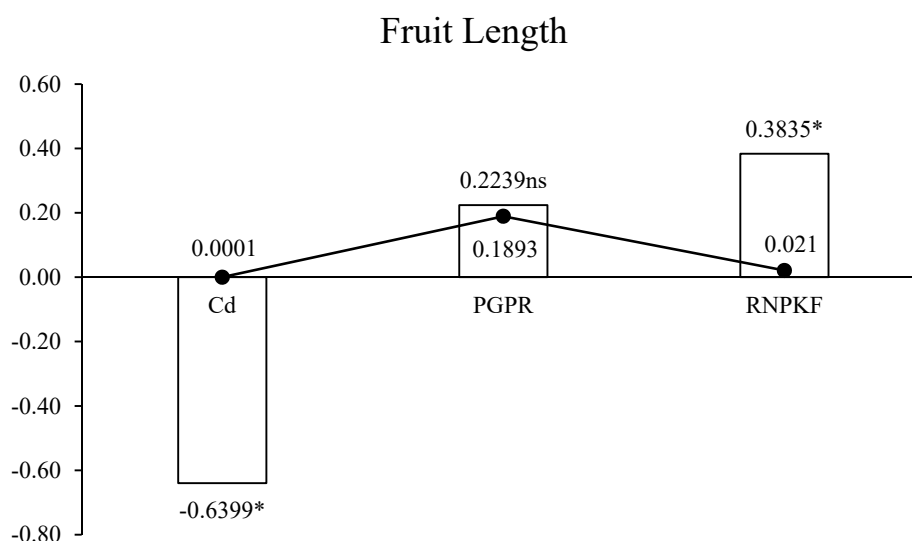
### 3.2. Bitter Gourd Fruit Length

Effects of PGPRs inoculation and application of RNPkF under various Cd levels were significant ( $p \leq 0.05$ ) on bitter gourd fruit length (FL). Application of RNPkF + *S. maltophilia* and RNPkF + *A. fabrum* were significantly different from control at 2 and 5 mg Cd kg<sup>-1</sup> soil for FL. It was observed that RNPkF showed a positive significantly better response at 2 mg Cd kg<sup>-1</sup> soil but remained non-significant at 5 mg Cd kg<sup>-1</sup> soil over control for FL (Figure 4). Main effects of PGPRs and RNPkF were significant, but their interaction remained non-significant for FL at 2 and 5 mg kg<sup>-1</sup> soil. Disordinal non-significant interaction was found between PGPRs and RNPkF at 2 mg Cd kg<sup>-1</sup> soil, but the interaction was non-significant ordinal at 5 mg Cd kg<sup>-1</sup> soil for FL. Cadmium showed significant but negative correlation ( $-0.6399$ ;  $p = 0.0001$ ) with FL. Inoculation of PGPRs ( $0.2239$ ;  $p = 0.1893$ ) gave non-significant positive correlation, while RNPkF ( $0.3835$ ;  $p = 0.021$ ) showed positive significant

correlation with FL (Figure 5). The maximum increase of 19 and 29% in FL was observed from control where RNPKEF + *A. fabrum* was applied at 2 and 5 mg Cd kg<sup>-1</sup> soil, respectively.



**Figure 4.** Bitter gourd fruit length (cm) treated with PGPRs, RNPKEF, and their combination under 2 and 5 mg Cd kg<sup>-1</sup> soil. Different small letters express significant differences at  $p \leq 0.05$ .

