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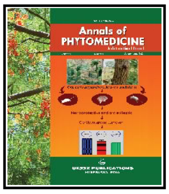

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Minimizing the allelopathy of *Solanum nigrum* L. in green gram (*Vigna radiata* (L.) Wilczek) through *Pseudomonas fluorescens*

Mohammad Salman, Deepti Prabha, Sony Grace, Gufran Ahmad*, S. Balaji Naik and Md. Mahtab Rashid***,◆**
Department of Seed Science and Technology, H.N.B. Garhwal University, Srinagar-246174, India
**K.V.K., Mahoba, Banda University of Agriculture and Technology, Banda-210001, India*
***Faculty of Forest Science and Forest Ecology, Georg-August-University of Göttingen-37073, Goettingen, Germany*
****Department of Plant Pathology, Bihar Agricultural University, Sabour-813210, India*

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Abstract

The main objective of this study was to find the allelopathic effect of a commonly found annual weed *Solanum nigrum* L. on the growth of green gram (*Vigna radiata* (L.) Wilczek) and its alleviation through seed treatment of plant growth-promoting microbes (PGPMs). It was evident that extract of *S. nigrum* significantly reduces the germination of the pulse with increasing concentration. The allelopathic effects remain pronounced throughout the life-cycle of green gram. It was, however, seen that *Pseudomonas fluorescens* effectively reduces such impact in all the growth parameters to the extent that it even outperformed the control. The increased root, shoot, and seedling length suggests that *P. fluorescens* aids in overcoming the allelopathic stress caused by extract of *S. nigrum* from initial stages itself which later can be seen extending to fruiting. A higher pod length and biomass in the same also indicates that the PGPM additionally also enhances the biological activity of plants which potentially contributes to the yield. Hence, it can be concluded that the application of potent PGPMs can efficiently reduce the negative effects of weeds in green gram and sustain the production while simultaneously reducing the cost of intercultural operations and the application of synthetic chemicals.

1. Introduction

Legumes are considered poor man's meat owing to their higher protein content. They are considered as the appropriate choice for combating nutritional insecurity of the growing population under shrinking agricultural land and changing climate. Green gram (*Vigna radiata* (L.) Wilczek) is one of the major legumes which is significantly preferred over others by consumers due to its palatability (Maji *et al.*, 2020). It is known that the production and productivity of all crops are influenced by various abiotic and biotic factors. In the case of green gram, weeds are the major biotic cause that reduces the production potential of the crop due to its short lifespan, slow growth at early stages, growing labour crisis, and frequent rainfall during the cropping season (Maji *et al.*, 2020). *S. nigrum* (common name: Black night shade) is a noxious annual weed worldwide which are resistant to synthetic herbicides and are known for their allelopathic effect on crop plants (Henriques *et al.*, 2006). Allelopathy is one of the mechanisms through which weeds compete with crop plants and is defined as the stimulatory effect of one plant on the growth of other plants within the same ecosystem through chemical substances called allelopathic chemicals. *S. nigrum* produces various secondary metabolites that are phytotoxic, but their effects are more prominent on crops than on weeds. The weed is proven to reduce the germination and growth of root, shoot, and seedlings in a few monocots and dicots (Sabh and Ali, 2010; Afolayan and Bvenura, 2017; Alam and Khan, 2020).

Since there is a shift towards organic and sustainable agriculture, the production of crops with no or minimum tillage and less application of inorganic pesticides is being promoted. Subsequently, interactions of crop plants with weeds are being explored to increase the production potential of crop plants under organic farming. Additionally, utilization of plant growth-promoting microbes (PGPMs) are also in prime focus for the same goal as they are proven of conferring resistance to crop plants against various abiotic and biotic stresses (Rani and Azmi, 2019). Although, *S. nigrum* is such an important annual weed and has widespread allelopathic effects on crop plants, its interaction with green gram has not been investigated. Moreover, the application of PGPMs for alleviation of allelopathic effects has also not been investigated thoroughly. Thus, this study was conducted to find the allelopathic effects of *S. nigrum* on green gram and to assess the potential of two PGPMs namely: *Pseudomonas* spp. and *Bacillus* spp. in the alleviation of allelopathic effects.

