

ENHANCING OF *CANDIDA TROPICALIS* AND THEIR POTENTIAL ON VEGETABLE SOYBEAN CV. CHIANG MAI 84-2 GROWTH AND YIELD AS INOCULANT BIOFERTILIZER

R. D. S. Risman
Researcher

K. Sungthongwises*
Assoc. Prof.

Agronomy Section, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand

* Correspondence: skiriy@kku.ac.th; Tel.: +66-0903241635

ABSTRACT

Since phosphorus (P) is lower in agricultural areas with acid sandy soil, it runs out in different parts of crop plants after harvesting. To boost growth and development in the next cropping system, only overuse of inorganic phosphate fertilizers can adversely affect agricultural sustainability. Phosphate-solubilizing fungi like *Candida tropicalis* play a role in plant P nutrition through soil P dynamics and growth hormones. Three PSF isolates (Sw01, Pu02, and Pu04) from bulk soil of fresh corn were tested for optimal media and incubation times. The results showed that Sw01 produced the highest population of 593.33×10^8 CFU L⁻¹ when cultured in TB media after five days. These PSFs were used to investigate the potential of *C. tropicalis* on yield components, chlorophyll pigments, and nutritional value of vegetable soybean cv. Chiang Mai 84-2. The result demonstrated that applying 50% inorganic fertilizer mixed with *C. tropicalis* enhanced leaf area, root length at the vegetative stage, and chlorophyll a and total chlorophyll contents at the flowering and harvest stage. This application increased pod fresh weight to 11,214 kg ha⁻¹, with an average of 28 pods plant⁻¹ and 47.50 seeds plant⁻¹ compared to fertilizer managements.

Keywords: auxin, economic yield, green bean, phosphate solubilizing fungi, phosphorus

ريسمان و سونغثونغويسيس

مجلة العلوم الزراعية العراقية- 2025 56: (2) 677-691

Chiang Mai 84-2 كسماد حيوي تلقيحي *Candida tropicalis* على نمو وإنتاجية فول الصويا النباتي صنف

كيريا سونغثونغويسيس
أستاذ مشارك

ريري ديانغ ساري ريسمان
باحث

المستخلص

نظراً لأن الفوسفور منخفض في المناطق الزراعية ذات التربة الرملية الحمضية، فإنه ينفد في أجزاء مختلفة من نباتات المحاصيل بعد الحصاد. لتعزيز النمو والتطور في النظام الزراعي التالي، يمكن أن يؤدي الإفراط في استخدام الأسمدة الفوسفورية غير العضوية إلى تأثيرات سلبية على استدامة الزراعة. تلعب الفطريات القابلة للذوبان في الفوسفور مثل *Candida tropicalis* دوراً في تغذية النبات بالفوسفور من خلال ديناميكيات الفوسفور في التربة وهرمونات النمو. تم اختبار ثلاث عزلات من الفطريات القابلة للذوبان في الفوسفور (Sw01 و Pu02 و Pu04) من التربة الأصلية للذرة الطازجة لتحديد الوسائط وأوقات الحضانة المثلى. أظهرت النتائج أن Sw01 أعلى عدد سكاني بلغ 593.33×10^8 وحدة تشكيل مستعمرات لكل لتر (CFU L⁻¹) عندما تم زراعتها في وسط TB بعد خمسة أيام. تم استخدام هذه الفطريات لدراسة إمكانات *C. tropicalis* في مكونات المحصول، وصبغات الكلوروفيل، والقيمة الغذائية لفول الصويا النباتي من صنف Chiang Mai 84-2. أظهرت النتيجة أن تطبيق 50% من الأسمدة غير العضوية مختلطة مع *C. tropicalis* عززت مساحة الأوراق، وطول الجذور في المرحلة النباتية، وكمية الكلوروفيل أ وإجمالي الكلوروفيل في مرحلة الإزهار ومرحلة الحصاد. هذا التطبيق زاد من وزن القرون الطازجة إلى 11,214 كجم/هـ، مع متوسط 28 قرناً لكل نبتة و 47.50 بذرة لكل نبتة مقارنة بإدارة الأسمدة

الكلمات المفتاحية: الأوكسين، العائد الاقتصادي، الفاصوليا الخضراء، الفطريات المذيبة للفوسفات، الفوسفور

Received:12/7/2024, Accepted:19/11/2024

INTRODUCTION

Vegetable soybean is popularly known as edamame in Japan, maodou in China, and green soybean in North America. The immature pods are boiled and the seeds are highly nutritious snack food. Nowadays, vegetable soybean demand is increasing as gains recognition for its nutritional value, paving the way for the expansion of the crop in developing and developed countries. China, Japan, Taiwan, Thailand, Indonesia, and Vietnam are the major vegetable soybean-producing countries (26). Vegetable soybeans are rich in protein (13%), cholesterol-free oil (5.7 %), phosphorus (158 mg 100 g⁻¹), calcium (78 mg 100 g⁻¹), vitamin B1 (0.4 mg 100 g⁻¹) and B2 (0.17 mg 100 g⁻¹). They also contain isoflavones, and vitamin E (21). Phosphorus (P) is an important nutrient for seed emergence and reproductive growth after nitrogen (N) plays a role in plant metabolism, structure, and energy transformation at vegetative growth (28). Plant metabolism as cellular transfer, respiration, and photosynthesis of the crop being involved with P. Plant up take P as either H₂PO₄⁻ or HPO₄²⁻ (orthophosphate ions), depending on soil pH (23). A phosphorus deficiency can lead to stunt crop growth rate by poor root development, delayed maturity, and decreased yield. Therefore, ensuring an adequate supply of P is crucial for optimizing plant growth and productivity. However, the availability of P is a serious issue due to its fixation and precipitation behavior in soil which lowers the efficiency of added P (45). Most P in the soil is tied up in an unavailable form for crop growth. The amount of available P for plant uptake is low compared to the total P in the soil. For example, total soil P exceeds 896.43-1,120 kg ha⁻¹, but the available P in soil solution might only be 0.043-0.143 kg ha⁻¹ (28). Phosphorus compounds more than 80% are immobile and are not readily soluble in soils which is not easily accessible for plant growth. Phosphorus is sequestered by adsorption to the soil surface and precipitation by reaction with soil cations, particularly iron (Fe), aluminum (Al) and calcium (Ca) (44). The response of soybeans to P primarily depends on the amount of available P in the soil. Shahid et al. (32) reported that the

