J. Plantarum., 6(2): 55-66

ISSN PRINT 2710-4079
ISSN ONLINE 2710-4087

ALLELOPATHIC EFFECT OF PSEUDOMONAS FLUORESCENS (PF 083) ON COTTON WEED *SORGHUM HALEPENSE* (L.) Pres

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Received on: 26-01-24; Reviewed on: 25-04-24; Accepted on: 23-05-2024; Published on: 10-12-2024

Abstract

Cotton (*Gossypium hirsutum* L.) belongs to the Malvaceae (mellow) family of plants. It is one among Pakistan's main crops. Cotton can also provide the raw materials for textiles, apparel, vegetable oil, and animal feed. Pakistan is the third-largest buyer of raw cotton in the world and the fourth-largest producer of cotton worldwide. By releasing specific chemical components into the environment, one plant can have both direct and indirect, distinct negative and positive impacts on another plant. This phenomenon is known as alleloopathy. One of the principal weeds with significant allelopathic power is Johnson grass (*Sorghum halepense (L.)* Pres). *S. halepense*, is one of the most pervasive and upsetting weeds. From low to high concentrations, allelochemicals in *S. halepense* leaf extract affect and inhibit cotton seed development rate, fresh and dry biomass and seedling length. Biological method in order to lessen the cotton allelopathic stress and to promote the growth of cotton seeds, *Pseudomonas fluorescene* (PF 083) acts as both a bioherbicide and a Plant Growth Promoting Rhizobacteria (PGPR). Cotton seed germination (70%), seedling length (20%), fresh seedling biomass (90%), and dried seedling biomass (45%) all increased with the addition of Pseudomonas PGPR PF083. The current study revealed that Johnson grass strong allelopathic effects significantly surpassed by *Pseudomonas fluorescene* (PF083).

Key words: Allelopathy, Biological tool *Pseudomonas fluorescene* (PF 083), *Gossypium hirsutum*, *Sorghum halepense*.

Introduction

Cotton (*Gossypium spp.)*, a major natural fiber, is one of the world's most significant cash crops and is grown-up commercially in over 50 international locations. It is belonging to the Malvacea family. Five countries included India, China, United States, Uzbekistan and Pakistan are the main cotton developing countries, with China maintaining the maximum production (1,265 kg/ha) (Anjum and Zia, 2020). During 2022-23, cotton area sown increased to 2,144 thousand hectares against 1,937 thousand hectares last year, revealing a growth of 10.7 percent. However, due to floods that swept away the entire crops in Sindh and Balochistan its production remained low at 4.910 million bales In Punjab, cotton producing districts Rajanpur, DG Khan and Taunsa were worst hit and damaged the cotton crop. Moreover, the insect pests, and weeds remained prevalent during the season. (Hussain, 2023) *Gossypium* has 50 recognized species, Forty Five diploid with Twenty Six chromosomes, arranged in 8 cytogenetic groups labeled AG (Chauhan and Mahajan, 2014).

Cotton provides the country's largest export revenue, and along with fluff, cottonseed for oil and flour accounts for 80 percent of the country's oilseed production. 10% of the nation's GDP and 55percent of its foreign exchange revenues come from cotton and items related to it (Naeem *et al.*, 2023).

Weeds in the field reduce both the quantity and quality of the crop. Weeds are considered undesirable plants that have a detrimental effect on the growth of desirable plants and reduce the productivity of those desirable plants (Choudhary *et al*., 2023). *Sorghum halepens* (L.) Pers. (Johnson grass), a commonplace weed in cotton fields, belongs to the Poaceae own family, is a perennial C4 glaminoid species, and is include one of the world worst weeds (Khan *et al*., 2024; Ramona *et al*., 2015). *Sorghum halepense* is known to have destructive consequences at the increase and improvement of neighboring vegetation. (Hussain *et al*., 2021; Uremis *et al*., 2009) Extensively stated in growing areas, it has triggered substantial yield reductions in economically crucial cotton vegetation (Nemr and Hammood 2023).

Allelopathy is occurrence in which plants inhibit the growth of other plants by releasing allelochemicals (Bennett and Klironomos 2019). An incidence when secondary metabolites generated by microbes and plants either promote or impede the growth and development of agricultural crops (Cheng and Cheng, 2015). Various plant sections release these substances into the environment through residual decomposition, root exudation, stem flow, and exudation (Khan *et al*., 2023).