2. Materials and Methods

2.1 Seeds, plant materials, and bacterial cultures

The seeds of green gram cultivar IPM 205-7 (Virat) were procured from the Indian Institute of Pulse Research (IIPR), Kanpur, Uttar Pradesh, India. Plant samples of *S. nigrum* were collected at their flowering and fruiting stages from the agricultural fields of H.N.B. Garhwal University, Srinagar, Uttarakhand, India. The isolates of

Corresponding author: Mr. Md. Mahtab Rashid
Department of Plant Pathology, Bihar Agricultural University, Sabour-813210, India
E-mail: mahtabrashid@gmail.com
Tel.: +91-8084009817

Pseudomonas fluorescens, SP-37 and *Bacillus* spp., BS-56, were also procured from H.N.B. Garhwal University, Srinagar, Uttarakhand, India. For reactivation of the bacterial isolates, King's B and YEMA media were used, respectively.

2.2 Preparation of aqueous extract

The leaves of collected *S. nigrum* plants were dried in a hot-air oven at 35^0C until a constant weight was obtained and then crushed to a powdered form. The dried powder was sieved through a 1 mm sieve and stored in sealed vials at 4^0C for further use. An aqueous solution of 3%, 6%, 10%, and 15% of *S. nigrum* was prepared by dissolving 3 g, 6 g, 10 g, and 15 g of dried powder, respectively in 100 ml of distilled sterilized water. The solution was then agitated for 24 h in a shaker at room temperature. The solution was then filtered using Whatman No. 1 filter paper and the extract was stored in a dark and cool place.

2.3 Germination of green gram under different concentrations of *S. nigrum* extract

The seeds of green gram were surface sterilized using 1% sodium hypochlorite (NaOCl) solution. 10 surface-sterilized seeds were placed in filter paper covered Petri dish. 5 ml of each *S. nigrum* aqueous solution (3%, 6%, 10%, and 15%) were applied to Petri dish in three replications. The seeds were then incubated at room temperature for 10 days and the germination percentage was calculated for each treatment.

2.4 Experimental setup and growth parameters

The surface-sterilized seeds of green gram were treated with bacterial suspension of *P. fluorescens* (SP-37) and *Bacillus* spp. (BS-56) @ 1×10^8 CFU ml^{-1}, individually and in a consortium. The seeds were then sown in plastic pots containing 1.5 kg of a sterilized mixture of garden soil and vermicompost (10%). 160 ml of 15% *S. nigrum* aqueous solutions was applied to the pots and plants were grown under natural conditions.The treatments were named as follows:

C: Plants without seed treatment.

Ext: Plants from seed treated with 15% *S. nigrum* extract.

PfExt: Plants from seed treated with *P. fluorescens* (SP-37) and 15% *S. nigrum* extract.

BExt: Plants from seed treated with *Bacillus* spp. (BS-56) and 15% *S. nigrum* extract.

ConExt: Plants from seed treated with a consortium of *P. fluorescens* (SP-37) and *Bacillus* spp. (BS-56) and 15% *S. nigrum* extract.

The following growth parameters were calculated from the data recorded as per ISTA rules (1985): germination percentage, speed of germination (Maguire's formula 1962, Wardle *et al.*,1991, and Bradbeer's formula 2013), root length, shoot length, seedling length, fresh and dry weight of seedling, and seedling vigour index 1 and 2.

2.5 Data analysis

All data in the present investigation were recorded in triplicate under a completely randomized design (CBD). The differences between the treatments were calculated using two-factor ANOVA using the software SPSS (v 16.0). The mean values of the treatments were compared using Duncan's new multiple range test at a $p> 0.05$ significance level.

3. Results

3.1 Germination of green gram under different concentrations of *S. nigrum* extract

There was an evident reduction in germination percentage of green gram cultivar IPM 205-7 (Virat) due to the presence of *S. nigrum* extract. The highest germination percentage of 91.6% was observed in control which reduced significantly with the increasing concentration of *S. nigrum* extract (Figure. 1). The lowest germination percentage of 61.3% was observed in seeds treated with 15% *S. nigrum* extract which was selected for further experimentation.