application of P 100 kg P₂O₅ ha⁻¹ significantly increases the grain yield of soybeans. Using phosphate solubilizing microorganisms (PSMs), such as bacteria, fungi, and yeast in agricultural ecosystems is viewed as an economical and energy-efficient strategy to improve the effectiveness and efficiency of various crops under harsh environments (4, 5, 6, 8, 15, 47). In particular; solubility of P fertilizers (7, 46, 48, 49, 50). In soils many species of microorganisms have been discovered, particularly in the rhizosphere of plant growth such as *Trichoderma harzianum* (CCTCC-RW0024), *Penicillium* sp. and *Aspergillus niger* (39), *Sarocladium zeae* sp. (TS-ASV) strains (11), *Candida tropicalis* isolated from the rhizosphere of rice (2). *Candida tropicalis* is important in utilizing easily available and more complex litter-derived carbon (C), more efficiently than bacteria, thus contributing actively to soil formation (40). Additionally, it can produce plant growth-promoting hormones that influence plant germination, root and shoot development, xylem differentiation, and flowering (30). Furthermore, it adapts to adverse abiotic conditions, including salinity, drought, heavy metals, and extreme pH (27). Studies have shown that *C. tropicalis*, particularly the non-pathogenic strain NCIM 3321, can enhance phosphorus availability in soil by producing extracellular phytase, thus promoting plant growth (29). *C. tropicalis* has been successfully used as a biofertilizer in various crops, including potatoes (17), soybeans (24), sugar beet (1), and rice (2). Furthermore, its rhizosphere strain *C. tropicalis* HY, included in the commercial biofertilizer BioGro, has positive on rice growth and nitrogen nutrition (13, 19). Optimizing the growth media and incubation time is crucial for cultivating PSF agriculture research. The right medium is essential, providing nutrients for *C. tropicalis* growth and activities. Common media used to cultivate, and study the phosphate solubilizing capabilities of PSF in the laboratory include Glucose-Phosphate Agar (GPA), Luria-Bertani (LB), Nutrient Broth (NB), Tryptic Soy Broth (TB), and Yeast Malt Broth (YMB) (25, 43). Equally important is finding the optimal

incubation time, as PSF grows more slowly than bacteria and often needs a longer incubation period. Depending on the fungal species and conditions, PSF typically takes 5-14 days to reach optimal growth and the fungi thrive at temperatures between 25-30°C. Phosphate solubilizing bacteria (PSB) showed 1-50% of the total microbial population, while phosphate solubilizing fungi (PSF) was found 0.1-0.5% (12). Bononi et al. (10) reported that the application of P 70 kg ha⁻¹ with *Trichoderma* spp (AMS 34.39) can increase the biomass of soybean plants. In addition, applying PSF can increase soybean growth from 2% to 41% with P uptake efficiency up to 141%. This enhances the availability of P to the soybean plants, potentially improving their growth and yield. Chiang Mai 84-2 (CM84-2) is a vegetable soybean cultivar released in 2012 by the Chiang Mai Field Crops Research Center (CMFCRC) (10). The Chiang Mai 84-2 is the first Thailand vegetable soybean variety in which pod has met the export standard. In addition, this variety provides pandan flavor while tasting the boiled pod and has been recommended to farmers for vegetable soybean production (20). This study aimed to investigate the potential of *C. tropicalis* on the growth and yield components of vegetable soybean cv. Chiang Mai 84-2. By assessing the effects of *C. tropicalis* on vegetable soybean growth and productivity in pot condition, this research seeks to provide valuable insights into the potential benefits of *C. tropicalis* in vegetable soybean production systems.

MATERIALS AND METHODS

Enhancing of PSF population through different media and incubation time:

Phosphate solubilizing fungi isolates (Sw01, Pu02, and Pu04) from bulk soil after growing sweet and purple waxy corn were used to determine the ability to grow on various culture media and incubation time. The following conditions were evaluated: 1) Five media formulas including Glucose Peptone Agar (GPA), Luria-Bertani (LB), Nutrient Broth (NB), Tryptic Broth (TB), and Yeast Malt Broth (YMB), and 2) The incubation times of 3, 5, 7 and 10 days. Each liquid media was prepared according to standard protocols

and sterilized by autoclaving at 121°C for 20 minutes (43). Erlenmeyer flasks containing 50 ml of the different media with PSF isolates were incubated for 3, 5, 7, and 10 days at 30 °C in an incubator shaker at 150 cycles min⁻¹. After incubation, aliquots of each dilution were diluted and spread over the NBRIP solid media with three replications. Samples were incubated at 25 °C for 5-7 days and colony-forming units (CFUs) were counted to determine PSF population density. Identification of PSF isolates was analysed by Thailand Bioresource Research Center (TBRC) National Center for Genetic Engineering and Biotechnology. The reports showed that Sw01, Pu02 and Pu04 were similarity with type strain of *C. tropicalis*.

Pot experiment

To determine the effect of *C. tropicalis* on vegetable soybean growth and yield, *C. tropicalis* inoculum was carried out in greenhouse conditions. Pots size with diameters top and bottom of 12 cm and 9 cm respectively, with a height of 10 cm, and a volume of 848.60 cm³ were used at germination until one month after germinated, with planting media only soil. One month after germination plant was transferred to the big pot with diameters top and bottom of 25 cm and 18 cm respectively, with a height of 19 cm, and a volume of 6,958.89 cm³. The planting media was sterilized in an autoclave at 121 °C for 20 min before filled in plastic pots (31). The treatments were: 1) Control (H₂O); 2) PSF solution (10⁸ CFU l⁻¹); 3) Plant growth promoting rhizobacteria (PGPR); 4) 100% inorganic fertilizer (152.50 kg N ha⁻¹ + 80.63 kg P ha⁻¹ + 95.63 kg K ha⁻¹); 5) 50% inorganic fertilizer + PSF solution and 6) 25% inorganic fertilizer + PSF solution, with three replication. Chang Mai 84-2 seeds were sterilized with 5% sodium hypochlorite (NaOCl) for 10 min and cleaned with sterile water 3 times. Three seeds were transferred into the pot for germination. Plants are watered with 400 ml pot⁻¹ uniformly, to raise the moisture content of the soil to field capacity (42). Phosphate solubilizing fungi inoculum was added two times per week until harvested while inorganic fertilizer was used at 1 week, 3 weeks, and 6 weeks after germination. Data

collection of plant height, number of leaves, SPAD value, leaf area, root length, number of roots were carried out for 2 weeks, 1 month, 45 days after germination (reproductive stage) and dry weight of leaves, stems and roots were carried out at 45 days after germination (reproductive stage), chlorophyll a, b, total chlorophyll and carotenoid were carried out at 45 days after germination (reproductive stage) and harvest stage.