Pseudomonas and other variety of vital microorganisms for agriculture, are utilized to control plant stressors and to promote plant growth. (Bouremani *et al*., 2023; Islam, Nain, Alam, and Banu, 2018). A diverse group of bacteria that mostly develops in the rhizosphere is known as bacteria that promote plant growth. Raouf *et al*., (2021). Pseudomonads can directly affect plant growth through a variety of ways, either by synthesizing chemicals that promote plant growth or by enabling the intake of nutrients from the environment (Kumawat *et al*., 2023; Viscardi *et al*., 2016). The purposes of this study to use *Pseudomonas fluorescene* (PF 083). as PGPR *and bioherbicide* to inhibit or control the cotton weed *sorghum halepense* allelopathic effects on the cotton seed growth.

Material and methods

Plant sample collection

Sorghum halepense plants were freshly harvested from fields, the margins of irrigation canals, and barren ground. Fresh, healthy leaves were provided after the plants had been cleansed twice or more with flowing tap water. Whole plants and separable leaves were dried for 3–4 days in the shade, ground into a fine powder with a metal blender, and kept in sealed bottles for extraction.

Preparation of Leaf Extract

I n preparation for leaf extraction, 100g of leaf powder was mixed with 150mL of distilled water and shake for 24 hours for even extraction. Aqueous extracts are kept at 4 °C in opaque bottles until use to prevent allelochemical deterioration.

Seeds sample collection

Healthy and vigorous seeds of (cotton) SS.32 was collected from Dost Seed Corporation. These seeds were in good physical shape, free of illness and in good state. Before use Seeds were sterilised with sodium hypochlorite (NaOCL) 2% (v/v) solution and rinsed with tap water incessantly to decrease any type of infection or to evade pollution. Prior to use these seeds were dipped in water for one day at room temperature.

Sample collection of PGPR

Pseudomonas ***fluorescene*** (PGPR) pure culture (PF-083) was collected from Department of Botany, Minhaj University Lahore.

Nutrient medium preparation for Bacteria

Nutrient medium broth was prepared by taking 250 ml of distilled water mixed by 5 g of dextrose, 5 g of calcium carbonate (CaCO3) and 5 gram yeast for bacterial growth. To prevent contamination, nutrient medium was autoclaved at 121°C for 30 minutes. After 30 minutes, *Pseudomonas fluorescene* bacteria (PF-083) were inoculated in a laminar flow, after the inoculation step was completed, the culture was kept in an incubator at 30° for 3-4 days.

Bacterial strain filtration

To acquire secondary metabolites, distilled water was used to filter bacterial cultures. The strain was filtered by using Whatman filter paper of grade 5 and micro filter paper 0.45 µm pore size is used to recover *Pseudomonas fluorescene*.

Laboratory experiment

The petri dish was washed with water to remove dust and dirt. The Petri dish was wrapped in aluminum foil and placed in a high temperature autoclave at 120 °C for 30 min to sterilize it. Assembled and split two sets of petri dishes. Set 1 included 11 treatments, T0 = control (distilled water), T1 = 5% foliar solution, T2 = 4.6% foliar solution, T3 = 4.2% foliar solution, T4 = 3.8% foliar solution , and T5 = 3.4% Leaf Solution T6 = 3% Leaf Solution T7 = 2.6% Leaf Solution T8 = 2.2% Leaf Solution T9 = 1.8% Leaf Solution T10 = 1.4% Leaf 5% Leaf Solution, T2 = 4.6% Leaf Solution, T3 = 4.2% leaf solution, T4 = 3.8% leaf solution and T5 = 3.4% Leaf Solution T6 = 3% Leaf Solution T7 = 2.6% Leaf Solution T8 = 2.2% Leaf Solution T9 = 1.8% Leaf Solution T10 = 1.4% Leaf Solution T11 = 1% Leaf Solution. Set 2 also has 11 treatments: T0+ = control (pathogen), T1 5% pathogen and leaf solution, T2 = 4.6% pathogen and leaf solution, T3 = 4.2% pathogen and leaf solution, T4 = 3.8% pathogen and leaf solution and T5 = 3.4% pathogen and leaf solution T6 = 3% pathogen and leaf solution T7 = 2.6% pathogen and leaf solution T8 = 2.2% pathogen and leaf solution T9 = 1.8% pathogen and leaf solution T10 = 1.4% pathogen and leaf solution T11 = 1% pathogen and leaf solution.