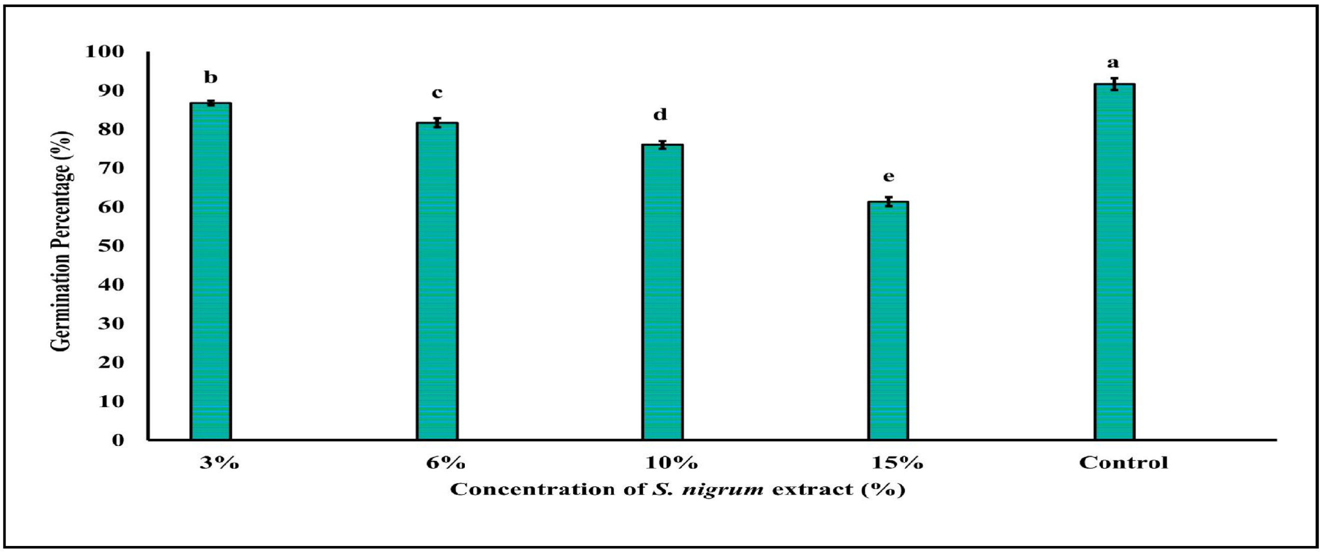


Figure 1: Seed germination (%) of green gram var. IPM 205-7 (Virat) at increasing concentration of *S. nigrum* extract.

3.2 Growth parameters

All the growth parameters were significantly different in each of the treatments. Maximum percentage of seed germination (93.0%) under the influence of 15% *S. nigrum* extract was observed in seeds treated with *P. fluorescens* (SP-37) (PfExt) which was interestingly even higher than in control (C), *Bacillus* spp. (BS-56) treated seeds (BExt), and consortium treated seeds (ConExt). As expected, the lowest percentage of seed germination (59.3%) was found in seeds treated only with *S. nigrum* extract (Ext) (Figure 2).The difference in germination speed was non-significant between control and seeds treated with *P. fluorescens* (SP-37). However, as above the lowest germination speed was of seeds treated only with *S. nigrum* extract at 1.80 (Figure 3). Root length was highest in control (6.84 cm), followed by PfExt and BExt treatments with non-significant differences, however, the shoot length was highest in PfExt treatment (12 cm), followed bycontrol and BExt treatments with non-significant differences. Both root and shoot length were lowest in plants from seeds treated only with *S. nigrum* extract at 4.6 cm and 6.6 cm, respectively (Figure 4a and b).