Determination of chlorophyll pigments

The photosynthetic parameters were determined by measuring Total Chlorophyll (TC, chl a + chl b) (1), chlorophyll a (chl a) (2), chlorophyll b (chl b) (3), and carotenoid (car) as described by (3). Determination of chlorophyll pigments by weighting the leaf samples 0.1 g, then the samples were grinding with 10 ml of 80% acetone using mortar and pestle. The solution was filtered with filter paper Whatman No.1 and the total extraction volume (V) was recorded and then put in a glass test tube. The absorbance was measured at wavelengths OD440, OD645, and OD663 using a UV-Vis spectrophotometer (Model i3, Jinan Hanon Instruments Co., Ltd, China) and 80% acetone as a blank. The determination of photosynthetic pigments was calculated using the equations as described by Arnon (28) and carotenoid (car) was calculated using the equations by Bajracharya (9). All the photosynthetic pigment contents were expressed in mg gFW⁻¹. The equations were calculated as follows:

$$\text{Total Chlorophyll (mg gFW}^{-1}) = \frac{[20.2(\text{OD645}) + 8.02(\text{OD663})]}{(100 \times V)} \quad (1)$$

$$\text{Chlorophyll a (mg gFW}^{-1}) = \frac{[12.7(\text{OD663}) + 2.69(\text{OD645})] \times V}{(1000 \times W)} \quad (2)$$

$$\text{Chlorophyll b (mg gFW}^{-1}) = \frac{[22.9(\text{OD645}) + 4.68(\text{OD663})] \times V}{(1000 \times W)} \quad (3)$$

$$\text{Carotenoid (mg gFW}^{-1}) = \frac{[4.69(\text{OD440}) - 0.268(20.2 \times \text{OD645}) + (8.02 \times \text{OD663}) \times V]}{(1000 \times W)} \quad (4)$$

Where,

V = volume of 80% acetone (mL)

W = sample fresh weight (g)

Statistical analysis

The research data were analyzed using the analysis of variance (ANOVA) performed for laboratory work and pot experiments with three replicates per treatment. A factorial in a completely randomized design was used for the laboratory work. A model of a completely randomized design (CRD) was used for pot experiments. The Statistix10 program software was used to conduct the least significant difference test (LSD) at $P \leq 0.05$ (37).

RESULTS AND DISCUSSION

Enhancing of PSF population by media and incubation time: Different media and incubation times were tested to increase the population of PSF. The results showed a significant difference in the PSF population, the population of Sw01 isolate within TB media showed the highest of 215.00×10^8 CFU l⁻¹ while Pu02 and Pu04 isolates within LB media showed the maximum population of 36.67 and 28.33×10^8 CFU l⁻¹ respectively (Table 1). The incubation time for 5 days enhance the higher Sw01 population than other times. On the other hand, the incubation time for 7 days showed the highest Pu02 and Pu04 population. The interaction between media and incubation time showed significant different of the PSF population. Sw01 isolate showed the highest population of 593.33×10^8 CFU l⁻¹ when culture in TB media for 5 days. In addition, Pu02 and Pu04 isolates showed the highest population of 146.67 and 113.33×10^8 CFU l⁻¹ after culture in LB media for 7 days respectively.

Table 1. The population of PSF isolates in different media and incubation time

Treatment	The population of PSF×10 ⁸ (CFU) l ⁻¹		
	Sw01	Pu02	Pu04
Media (M)			
GPA (1)	10.83 ^b	20.83 ^b	0.00 ^b
LB (2)	18.33 ^b	36.67 ^a	28.33 ^a
NB (3)	28.33 ^b	0.00 ^c	22.50 ^a
TB (4)	215.00 ^a	0.92 ^c	0.83 ^b
YMB (5)	17.50 ^b	2.50 ^c	0.00 ^b
F-test	**	**	**
Time (D)			
3 days (1)	76.00 ^{ab}	1.33 ^b	0.00 ^b
5 days (2)	128.67 ^a	0.00 ^b	0.00 ^b
7 days (3)	20.00 ^b	46.06 ^a	40.67 ^a
10 days (4)	7.33 ^b	1.33 ^b	0.66 ^b
F-test	**	**	**
Media × Time			
M1 × D1	13.33 ^c	0.00 ^c	0.00 ^c
M1 × D2	0.00 ^c	0.00 ^c	0.00 ^c
M1 × D3	30.00 ^c	83.33 ^b	0.00 ^c
M1 × D4	0.00 ^c	0.00 ^c	0.00 ^c
M2 × D1	73.33 ^c	0.00 ^c	0.00 ^c
M2 × D2	0.00 ^c	0.00 ^c	0.00 ^c
M2 × D3	0.00 ^c	146.67 ^a	113.33 ^a
M2 × D4	0.00 ^c	0.00 ^c	0.00 ^c
M3 × D1	23.33 ^c	0.00 ^c	0.00 ^c
M3 × D2	23.33 ^c	0.00 ^c	0.00 ^c
M3 × D3	56.67 ^c	0.00 ^c	90.00 ^b
M3 × D4	10.00 ^c	0.00 ^c	0.00 ^c
M4 × D1	256.67 ^b	0.00 ^c	0.00 ^c
M4 × D2	593.33 ^a	0.00 ^c	0.00 ^c
M4 × D3	10.00 ^c	0.33 ^c	0.00 ^c
M4 × D4	0.00 ^c	3.33 ^c	3.33 ^c
M5 × D1	13.33 ^c	6.67 ^c	0.00 ^c
M5 × D2	26.67 ^c	0.00 ^c	0.00 ^c
M5 × D3	3.33 ^c	0.00 ^c	0.00 ^c
M5 × D4	26.67 ^c	3.33 ^c	0.00 ^c
F-test	**	**	**