Pot preparation

A pot experiment was designed to access the impact of *S.halepense* and *Pseudomonas fluorescene* PF083 bacteria on cotton seeds (*G.hirsutum*). The same size pots were taken. The soil was sterilized with formalin and evenally filled in each pot. Soil used in this experiment was loam in texture with ph 7.8. Following soil filling, water was added in each pot to moistened the soil and left over night for uniform soil texture. Cotton seeds were coated with *Pseudomonas fluorescene* (PF-083) and keep soaked in in bacterial filtrate for one day in incubator at 30°C (Wu *et al*., 2012).

Pot experiment Assay

Pots were settled in two sets. Set 1 is negative and consist of only dry biomass of Johnson grass shoot of 0.5%, 1%, 1.5%, 2%, 2.5% and 3%. Set 2 positive consist of bacteria Pseudomonas *fluorescene* (PF-083) and dried powder of shoot 0.5%, 1%, 1.5%, 2%, 2.5% and 3%. Each set has same concentration of Johnson grass shoot dried powder.

Statistical analysis

Standard errors of mean in laboratory and pot bioassays trials were calculated using computer software Microsoft Excel. All the data were analyzed by analysis of variance (ANOVA) followed by LSD Test at 5% level of significance using computer software Statistics 8.1.

Results

In Vitro Bioassay

Pseudomonas (PF-083)'s bioherbicidal effects on cotton seed germination in the presence of *Sorghum halepense* Shoot allelopathy

In response to the bioherbicidal activity of *Pseudomonas spp*. (PF-083) and the allelopathic stress of *S. halepense* shoot, a significant amount of *Gossypium hirsutum* seeds germinated at ($P \leq 0.05$) as shown in Figure 4.1. Control (+ve) (95.3%) had a meaningfully greater germination percentage than Control (-ve) (81%). All -ve concentrations (1%, 1.4%, 1.8%, 2.2%, 2.6%, 3%, 3.4%, 4.2%, 4.6%, and 5%) altered with solely allelopathic *S. halepense* shoot significantly reduce the percentage of germination (66% to 23%) by boosting allelopathy. The percentage of germination increased (from 7% to 49%) when treated with the growth regulator Pseudomonas spp. (PF-083) at all positive concentrations (1%, 1.4%, 1.8%, 2.2%, 2.6%, 3%, 3.4%, 4.2%, 4.6%, and 5%). In comparison to 4.6% of negative treatments, the positive treatment group's cotton seed germination significantly increased (by 49.98%). According to Figure (Fig. 1), the other therapies were all significantly different from one another.

Pseudomonas (PF-083)'s bioherbicidal effects on cotton seedling length under the allelopathic influence of *Sorghum halepense* shoots

When *S. halepense* shoot stress was present, Pseudomonas species (PF-083) had a substantial effect on the length of *Gossypium hirsutum* seedlings at ($P \leq 0.05$) as shown in Figure 2. The seedling length of the control positive (3.1267 cm) was significantly increased (29.91%) over the control negative (2.4067 cm). The seedling length decreased significantly from 3.1067 cm to 3.033 cm due to the concentrations of all negative factors (1%, 1.4%, 1.8%, 2.2%, 2.6%, 3%, 3.4%, 4.2%, 4.6%, and 5%), which increased the *S. halepense* shoot allelopathy activity. The values for each concentration from all of the positive groups (1%, 1.4%, 1.8%, 2.2%, 2.6%, 3%, 3.4%, 4.2%, 4.6%, and 5%) lengthen seedlings modified with growth regulator (0.32% to 14.58%). Compared to 2% negative treatments altered with simply *S. halepense* shoot, the 2% positive treatment significantly lengthens seedlings (14.58%). Treatment's 4.6% and 5% negative and positive effects have no bearing on one another. The other treatments were all very distinct from one another and shown in graph (Fig. 2).

Pseudomonas (PF-083)'s bioherbicidal effects on cotton seedling weight under the allelopathic influence of *Sorghum halepense* shoots

S. halepense shoot pressure exerted on the newly planted *G. hirsutum* seedlings Pseudomonas sp. (PF-083) played a significant role as a bio herbicide at ($P \leq 0.05$). The seedling fresh weight of the positive control (6.333 g) was significantly increased over the negative control (5.667 g). The negative group of concentrations comprised solely with allelopathic *S. halepense* shoot extract (1%, 1.4%, 1.8%, 2.2%, 2.6%, 3%, 3.4%, 4.2%, 4.6%, and 5%) considerably reduce the seedling's fresh weight (5.667 g to 3.603 g) by enhancing the allelopathic activity. The seedling fresh weight increases (0.60% to 11.75%) with the addition of Pseudomonas sp. (PF-083) at concentrations of (1%, 1.4%, 1.8%, 2.2%, 2.6%, 3%, 3.4%, 4.2%, and 5%. The seedling length (11.75%) under the 2% positive treatment is significantly increased compared to the 2% negative treatment. Treatment 1.4%, 3.4%, 4.2% favourable and negative outcomes were not statistically significant. Each of these treatments varied greatly from the others presented in Figure (Fig. 3).