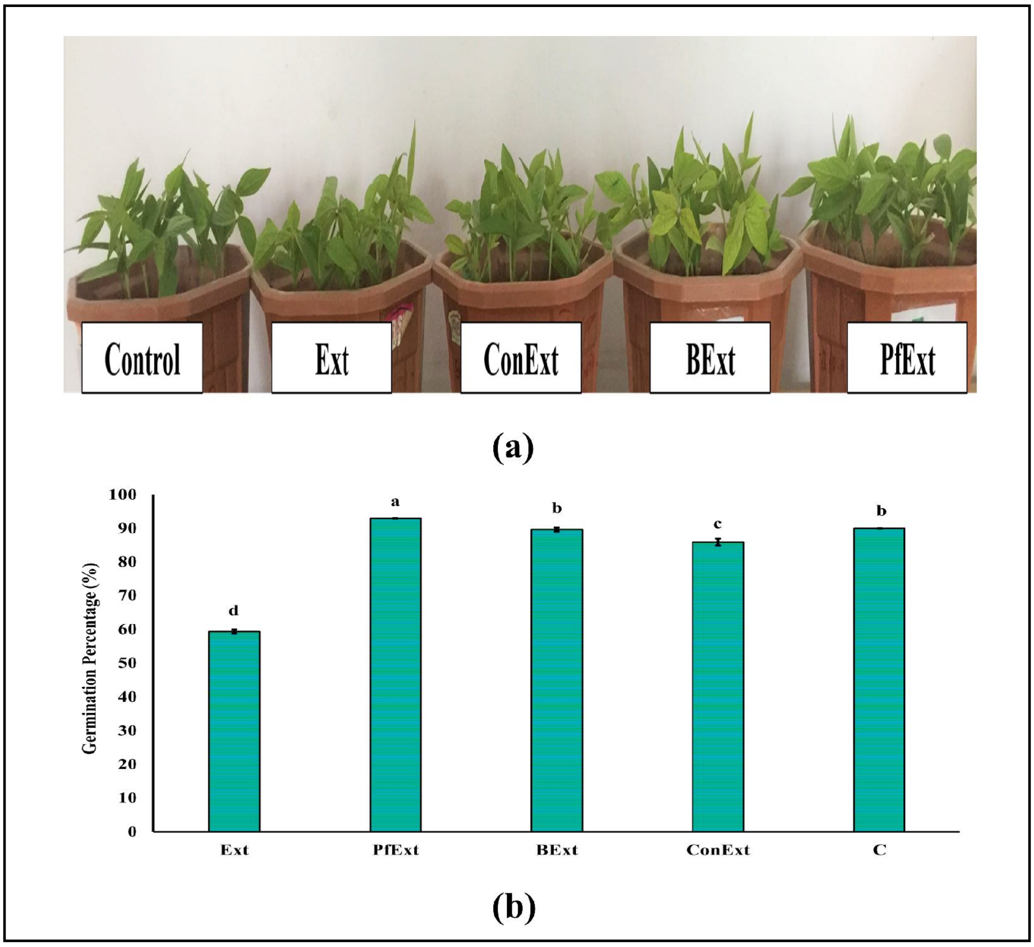
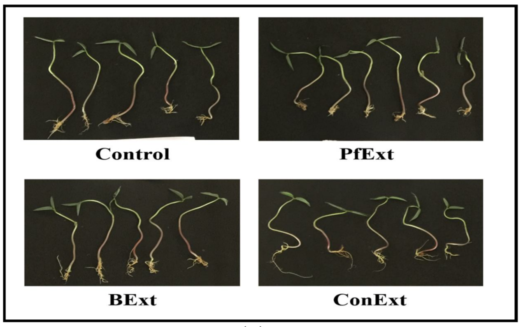


Figure 2: (a) Response of seedlings and (b) germination (%) of *P. fluorescens* (SP-37), *Bacillus* spp. (BS-56), and their consortium treated green gram seeds at 15% *S. nigrum* extract.



(a)

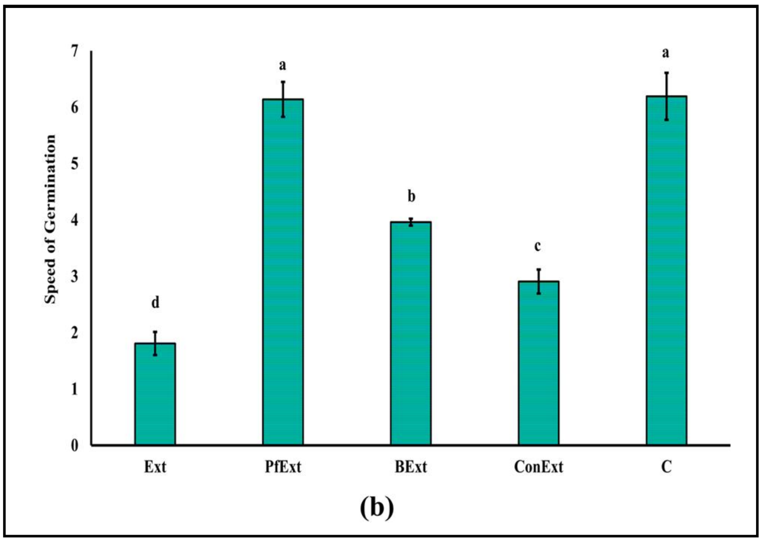


Figure 3: Germination speed of *P. fluorescens* (SP-37), *Bacillus* spp. (BS-56), and their consortium treated green gram seeds at 15% *S. nigrum* extract.