Enhancing the population of PSF involves optimizing various factors, including the type of media and incubation time. Our result found that TB media provided the higher

population of Sw01 isolate at 215.00×10⁸ CFU l⁻¹ than others media. Tartoff and Hobbs (38) reported that TB media is designed to be nutrient rich, providing ample resources for

the growth of a wide variety of microorganisms, including fungi. The presence of essential nutrients such as amino acids, vitamins and minerals supports rapid fungal proliferation. Fungi are highly adaptable organisms capable of thriving in a variety of environments. On the other hand, Pu02 and Pu04 isolates within LB media showed the maximum population of 36.67 and 28.33×10^8 CFU l^{-1} respectively. Wang et al. (43) studied that the nutrient composition, concentration and culture conditions of LB medium can influence the growth of microbial strains, commonly used for bacterial culture medium in the laboratory. While the incubation time for 5 days enhance the higher Sw01 isolate population than other times. In addition, the incubation time for 7 days showed the highest Pu02 and Pu04 population. Lacaz (22) evaluated that the exponential growth of *Paracoccidioides brasiliensis* occurs between 5 and 7 days of incubation in a complete medium, this period allows fungi to go through the lag phase, exponential phase, and reach the stationary phase where the population stabilizes at its maximum density. During this time, fungi can utilize the available nutrients and grow to their fullest potential. Opposite to

Cruz et al. (14) there was not sufficient growth after 7 days of incubation for several *Paracoccidioides* isolates, and better results were obtained after 15 days of incubation for all of the experimental conditions when initial inoculum of $105 \text{ cells ml}^{-1}$ were used.

The effects of *C. tropicalis* solution on vegetable soybean growth in pot experiments

The results from pot experiments showed that leaf area and root length were significantly different at two weeks after germination (Figure 1). While, plant height, SPAD value, and No. of root plant⁻¹ showed no significant difference. The application of *C. tropicalis* showed the highest significant difference in leaf area of 668.00 cm^2 and root length of 26.82 cm . Furthermore, the growth at one month of vegetable soybean showed a significant difference in all growth parameters (Figure 2) especially, plant height, leaf area, and No. of root plant⁻¹ increase from two weeks. The application of *C. tropicalis* and PGPR showed significant differences in No. of root plant⁻¹ at 24.75 and 23.00 respectively. In addition, vegetable soybeans under *C. tropicalis* with and without inorganic fertilizer application showed the highest significant difference in leaf area.