Pseudomonas (PF-083) bio herbicidal effects on cotton plant length under the allelopathic influence of *sorghum halepense*

Under the stress of *S. halepense*, Pseudomonas spp (PF-083) shown strong herbicidal effects on the cotton plant height in a pot at (P≤0.05). Cotton plants in the control -ve had a longer length (11.6 cm) than cotton plants in the control positive (13.16 g). The length of the control positive and negative cotton plants differed by 13.16%. The cotton plant length was significantly reduced by the concentrations of *S. halepense* changed (0.5%, 1%, 1.5%, 2%, 2.5%, and 3%) by increasing the movement of allelopathy. All positive absorptions were changed with a growth regulator. In comparison to each negative concentration, the dry biomass of plants increased from 0.5% to 1%, 1.5%, 2%, 2.5%, and 3% (1.644% to 26.654%). 2.5% concentration was observed to have a maximum dry weight percentage difference of 26.654%. Each treatment varied greatly from the others and revealed in (Fig. 4.).

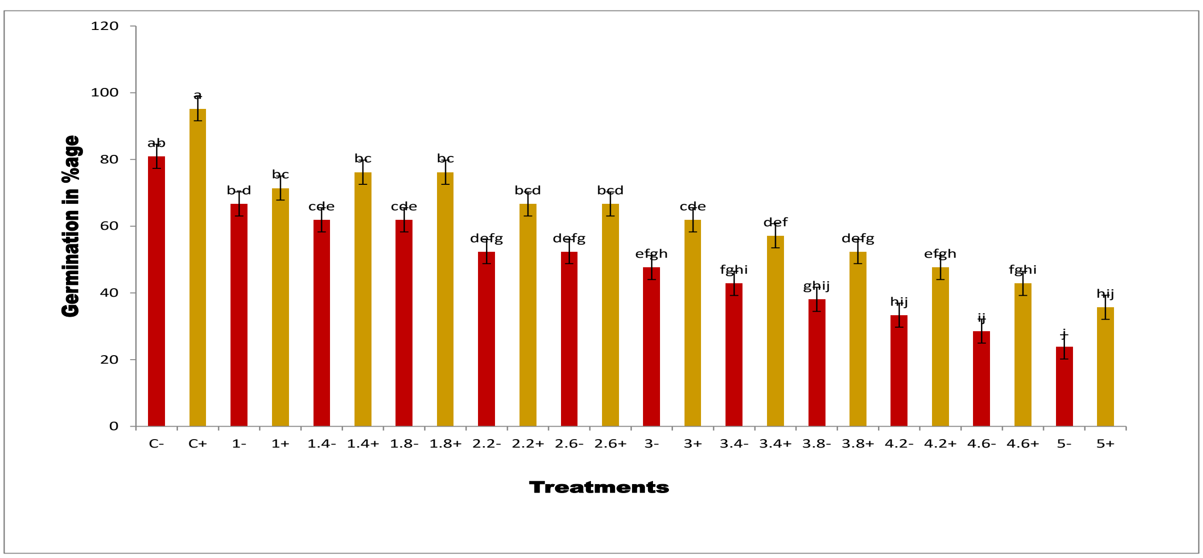

Figure 1: Consequence of Pseudomonas (PF-083) and aqueous shoot extract of *Sorghum halepense* on cotton (*Gossypium hirsutum*) germination (%). Different values reveal a considerable difference at the top of the bars (P≤ 0.05) as analyzed by LSD test