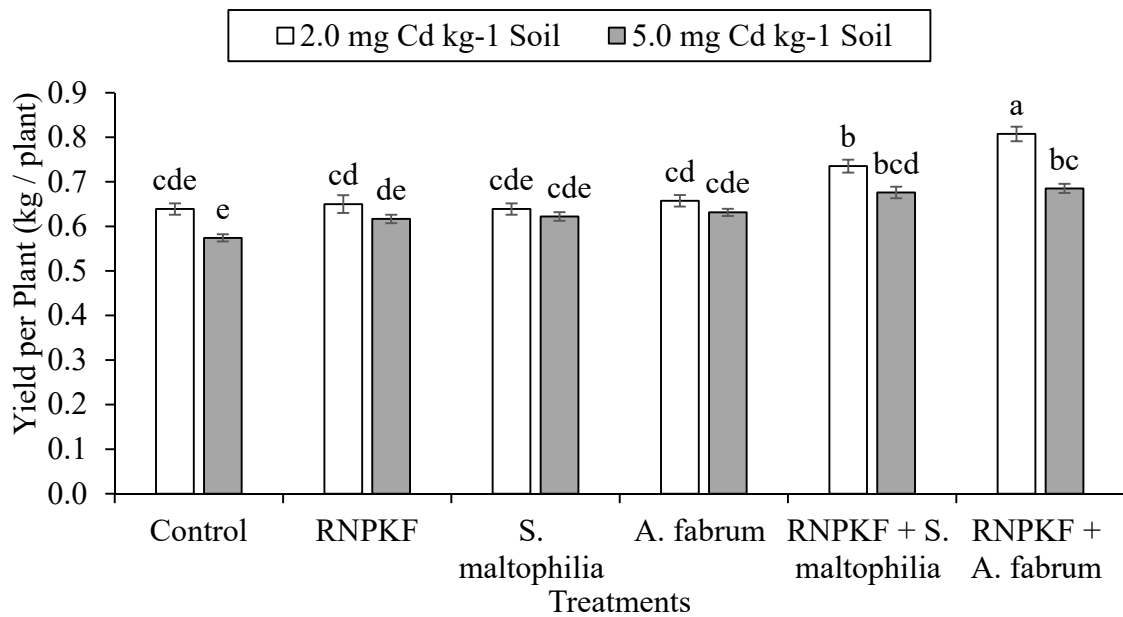


**Figure 5.** Pearson correlation of Cd, PGPRs, and RNPKEF for fruit length (FL). \* = significant ( $p \leq 0.05$ ); ns = non-significant.