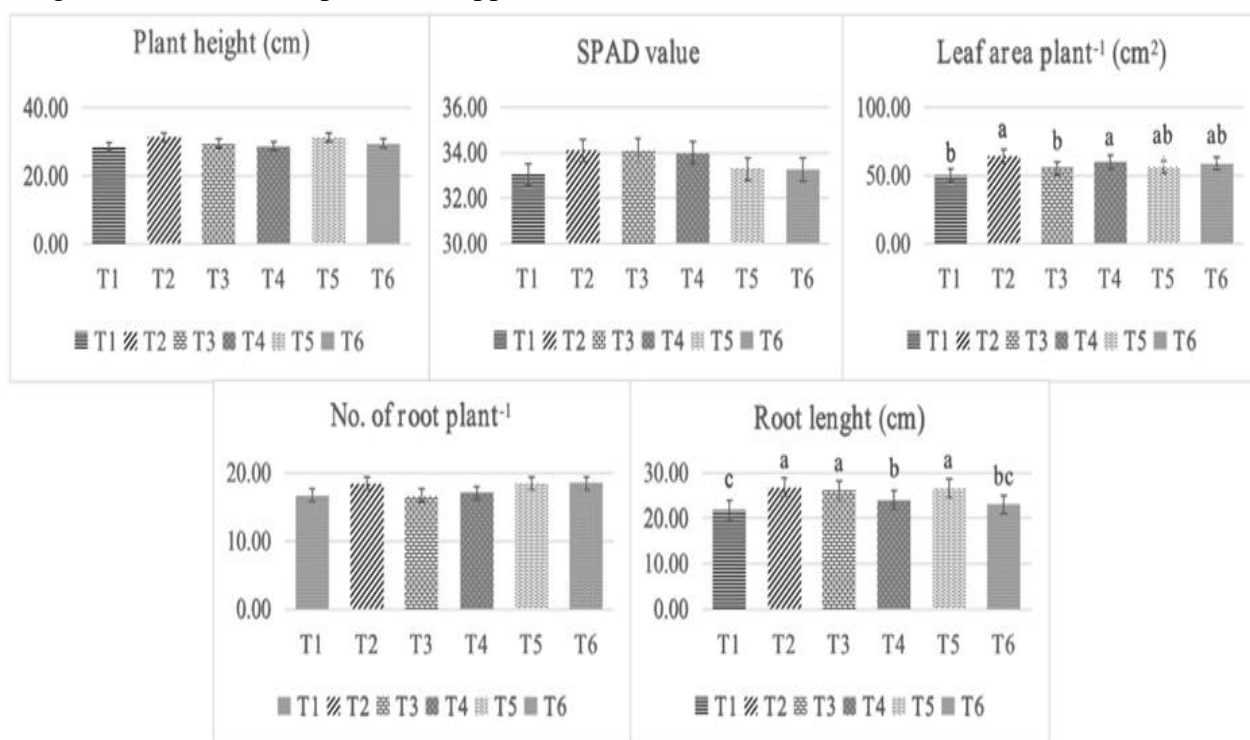


Figure 1. Plant growth of vegetable soybean at two weeks after germination

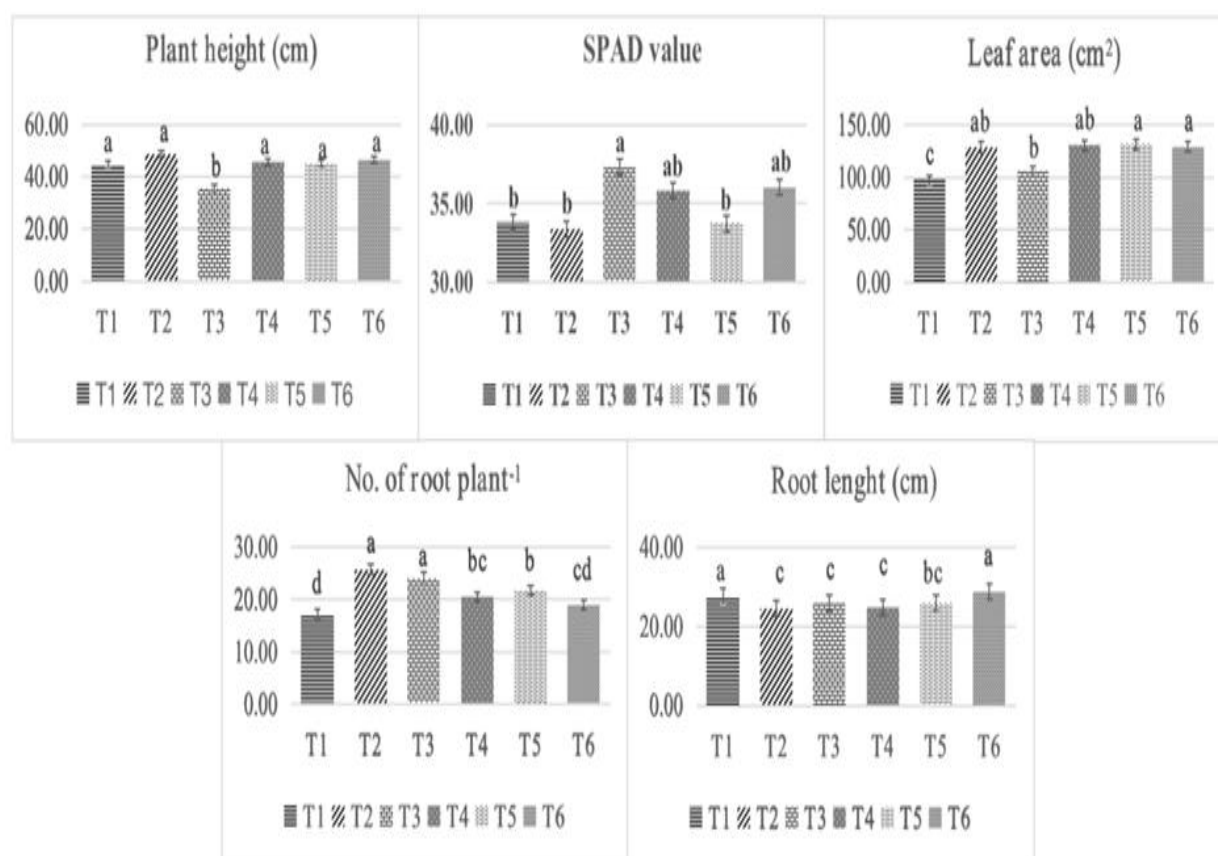


Figure 2. Plant growth of vegetable soybean at one month after germination

Plant growth and photosynthetic pigments of vegetable soybean at reproductive stage

The results indicate that significant differences were observed in plant height, leaf area, the No. of roots plant⁻¹, and root dry weight (Figure 3). While, plant height, leaf area, No. of root plant⁻¹, and root dry weight increased from one month. The application of only *C. tropicalis* solution and 50% inorganic fertilizer + *C. tropicalis* showed the highest significant differences in plant height, leaf area, and root dry weight and not significant differences from 100% inorganic fertilizer application. Regarding the photosynthetic pigments of

vegetable soybean (Figure 4), significant differences were found in chlorophyll a (Chl a) and total chlorophyll (TC) contents, while chlorophyll b (Chl b) and carotenoid (Car) contents did not show significant differences. The combination of 25 and 50% inorganic fertilizer + *C. tropicalis* showed the highest chlorophyll a and total chlorophyll compared to other treatments. This finding is consistent with the observation of enhanced plant growth at the reproductive stage, where the highest leaf area was recorded under the 25 and 50% inorganic fertilizer + *C. tropicalis* application.