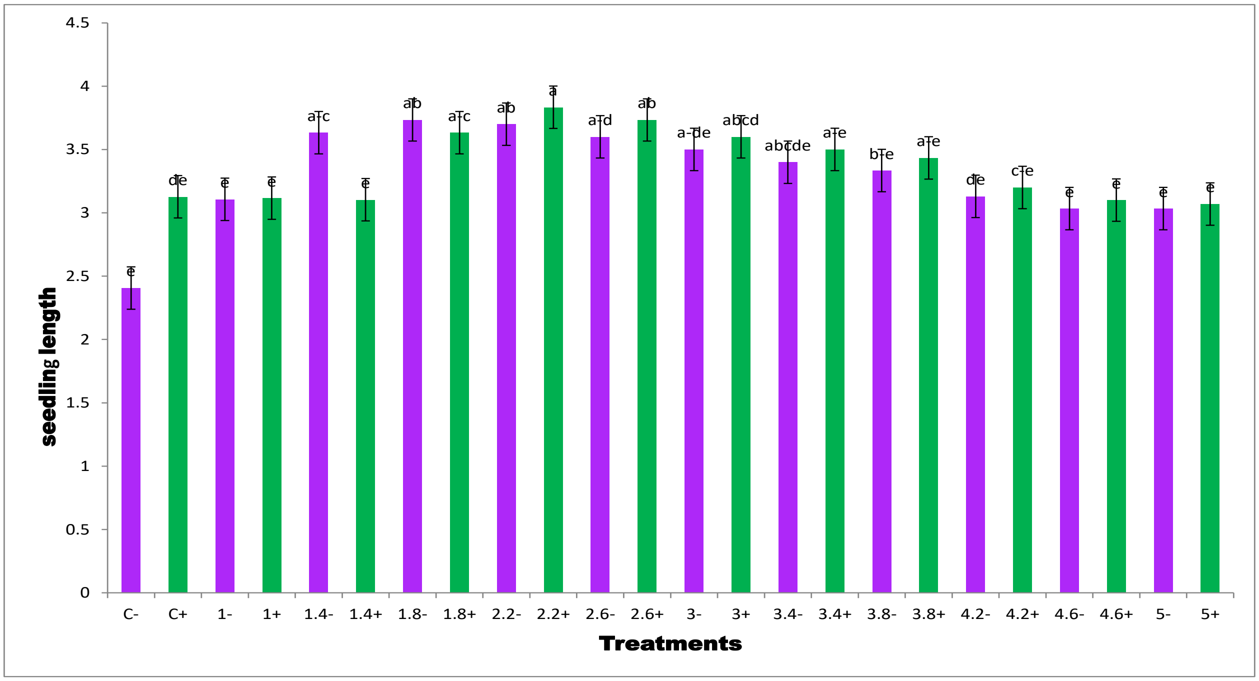

Figure 2: Consequence of Pseudomonas (PF-083) and aqueous shoot extract of *Sorghum halepense* on Cotton (*Gossypium hirsutum*) Seedling Length. Different values reveal a considerable difference at the top of the bars ($P \leq 0.05$) as analyzed by LSD test