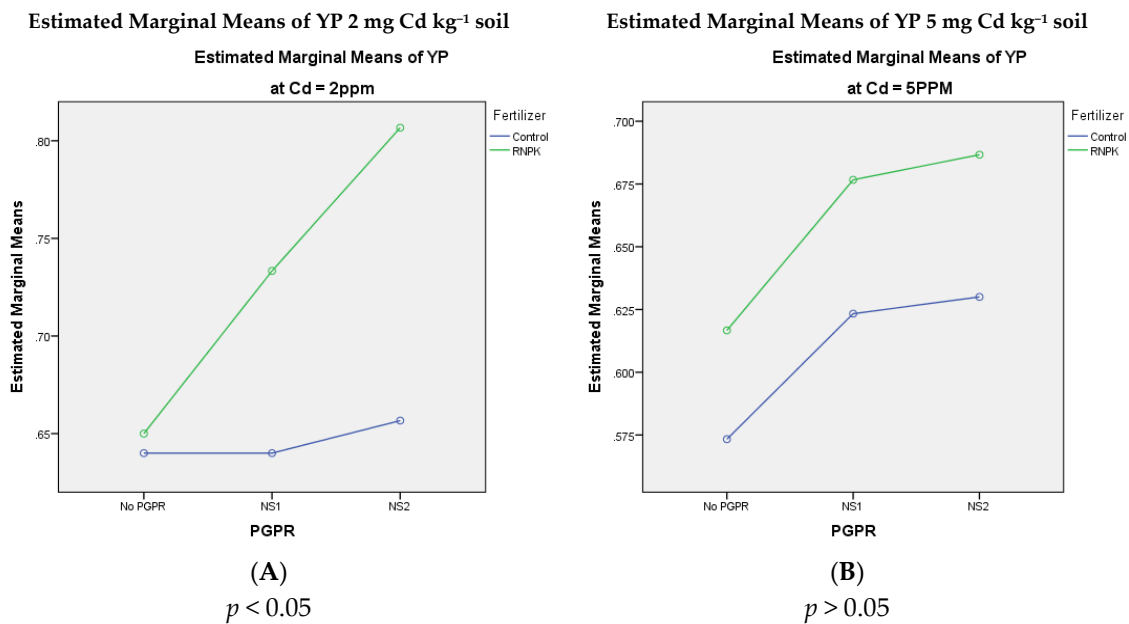
### 3.3. Bitter Gourd Yield per Plant

PGPRs *S. maltophilia* and *A. fabrum* and RNPKEF under 2 and 5 mg Cd kg<sup>-1</sup> soil significantly ( $p \leq 0.05$ ) affect bitter gourd yield per plant (YP). At 2 mg Cd kg<sup>-1</sup> soil, inoculation of PGPRs has significant main and interactive effects on YP. Application of RNPKEF has a significant main effect on YP at 5 mg Cd kg<sup>-1</sup> soil. Treatment RNPKEF + *A. fabrum* was significantly different as compared to control at 2 and 5 mg Cd kg<sup>-1</sup> soil Cd for YP (Figure 6). It was observed that the interaction of PGPRs and RNPKEF was significant ordinal at 2 mg Cd kg<sup>-1</sup> soil (Figure 7A) but non-significant ordinal at 5 mg Cd kg<sup>-1</sup> soil for YP (Figure 7B). Heavy metal Cd showed significant negative correlation ( $-0.4385$ ;  $p = 0.0075$ ) with YP. However, PGPRs ( $0.5035$ ;  $p = 0.0017$ ) and RNPKEF ( $0.3829$ ;  $p = 0.0212$ ) showed

positive significant correlation with YP (Figure 8). The maximum increase of 26.5 and 21.1% in YP was observed from control, where RNPKF + *A. fabrum* was applied at 2 and 5 mg Cd kg<sup>-1</sup> soil, respectively.

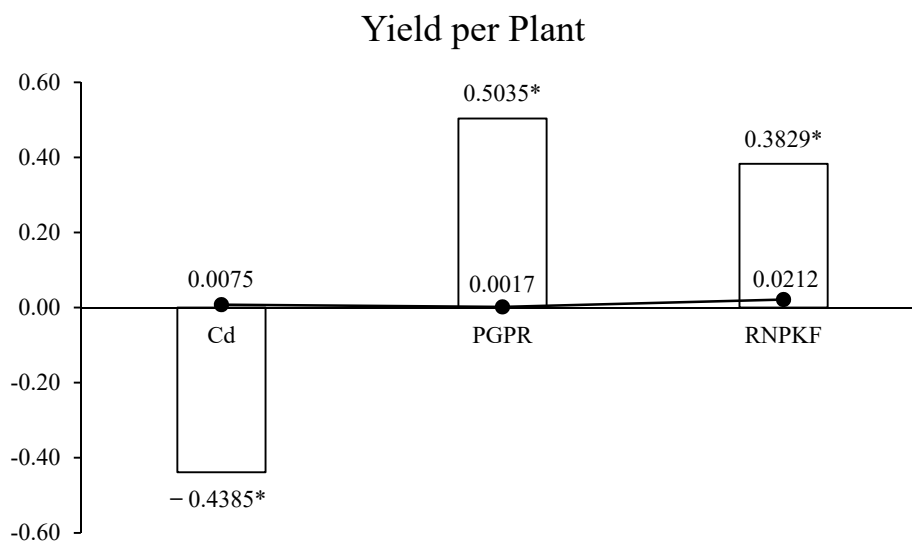


**Figure 6.** Bitter gourd yield (kg) per plant treated with PGPRs, RNPKF, and their combination under 2 and 5 mg Cd kg<sup>-1</sup> soil. Different small letters express significant differences at  $p \leq 0.05$ .



**Figure 7.** Interaction graph of *S. maltophilia* (NS1), *A. fabrum* (NS2), and RNPKF at 2 (A) and 5 mg Cd kg<sup>-1</sup> soil (B) for bitter gourd yield per plant (YP).