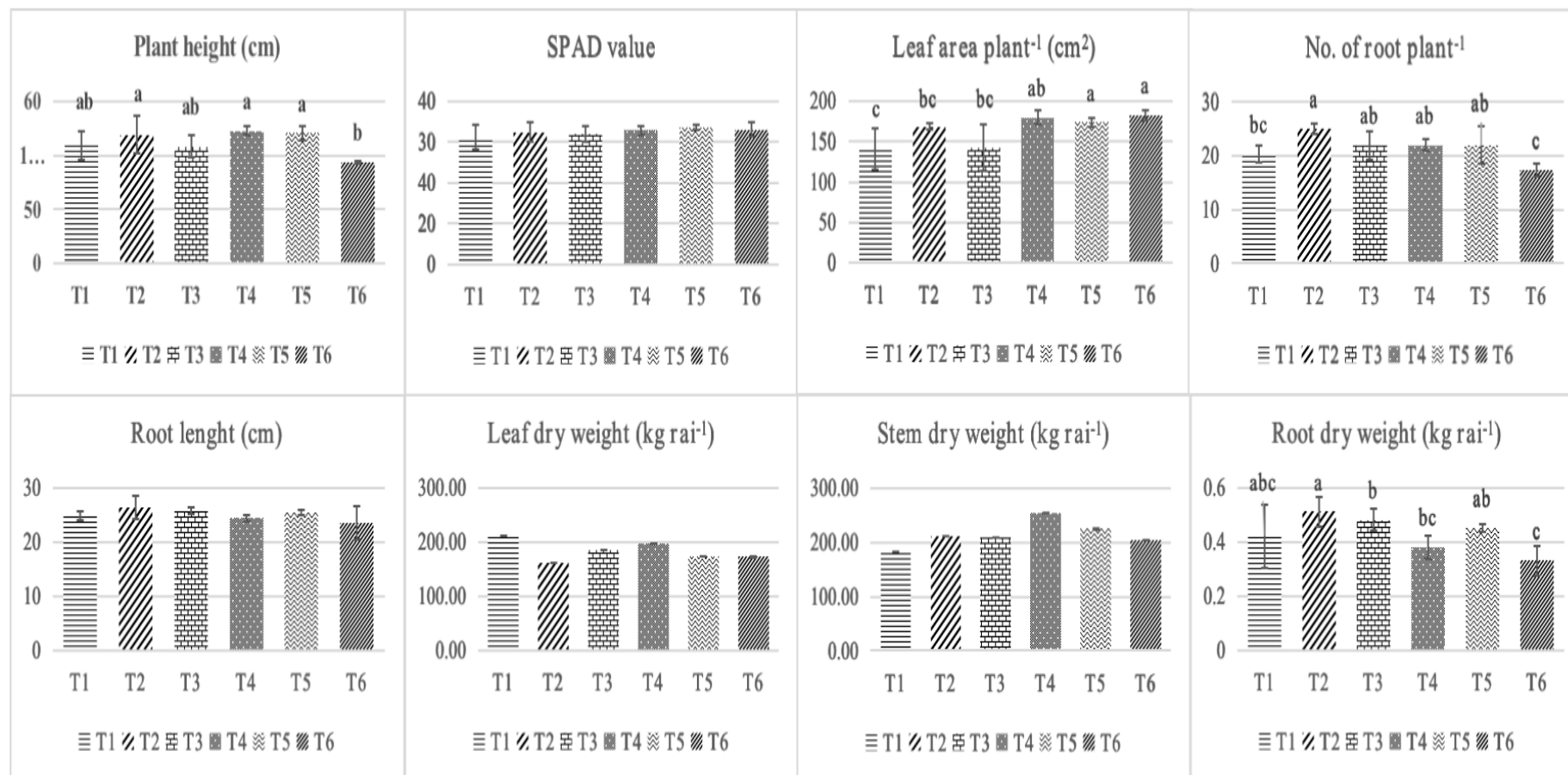


Figure 3. Plant growth of vegetable soybean at reproductive stage

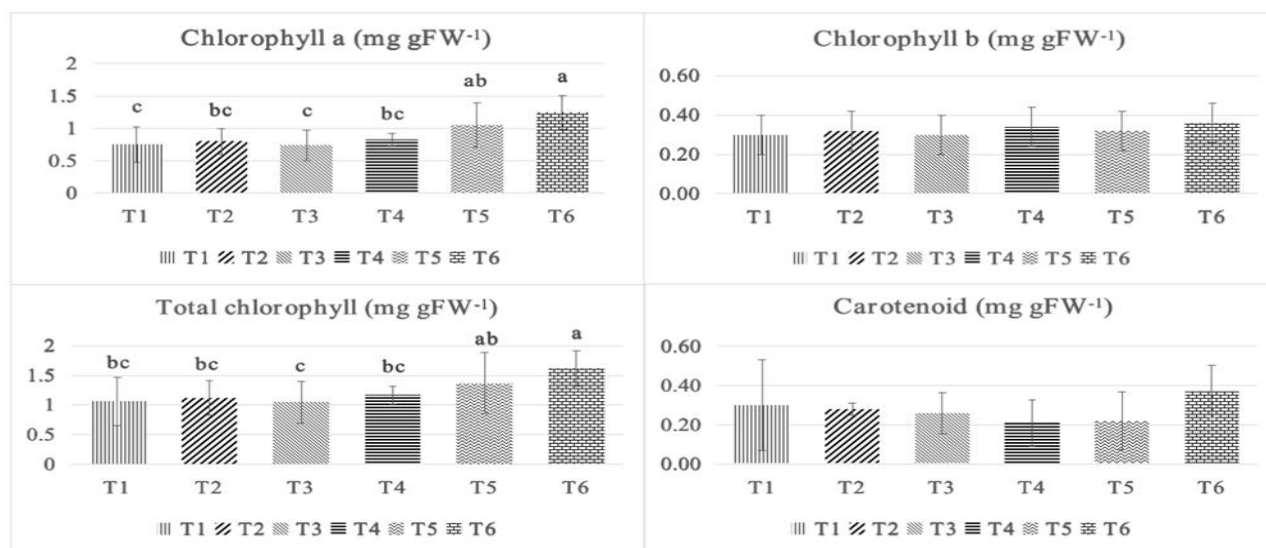


Figure 4. Photosynthetic pigments (Chlorophyll a, chlorophyll b, total chlorophyll and carotenoid) of vegetable soybean at reproductive stage

Yield components and photosynthetic pigments of vegetable soybean at harvesting stage: The results showed that the length of pod plant⁻¹, No. of pod plant⁻¹, No. of seed plant⁻¹, and pod fresh weight were significant differences at the harvesting stage (Figure 5). While No. of seed pod⁻¹ and 100 seed weight had no significant difference. The application of 50% inorganic fertilizer + *C. tropicalis* showed the highest significant difference in all parameters followed by the application of 25% inorganic fertilizer + *C. tropicalis*. In addition,

the study results indicate significant differences in the chlorophyll a (Chl a), total chlorophyll (TC), and carotenoid (Car) contents, but no significant differences in chlorophyll b (Chl b) (Figure 6). The treatments with 50 and 25% inorganic fertilizer combined with *C. tropicalis* resulted in the highest levels of chlorophyll a (Chl a), total chlorophyll (TC), and carotenoid (Car) contents compared to other treatments.