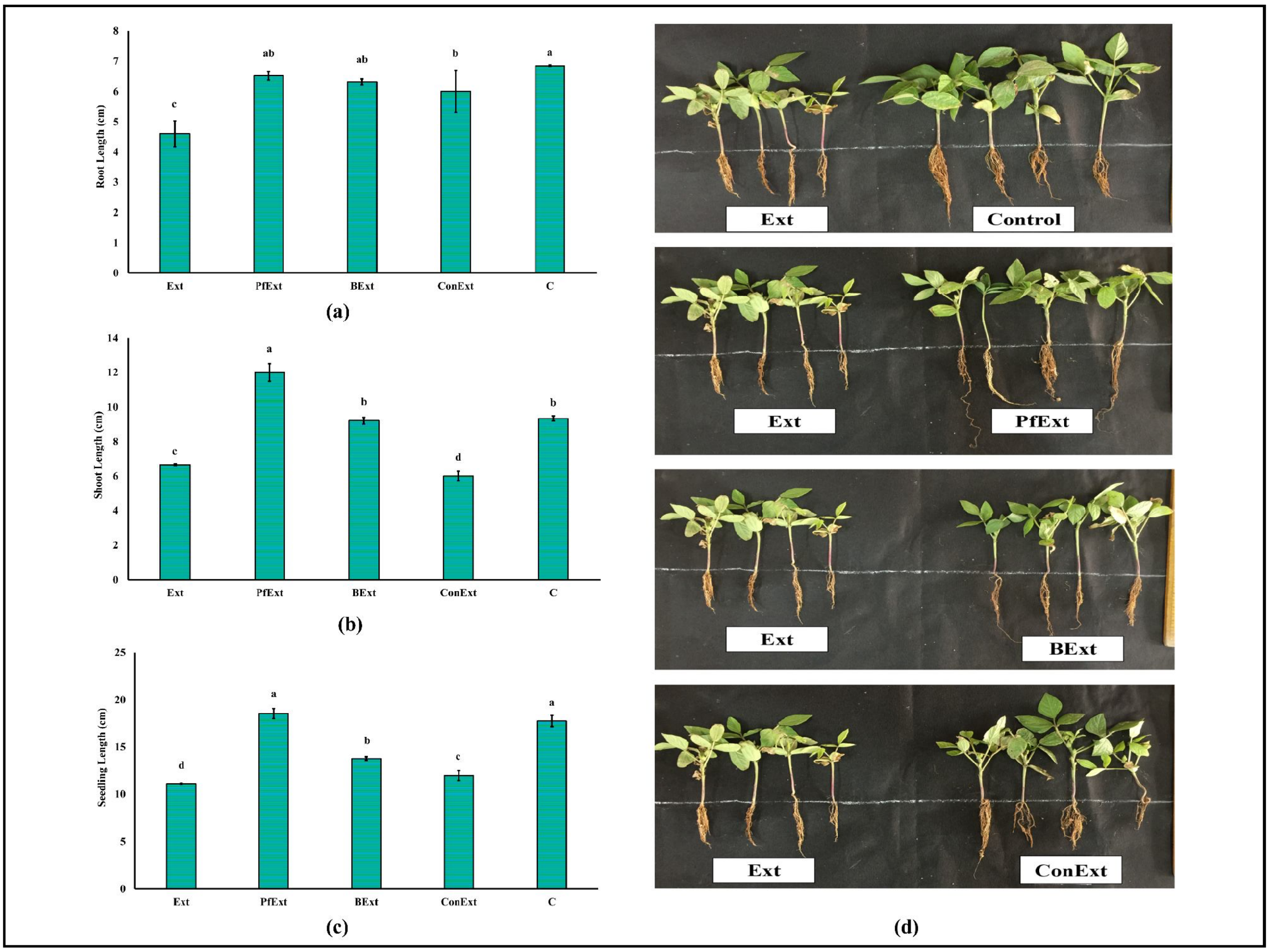


Figure 4: (a) Root length, (b) Shoot length, and (c) Seedling length of *P. fluorescens* (SP-37), *Bacillus* spp. (BS-56), and their consortium treated green gram seeds at 15% *S. nigrum* extract.