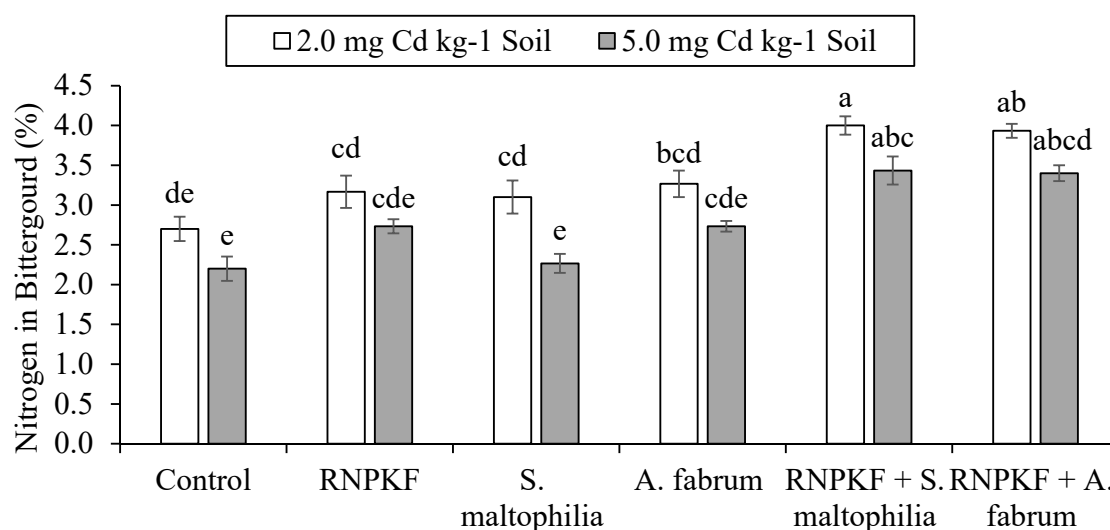




**Figure 8.** Pearson correlation of Cd, PGPRs, and RNPKE for yield per plant (YP)\* = significant ( $p \leq 0.05$ ); ns = non-significant.

### 3.4. Nitrogen Concentration in Bitter Gourd

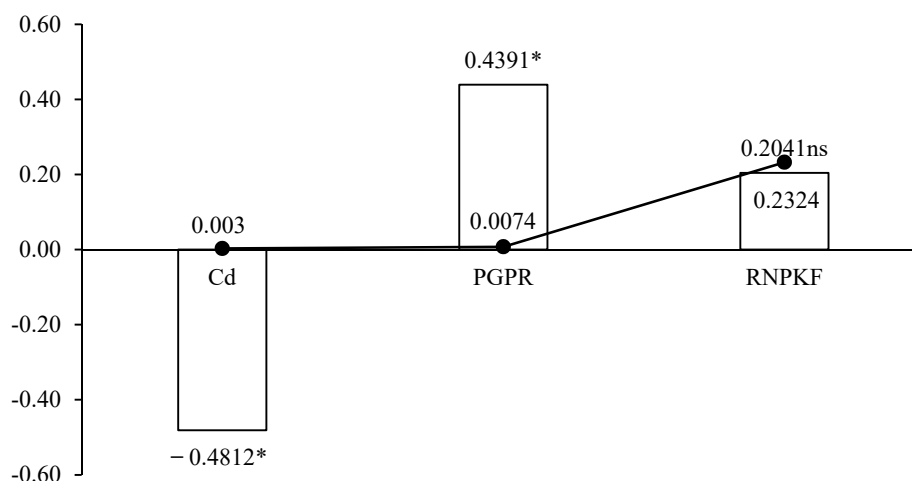
PGPRs and RNPKE significantly ( $p \leq 0.05$ ) changed the nitrogen concentration of bitter gourd (NB) under different levels of Cd. Main effects of PGPRs and RNPKE were significant on NB at 2 and 5 mg kg<sup>-1</sup> soil. However, the interaction of PGPRs and RNPKE was non-significant, ordinal at 2 and 5 mg Cd kg<sup>-1</sup> soil for NB. It was observed that RNPKE + *S. maltophilia* and RNPKE + *A. fabrum* were significantly different as compared to control at 2 and 5 mg Cd kg<sup>-1</sup> soil for NB (Figure 9). Heavy metal Cd showed significant negative correlation ( $-0.4812$ ;  $p = 0.0030$ ) with NB. However, PGPRs ( $0.4391$ ;  $p = 0.0074$ ) showed significant and RNPKE ( $0.2041$ ;  $p = 0.2324$ ) showed non-significant positive correlation with NB (Figure 10). The maximum increase of 48 and 56% in NB was observed from control where RNPKE + *S. maltophilia* was applied at 2 and 5 mg Cd kg<sup>-1</sup> soil, respectively.



**Figure 9.** Nitrogen concentration in bitter gourd (%) treated with PGPRs, RNPKE, and their combination under 2 and 5 mg Cd kg<sup>-1</sup> soil. Different small letters express significant differences ( $p \leq 0.05$ ).