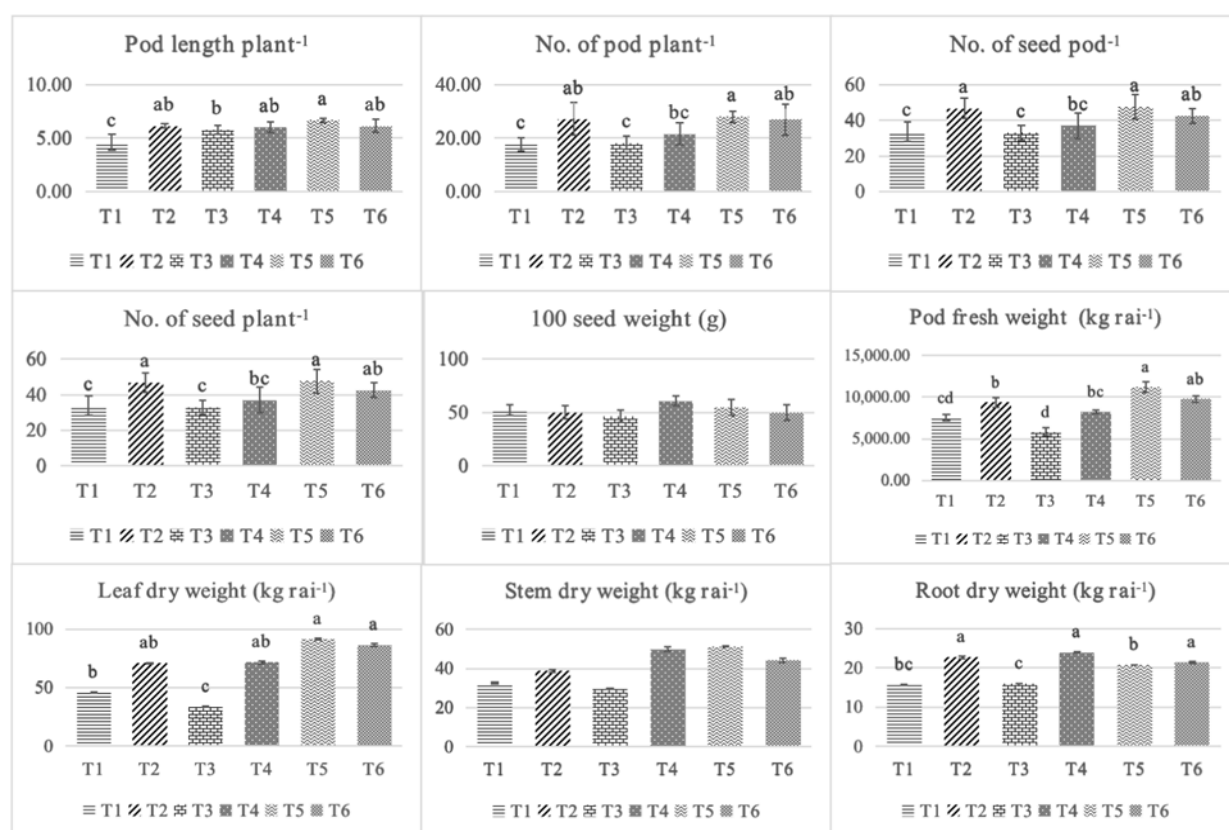


Figure 5. Yield components of vegetable soybean at harvesting stage

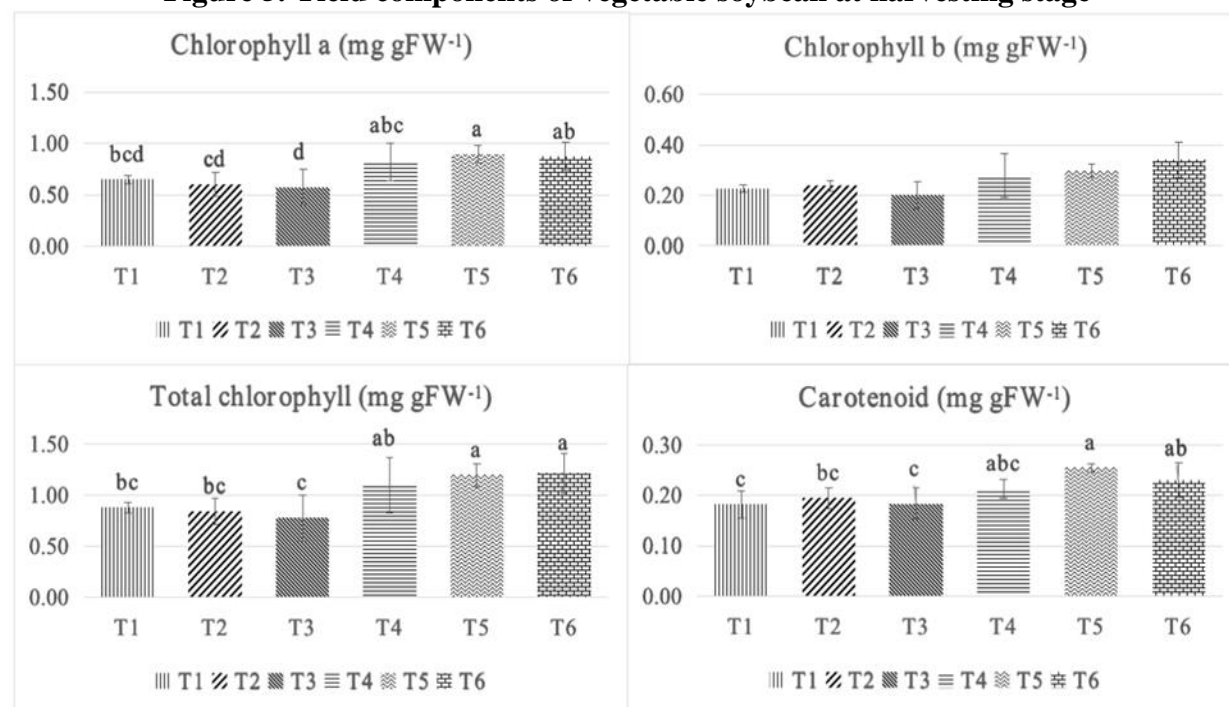


Figure 6. Photosynthetic pigments (Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid) of vegetable soybean at harvesting stage

The application of *C. tropicalis* solution has an effect on the growth of soybean vegetable. While, root length of vegetable soybean showed highest under no fertilizer and 25% inorganic fertilizer + *C. tropicalis* at one month after germination. Limited fertilizer

conditions can sometimes induce a stress response in plants that promotes root growth. This is a strategy to increase the plants ability to explore a larger soil volume for nutrients and water. Mild nutrient stress due to the absence of fertilizer might trigger a response

in plants to enhance root growth to compensate for limited nutrient availability (16). In addition, at the reproductive stage the results showed that under 25 and 50% inorganic fertilizer + *C. tropicalis* have a highest leaf area and photosynthetic pigment (chl a and TC) of vegetable soybean. Higher chlorophyll content generally indicates greater photosynthetic capacity, which can lead to increased plant growth and larger leaf area. Similar to Song et al. (36), reported that the treatment inoculated with *Trichoderma viride* had higher chlorophyll content and carotenoid content than the uninoculated treatment. Shome et al. (33) studied that inoculation of *Rhizobium japonicum* with 50% of the recommended N fertilizer produced the tallest plant and was also significant for leaf area

index and total chlorophyll content of the leaf (8.39 mg gFW^{-1}).

Effect of *C. tropicalis* application on nutritional parameters of vegetable soybeans: The results showed significant differences in the nutritional value of vegetable soybeans in all parameters (Figure 7). Applying 25% inorganic fertilizer + *C. tropicalis* showed substantial increases in total protein at 42.13%, calcium at 0.30%, and moisture content at 70.32% compared to only *C. tropicalis* solution. Additionally, the application of *C. tropicalis* solution alone resulted in the highest significant increase in fat and fiber content at 19.07% and 12.27% respectively. However, PGPR application led to a significantly higher carbohydrate and ash content at 34.45% and 2.17% respectively.