In a similar trend, the differences in seedling length were also non-significant between PfExt treatment (18.55 cm) and control (17.76 cm) while the lowest seedling length was observed in plants from seeds treated only with *S. nigrum* extract (11.15 cm) (Figure 4 c). The fresh weight was highest in seedlings from control while the dry weight of seedlings was highest in *P. fluorescens* (SP-37) treatment. Theseedlings from seeds treated only with *S. nigrum* extract had the lowest fresh weight and dry weight (Figures 5a and b). The seedling vigour indices 1 and 2 were highest in seedlings from seeds treated with *P. fluorescens* (SP-37), followed by control. The lowest seedling vigour indices 1 and 2 were observed in seedlings from seeds treated only with *S. nigrum* extract (Figure 6a).The highest pod length was observed in *P. fluorescens* (SP-37) treated plants followed by *Bacillus* spp. (BS-56) treated seeds and control with non-significant differences while, lowest in plants treated with *S. nigrum* extract (Figures 6b and c).

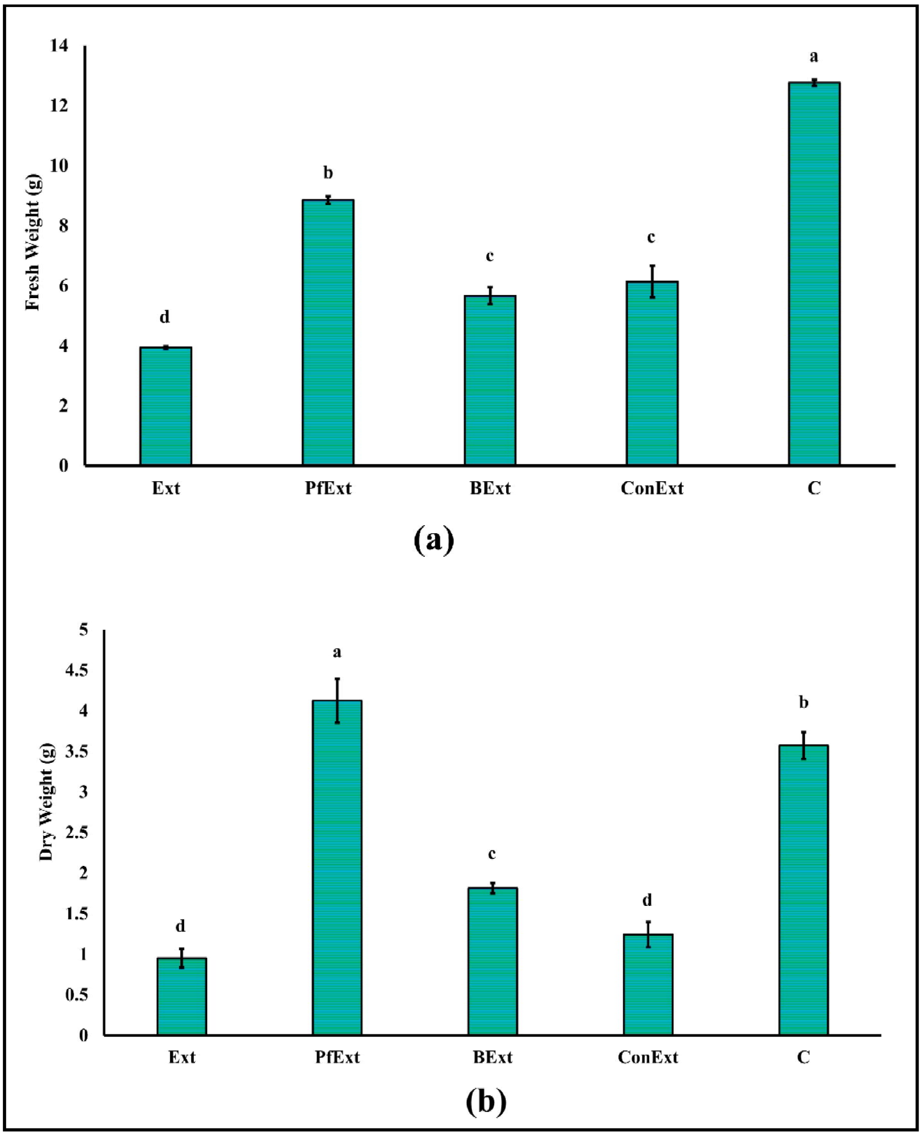


Figure 5: (a) Fresh weight and (b) Dry weight of *P. fluorescens* (SP-37), *Bacillus* spp. (BS-56), and their consortium treated green gram seeds at 15% *S. nigrum* extract.