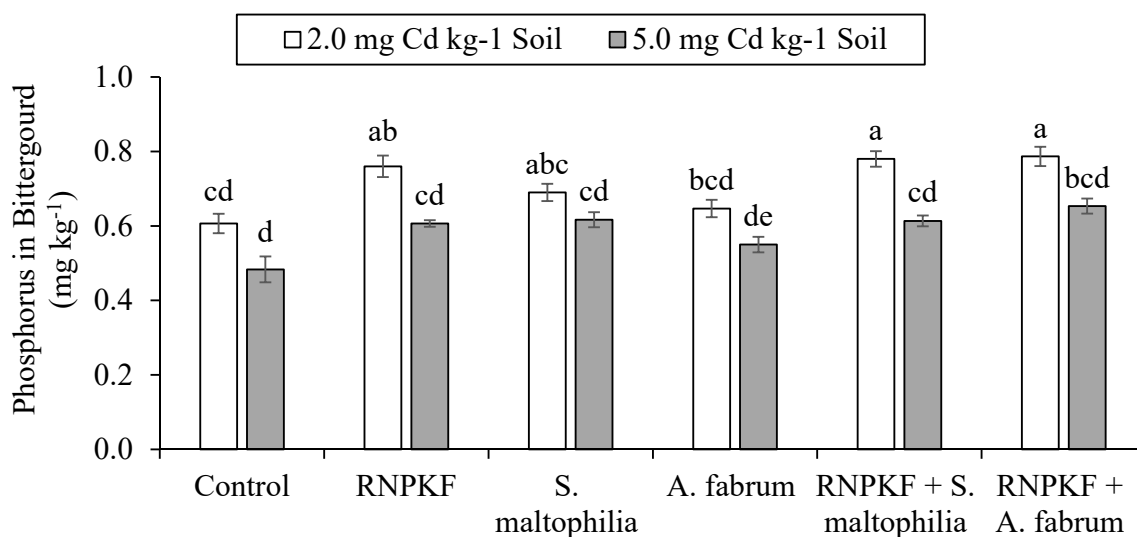
### Nitrogen concentration



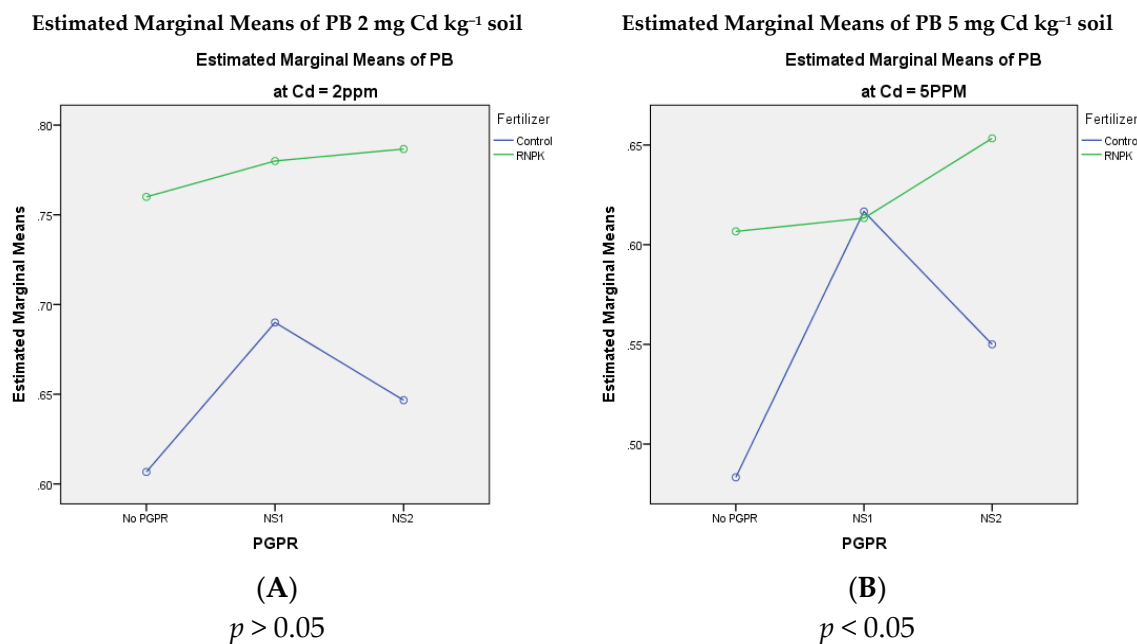
**Figure 10.** Pearson correlation of Cd, PGPRs, and RNPKE for bitter gourd nitrogen concentration (NB) \* = significant ( $p \leq 0.05$ ); ns = non-significant.