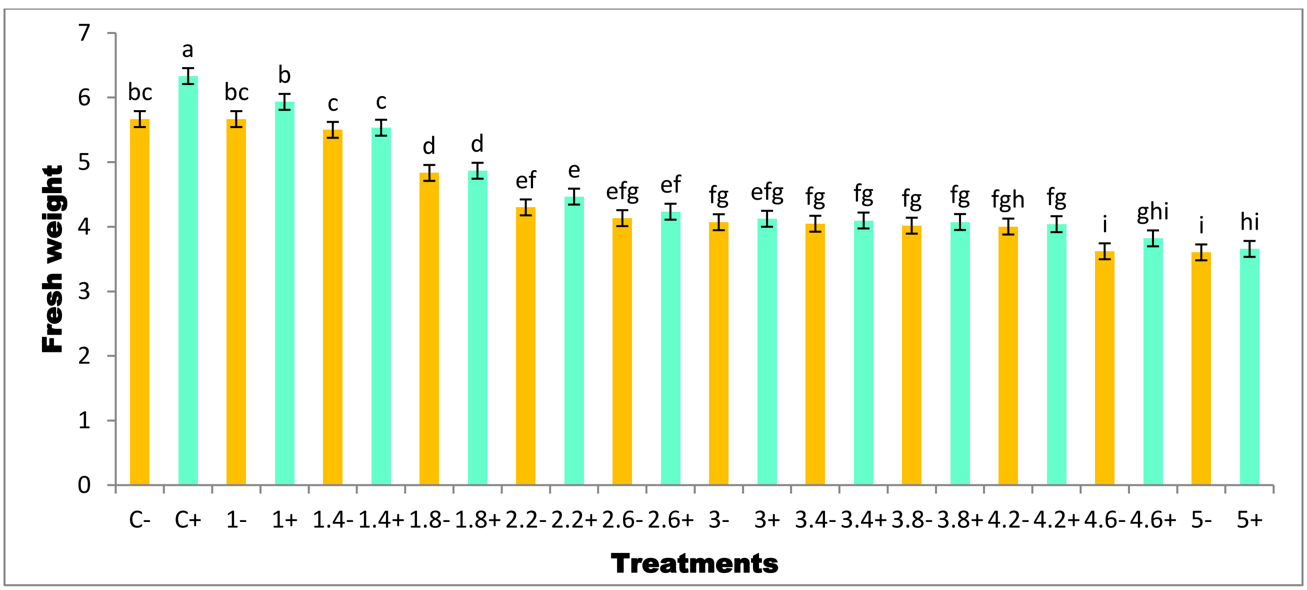

Figure 3: Effect of *Sorghum halepense* shoot extract and Pseudomonas (PF-083) on Cotton (*Gossypium hirsutum*) Seedling Fresh Weight. The values are displayed in full size differentiation at the top of the bars ($P \leq 0.05$) as analyzed by using LSD

Pot Bioassay

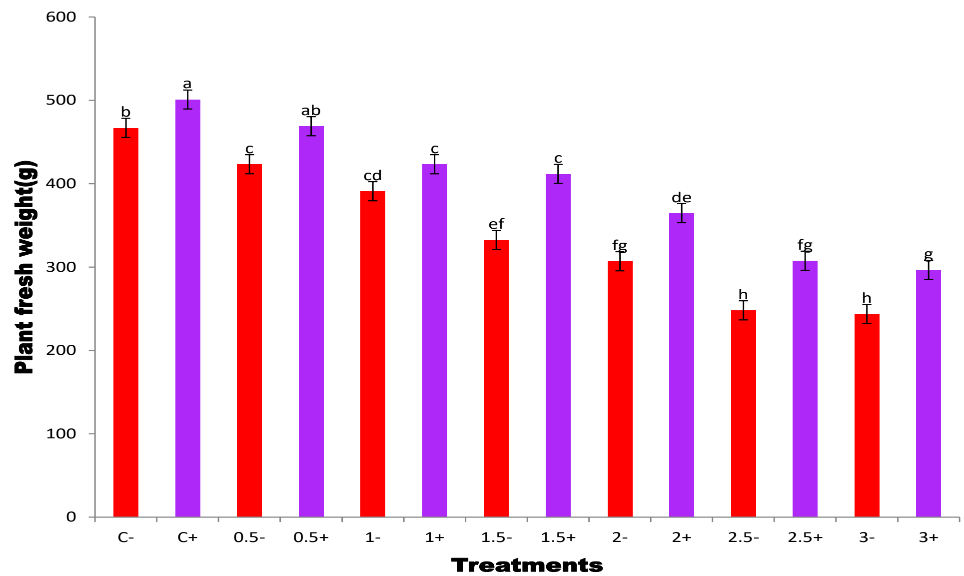

Figure 4: Effect of aqueous *Sorghum Halepense* and Pseudomonas (PF-083) shoot extract on cotton (*Gossypium hirsutum*) plant length. The values are displayed in full size differentiation at the top of the bars (P≤ 0.05) as examined by using LSD.

Fresh weight of the cotton plant Pseudomonas (PF-083) bio herbicidal activities under the allelopathic action of *sorghum halepense*

Under the stress of *S.halepense*, the bio herbicidal action of *Pseudomonas spp*. (PF-083) considerably increases the fresh weight *of Gossypium hirsutum* plant in pot at (P≤0.05). Cotton plants in the control -ve had a fresh biomass of 467 g, which was significantly greater than the control positive's 501 g. By increasing the activity of allelopathy, the applications changed with *S.halepense* from (3%, 2.5%, 2%, 1.5%, 1%, and 0.5%) significantly reduce the cotton plant fresh weight (469 g to 243.67 g). All positive concentrations were changed with a growth regulator. The fresh biomass of the plant increased from each negative concentration of (0.5%, 1%, 1.5%, 2%, 2.5%, and 3%) to (8.268% to 24.060%). Maximum percentage variation in fresh weight is 24.060% was observed 2.5% concentration. Each analysis varied greatly from the others, as indicated in (Fig. 5).

Pseudomonas (PF-083) bioherbicide effects on the cotton plant Dry weight under the allelopathic influence of *sorghum halepense*

Under the stress of *S.halepense*, Pseudomonas spp. (PF-083) demonstrated strong herbicidal properties on the dry biomass of cotton plants at (P≤0.05). Cotton plants in the control -ive had a dry biomass that was much greater (87.33 g) than that of the control positive (102 g).