4. Discussion

Legumes are highly nutritious and proteinaceous crops that are important in the prospect of providing food to the growing population of the world. Green gram is a major legume that has severe issues in intercultural operations especially weeding (Kumara, 2021). Weeds cause a consequential yield loss in crops through their direct and indirect effects. The allelopathy of weeds has serious negative effects on other crops (Li *et al.*, 2021) for which they are considered as the substitute for synthetic herbicides (Cheng and Cheng, 2015). However, agricultural scientists must evaluate their effect on common field cropsunder field conditions before their utilization as herbicides. *S. nigrum* is a major annual weed that is found all over the globe and has a prominent allelopathic effect on many crops (Ismaiel and Salama, 2021) and is commonly found as weeds during the cultivation of green gram (Kaur *et al*., 2010). PGPMs have shown immense potential in enhancing crop yield during different stresses and among them *Pseudomonas* spp. and *Bacillus* spp. of plant growth-promoting rhizobacteria (PGPR) have come out as torch-bearers (Cheng and Cheng, 2015). Their yield contributing mechanisms in plant-microbe interactions have shown their potential usability in plant-plant allelopathic interactions (Mishra *et al*., 2012). Therefore, our study was to find the potential of PGPMs in reducing the allelopathic effect of *S. nigrum* on legumes.