### 3.5. Phosphorus Concentration in Bitter Gourd

Effect of PGPRs and RNPKE under 2 and 5 mg kg<sup>-1</sup> soil was significant ( $p \leq 0.05$ ) on phosphorus concentration of bitter gourd (PB). Treatments RNPKE, RNPKE + *S. maltophilia*, RNPKE + *A. fabrum*, and RNPKE differed significantly at 5 mg Cd kg<sup>-1</sup> soil over control for PB (Figure 11). Application of RNPKE and PGPRs showed a significant main effect on PB at 2 mg Cd kg<sup>-1</sup> soil. At 5 mg Cd kg<sup>-1</sup> soil, PGPRs and RNPKE have a significant main and interactive effect on PB. Ordinal interaction was found between PGPRs and RNPKE at 2 mg Cd kg<sup>-1</sup> soil but significant ordinal interaction was observed at 5 mg Cd kg<sup>-1</sup> soil (Figure 12A,B) for PB. Cadmium showed a significant negative correlation ( $-0.6614$ ;  $p = 0.0001$ ) with BDP. However, PGPR ( $0.2537$ ;  $p = 0.1953$ ) showed non-significant and RNPKE ( $0.4422$ ;  $p = 0.0069$ ) showed significant positive correlation with PB (Figure 13). The maximum increase of 29.5% in PB was observed from control where RNPKE + *A. fabrum* was applied at 2 mg Cd kg<sup>-1</sup> soil.

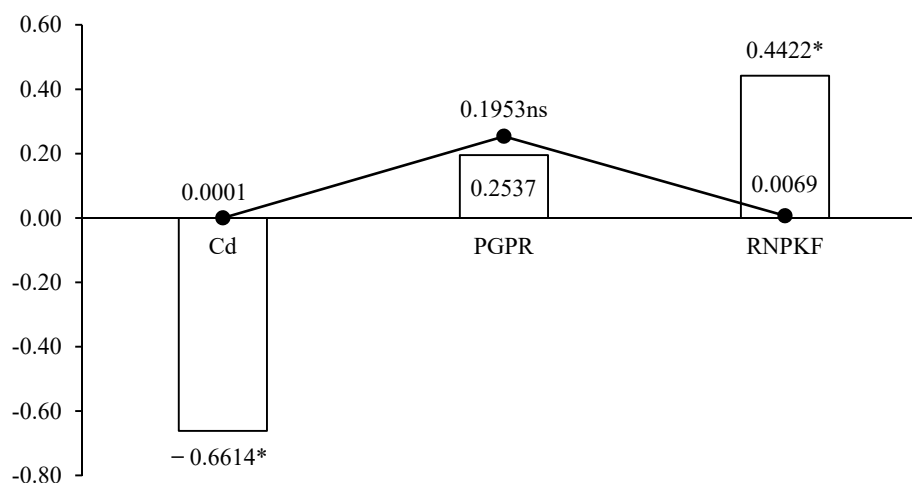


**Figure 11.** Phosphorus concentration in bitter gourd (%) treated with PGPRs, RNPKE, and their combination under 2 and 5 mg Cd kg<sup>-1</sup> soil. Different small letters express significant differences ( $p \leq 0.05$ ).



**Figure 12.** Interaction graphs of *S. maltophilia* (NS1), *A. fabrum* (NS2), and RNPKF at 2 (A) and 5 mg Cd kg<sup>-1</sup> soil (B) for phosphorus concentration in bitter gourd (PB).