The dry weight of the cotton plant is significantly reduced by the concentrations changed with *S.halepense* (0.5%, 1%, 1.5%, 2%, 2.5%, and 3%) by growing the activity of allelopathy. All +ive concentrations were changed with a growth regulator. In comparison to each negative concentration, the dry biomass of plants increased from (0.5%, 1%, 1.5%, 2%, 2.5%, and 3% to 16.79% to 40.157%). 2.5% concentration was found to have the largest dry weight percentage change, 40.157%. Each treatment varied greatly from the others and revealed in (Fig. 6).

Discussion

Allelopathic substances are to blame for the slowing down of cell division and cell elongation, the reduction of photosynthesis, and the uptake of water and nutrients and the reduce protein metabolisms and the translational process (Khangura *et al*., 2023). Allelopathy can function in some ways as a stressor, affecting the species and causing a significant number of allelochemicals to be produced by that species. It is an amazing characteristic of some plants to exert allelopathic pressure on rival plants .The term "allelopathic plants" refers to these. This might raise the possibility of allelopathic pyramid. The highly allelopathic species respond to environmental stress from other plant species much less (Choudhary *et al*., 2023)

The recent study, which found that cotton's development and germination were reduced, suggests that sorghum's allelochemicals may have an impact on cotton's leaves curling, roots growing, and the vital method of transpiration (Asgharipour and Heidari, 2011). *Sorghum halepense* is regarded as one of the most hazardous and destructive weeds (Ravlic *et al*., 2016). In many ways, this weed is damaging to other plant species. *Sorghum halepense* contains the following chemical compounds most frequently: phenolic acid derivative’s and chlorogenic acid, P-hydroxybenzeldehyde, P Coumaric acid, sorgoleone, and di-hydrosorgoleone (Matković, *et al*., 2018). Movahedpour *et al*. (2010) found that the most crucial enzymes' activity is lowered or inhibited by chlorogenic acid, which is contained in *sorghum halepense*. The primary one is (-phosphorylase), an enzyme involved in seed germination . The primary plant disasters caused by weeds are the production of allelopathic chemicals and phytotoxins, which serve as explosives to the most crucial plant components, including Shoot growth reduction, root rotting and decaying of plants sections (Zheng and Xu 2023). Weeds release compounds that lower photosynthetic rate , lower chlorophyll synthesis occurs owing to reduced light absorption and cause a significant slow growth of the affected plant's and decrease the shoot weight and shoot length (Peerzada *et al*., (2019). Additionally, the regulation of stomata opening is the most significant mechanism impacted by allelochemicals. This is because CO_2 concentrations have changed, as documented in *Vicia faba*. (Rai *et al*., 2003). Damage to the chlorophyll has also been noted as a significant contributor under allelopathic conditions. (Lorenzo *et al*., 2011).

ISSN PRINT 2710-4079
ISSN ONLINE 2710-4087

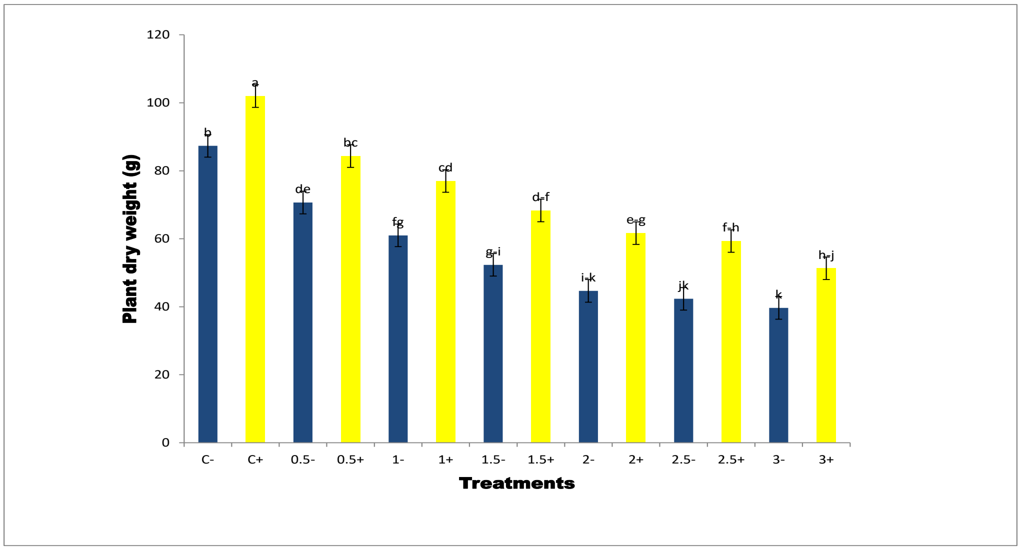

Figure 5: Effect of Pseudomonas (PF-083) and shoot extract of *Sorghum halepense* on fresh cotton (*Gossypium hirsutum*) weight. Extraordinary values are displayed in full size differentiation at the top of the bars (P≤ 0.05) as analyzed by using LSD