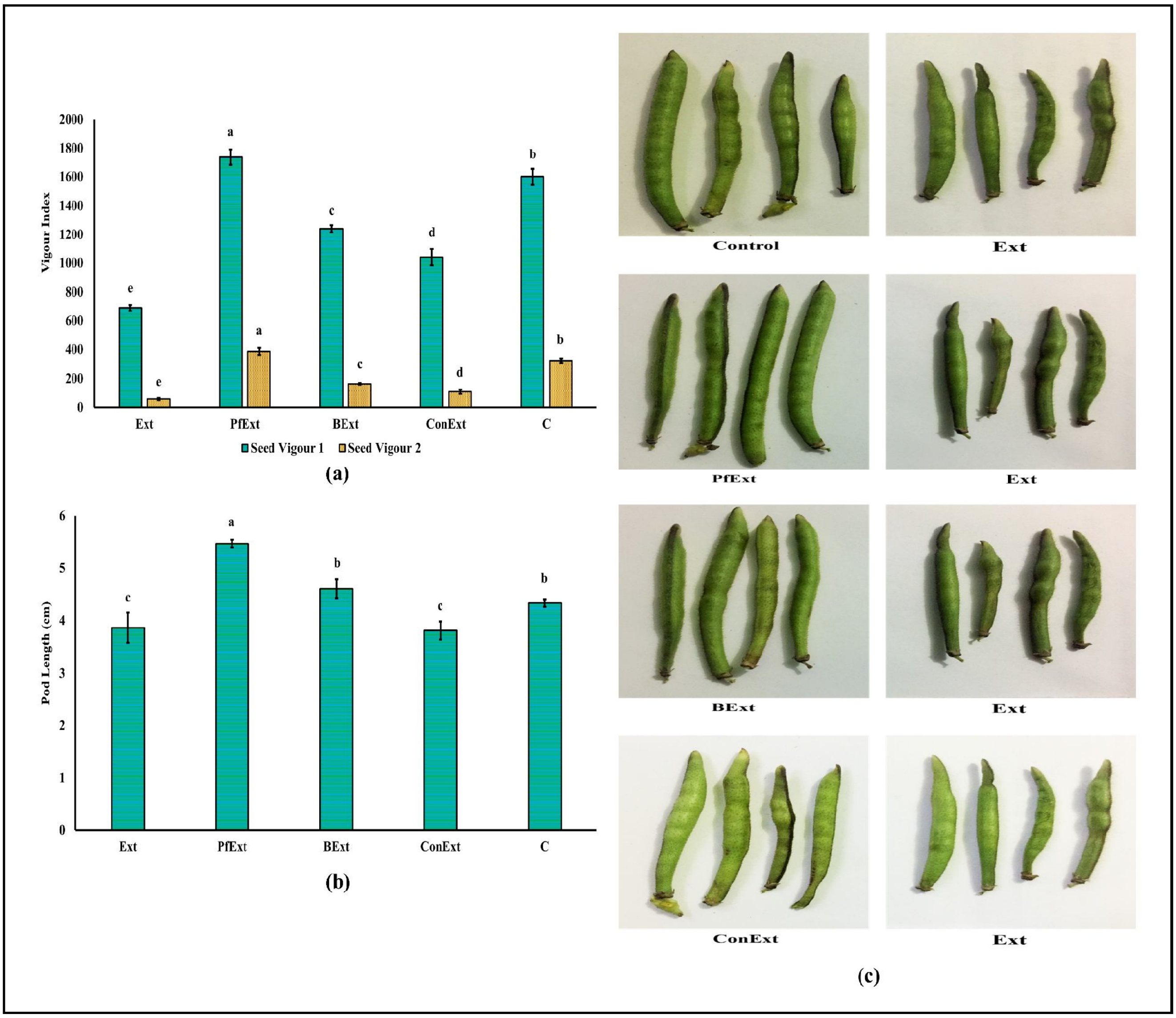


Figure 6: (a) Seed vigour 1 and 2 and (b, c) Pod length of *P. fluorescens* (SP-37), *Bacillus* spp. (BS-56), and their consortium treated green gram seeds at 15% *S. nigrum* extract.

From the results, it can be easily stated that extract of *S. nigrum* significantly has negative effects on the growth parameters of green gram. As observed, it reduces the germination percentage and speed, root length, soot length, seedling length and vigour, and pod length. The potential cause behind these effects is negative allelopathy by the bioactive metabolites of *S. nigrum*. Sabh and Ali (2010) and Ismaiel and Salama (2021) reported the same and stated that the alkaloids in the extract were the primary inhibitory molecule. The alkaloidal content in a plant varies in root, shoot, leaves, and fruits (Aburjai *et al*., 2014) and the increasing concentration of alkaloids is directly proportional to the decline in growth parameters. The application of *P. fluorescens* (SP-37) as seed treatment clearly alleviates plants from the negative allelopathic effects of *S. nigrum* extract. Since the genus, *Pseudomonas* is proven to degrade the alkaloidal compounds through different mechanisms (Bruce *et al*., 2010; Nogales *et al*., 2017), it can be possibly said that in the present case, *P. fluorescens* (SP-37) may decompose the alkaloids of *S. nigrum* extract through different catabolic pathways. Additionally, species of *Pseudomonas* have been reported to protect the crops from allelochemicals of weeds through biofilm formation and enhanced expression of stress-responsive gene(s) in the crop (Mishra and Nautiyal, 2012; Kanwar *et al*., 2019). Moreover, in field conditions, *Pseudomonas* spp. change the rhizospheric microbial community of crops in a manner that increases the utilization of phytotoxic allelochemicals produced by the weeds along with increased nutrient mobilization in soil (Mishra and Nautiyal, 2012; Mishra *et al*., 2012). As in our case, *P. fluorescens* (SP-37) showed the significant ability to reduce the allelopathic effect of *S. nigrum* under greenhouse conditions. The weaker performance of *Bacillus* spp. in a single application (BExt) and a consortium of both PGPMs (ConExt) in presence of *S. nigrum* could be due to incompatibility issues.

5. Conclusion

From the present study, it can be concluded that *S. nigrum* has significant allelopathic effects on the green gram as it prominently inhibits the germination, growth, and development of the same. However, the application of *P. fluorescens* (SP-37) as seed treatment significantly enhances the resistance of green gram against the weed. Additionally, application of the same outperformed even the control plants which establish its potential in negating the allelopathic effect of *S. nigrum*, if the latter is used for management of weeds in green gram. Thus, the strategy of using extracts of *S. nigrum* to control the weeds and simultaneously utilization of *P. fluorescens* or any other PGPMs for negating the effect on crop plants can be implemented for the promotion of organic and sustainable agriculture. Moreover, the application of PGPMs can also be an eco-friendly method of improving crop health in weed-infested agricultural lands.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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