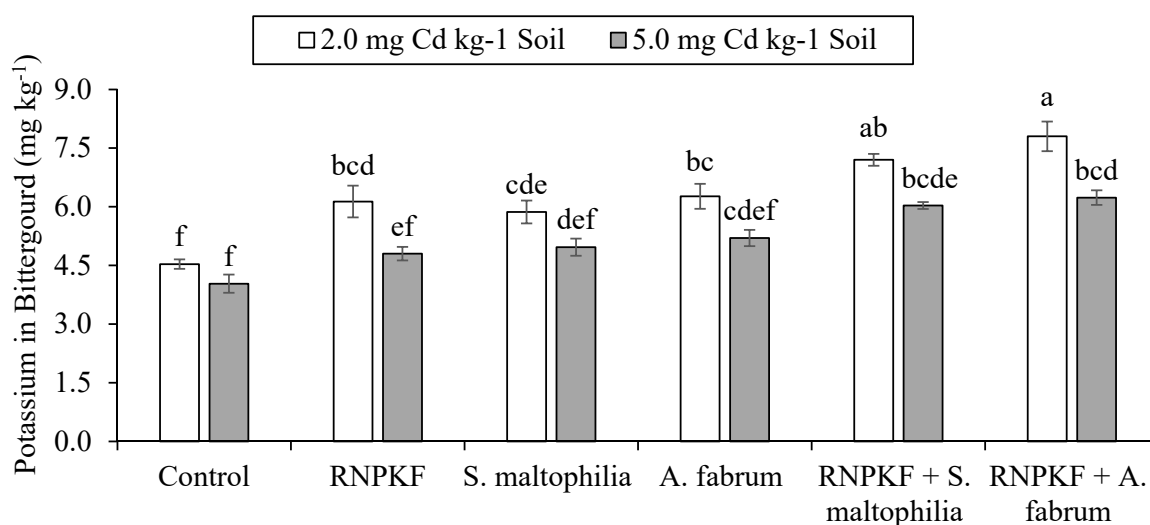
### Phosphorus concentration



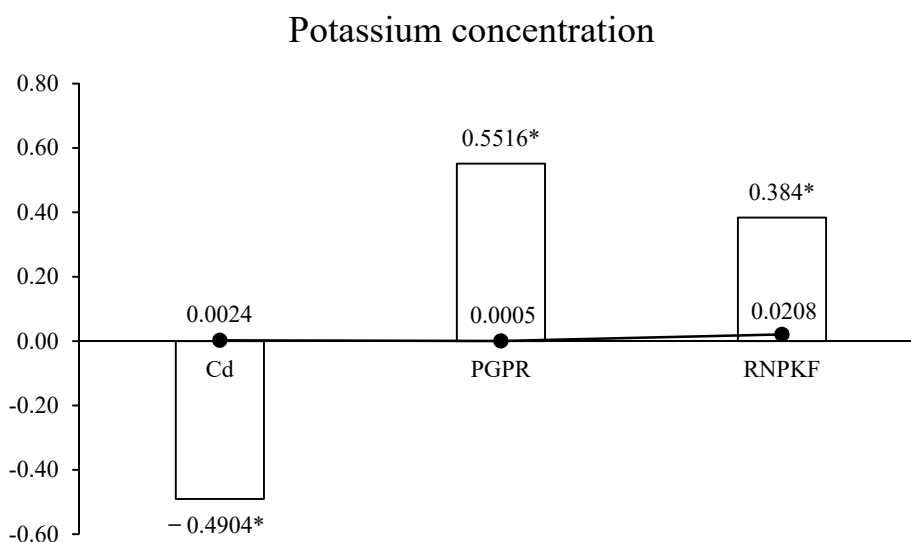
**Figure 13.** Pearson correlation of Cd, PGPRs, and RNPKF for bitter gourd phosphorus concentration (NB). \* = significant ( $p \leq 0.05$ ); ns = non-significant.

### 3.6. Potassium Concentration in Bitter Gourd

Influence of PGPRs and RNPKF 2 and 5 mg kg<sup>-1</sup> soil was significant ( $p \leq 0.05$ ) on potassium concentration of bitter gourd (KB). It was also observed that RNPKF + *S. maltophilia* and RNPKF + *A. fabrum* differed significantly for KB at 5 mg Cd kg<sup>-1</sup> soil (Figure 14). Both PGPRs and RNPKF have a significant main effect on KB at 2 and 5 mg Cd kg<sup>-1</sup> soil. Disordinal non-significant interaction was found between PGPRs and RNPKF at 2 mg Cd kg<sup>-1</sup> soil and but ordinal interaction was observed at 5 mg Cd kg<sup>-1</sup> soil for KB. Different levels of Cd showed significant negative correlation ( $-0.4904$ ;  $p = 0.0024$ ) with KB. However, PGPR ( $0.5516$ ;  $p = 0.0005$ ) and RNPKF ( $0.3840$ ;  $p = 0.0208$ ) showed significant positive correlation with KB (Figure 15). Application of RNPKF + *S. maltophilia*, RNPKF + *A. fabrum*, RNPKF, *S. maltophilia* and *A. fabrum* were significantly different as compared to control at 2 mg Cd kg<sup>-1</sup> soil for KB. The maximum increase of 72 and 55% in KB was observed from control where RNPKF + *A. fabrum* was applied at 2 and 5 mg Cd kg<sup>-1</sup> soil, respectively.