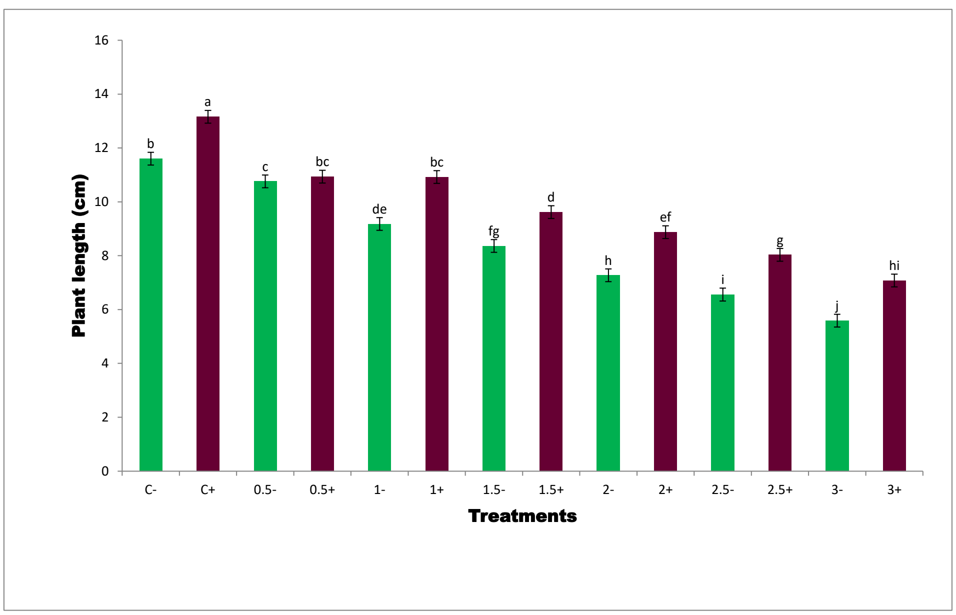

Figure 6: Outcome of Pseudomonas (PF-083) and aqueous Sorghum halepense shoot extract on Cotton (*Gossypium hirsutum*) Dry Weight. Different values reveal a considerable difference at the top of the bars (P≤ 0.05) as investigated by LSD test

The activity of allochemicals could not be clarified or explained by a single mechanism. The activity of allelochemicals stops basic plant activities like seed germination and seed growth. Additionally, many other biochemical, morphological, physiological, and anatomical problems in plants occur when allelochemicals interact with cellular and molecular processes (Kong *et al*., 2019). Huang *et al*. (2015) found that *Sorghum* weeds are widespread in cotton crop areas and frequently have an adverse impact on the growth and development of plants. *Sorghum* is a very strong competitor plant that competes for resources in fields and stresses crops in an allelopathic way when it grows in cotton fields. Ashrafi *et al*. (2009) and Xia *et al*. (2019) clarified that the presence of allelochemicals is the primary cause of the decrease in plant development.

It is also observed that the results of the recent study demonstrated that spraying plant roots and shoots with shoot extract were very effective or capable (Samad *et al*., 2023). The chemical sorgoleone, which is found in *sorghum*, can significantly reduce the height, leaf area, and weight of plants that come into contact with *sorghum* weeds in natural settings. Sorgoleone carries out many tasks in various ways. Sorgoleone has numerous deals has been shown in the most recent laboratory investigation (Jamro *et al*., 2022).

Conclusion

According to the results of the current study, the length, germination rate, and fresh and dried seedlings were all more inhibited by *sorghum* leaf extract. This study discovered that *sorghum* leaf extract significantly decreased the germination rate, length, and biomass of fresh and dried cotton seedlings. Cotton seed leaf germination rate was enhanced by *sorghum halepense* leaf extract Pseudomonas *fluorescene* (PF-083) PGPR enhanced cotton leaf germination, seedling length, and fresh and dry biomass can be utilized as bioherbicide. Evaluation of allelochemicals and their separation, identification, release, and translocation in field circumstances is crucial.

Conflicts of interest

There is no conflict of interest related to this article.

Author's contribution

All authors have contributed equally

Funding statement

There is no funding to show

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