**Figure 14.** Potassium concentration in bitter gourd (%) treated with PGPRs, RNPKF, and their combination under 2 and 5 mg Cd kg<sup>-1</sup> soil. Different small letters express significant differences ( $p \leq 0.05$ ).



**Figure 15.** Pearson correlation of Cd, PGPRs, and RNPKF for bitter gourd potassium concentration (NB). \* = significant ( $p \leq 0.05$ ); ns = non-significant.

#### 4. Discussion

A significant decrease in fruit length, fresh weight, and yield per plant of bitter gourd were observed in control at 5 mg Cd kg<sup>-1</sup> soil. Low uptake of N, P, and K in bitter gourd under Cd toxicity might be a major factor for reduction in yield, fruit length, and fresh weight. Higher biosynthesis of stress ethylene might be an allied factor responsible for a significant decline in yield of bitter gourd under Cd stress. According to Sanita di Toppi and Gabbrielli [7], accumulation of Cd beyond safe limit disturbed the nutrients homeostasis which played an imperative role in reduction of root and shoot elongation. Cadmium in plants also competes with divalent nutrients ions and decreases their uptake in plants [16]. Under Cd toxicity, transmembrane carriers in roots become unable to distinguish between non-essential Cd and essential divalent nutrients during their uptake [53,54]. Glick et al. [55] also documented that biosynthesis of endogenous stress ethylene under abiotic stress conditions, negatively affects the productivity of the crop. Toxicity of heavy metals causes abnormal division of cell thus induced chromosomal aberration in plants [56]. This resulted in a decrease of protochlorophyllide reductase activity. Such disturbance in plants also induced chlorosis in leaves [57].

Furthermore, Matile et al. [58] suggested the decomposition of lipids in cell wall when ethylene concentration is increased. They argued that ethylene when contact with chlorophyllase (chlase) gene it degrades chlorophyll caused in chlorosis. Furthermore, application of RNPKE + *A. fabrum* differed significantly better from the sole application of control for improvement in N, P and K. The improvement in N, P, and K mitigate the adverse impacts of Cd in bitter gourd. Panković et al. [59] observed that improvement in N uptake of sunflower alleviates the inhibitory influences of Cd [22,23,27,28,32]. Higher N facilitates in activity of Rubisco by an increase in soluble protein contents. Application of N in NH<sub>4</sub> form is efficacious in decreasing the Cd uptake due to antagonistic relationship [60]. Findings of the current experiment also support the above argument. Better N in bitter gourd was observed where yield was improved over control even under Cd toxicity. Under Cd stress, plants start producing N metabolites, i.e., proline that causes phytochelation and decreases the intake of Cd [61]. Application of phosphorus neutralizes the adverse impacts of Cd and improve the yield of crops [62]. Improvement of P uptake in plants enhances the synthesis of glutathione that prevents membrane damages caused by Cd [63]. Balance K concentration decreases the generation of reactive oxidative species (ROS) and inhibits the NADPH oxidase [64]. Moreover, less generation of stress ethylene by inoculation of *A. fabrum* and RNPKE + *A. fabrum* might be another major factor responsible for the enhancement in bitter gourd growth and yield in the current study. Both PGPRs were capable to produce ACC deaminase, which cleaves ethylene into intermediate compounds. Similar kinds of results were also documented by many scientists [25,26,30,31]. Glick et al. [44] proposed that enzyme ACC deaminase break ethylene into  $\alpha$ -ketobutyrate and ammonia [65,66]. Accumulated ethylene in roots moved towards rhizosphere; thus, ethylene becomes low in plant roots, and stress is alleviated. Similarly, Tripathi et al. [67] reported growth hormones, indole acetic acid, improved the root elongation for better uptake of nutrients [24].

## 5. Conclusions

It is concluded that PGPR, *A. fabrum* has more potential over *S. maltophilia* to alleviate Cd induced stress in bitter gourd. Inoculation of *A. fabrum* with RNPKE is an efficacious approach to improve N, P, and K concentration in bitter gourd. The combined use of RNPKE and *A. fabrum* can increase the number of bitter gourds per plant, bitter gourd fruit length, and yield per plant by alleviating 5 mg Cd kg<sup>-1</sup> soil induced toxicity. However, more investigations are suggested at field level to declare *A. fabrum* + RNPKE as an efficacious technique to mitigate Cd stress in bitter gourd.

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