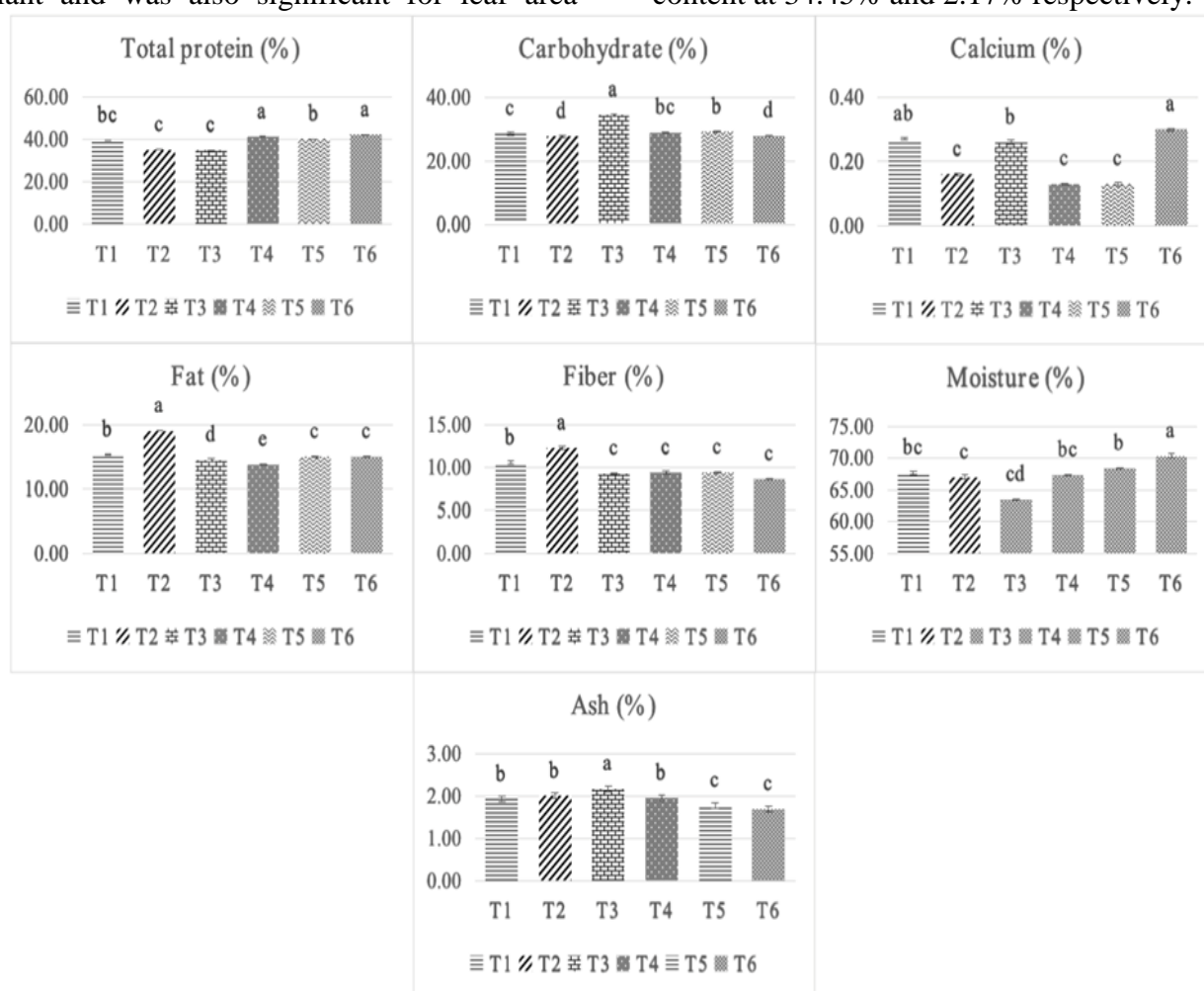


Figure 7. Nutritional values of vegetable soybean after inoculation of *C. tropicalis* solution

The results indicate that the application of *C. tropicalis*, alone or mixed with inorganic fertilizers, significantly impacts the nutritional value of vegetable soybeans. The application of *C. tropicalis* significant increase in fat and

fiber content which aligns with research by Verma et al. (41), who demonstrated that certain strains of PSF can enhance plant structural integrity and nutrient uptake, particularly lipids and fibers of rice, by

improving nutrient availability and promoting metabolic pathways associated with cell wall development. The synergistic effect of combining inorganic fertilizer + *C. tropicalis*, resulting in higher total protein, calcium, and moisture content, is consistent with studies such as that by Singh et al. (34), who demonstrated that microbial inoculants can improve nutrient uptake efficiency and nitrogen assimilation when used in conjunction with inorganic fertilizers. Haridy et al. (18) reported that treating soybean seeds with fresh yeast cells of *Saccharomyces cerevisiae* significantly increased the protein content of the resulting seeds. The treatment enhanced total protein (16.47%), soluble protein (22.9%), and insoluble protein (15.2%). This demonstrates the positive impact of PSF solution on soybean seed protein composition. This underscores the potential for integrating microbial solutions with low-level chemical inputs to achieve higher nutritional value in crops. *Candida tropicalis* from the bulk soil of fresh corn has demonstrated the potential to enhance the growth and yield of vegetable soybeans. When cultivated on TB media for five days, these PSF isolates are capable of producing a significant population, which may contribute to improved plant nutrient uptake and growth performance. Both applications of *C. tropicalis*, alone and in combination with reduced levels of inorganic fertilizers, significantly enhanced the growth and physiological parameters of vegetable soybeans. The study demonstrated that *C. tropicalis* improved leaf area, root length, and overall plant growth at two weeks and one month after germination, with the highest growth observed in treatments with 50% inorganic fertilizer + *C. tropicalis*. Additionally, *C. tropicalis* positively influenced chlorophyll and total chlorophyll content, particularly in combination with 25% and 50% inorganic fertilizer. At the harvesting stage, significant improvements were observed in the length of pod plant⁻¹, No. of pod plant⁻¹, No. of seed plant⁻¹, and pod fresh weight, with the best results found in the 50% inorganic fertilizer + *C. tropicalis* treatment. These findings suggest that *C. tropicalis* can enhance

plant growth and productivity, allowing for reduced inorganic fertilizer application without compromising crop yield. In addition, the application of *C. tropicalis* and its combination with inorganic fertilizers had a significant effect on the nutritional value of vegetable soybeans. However, more research is needed on *C. tropicalis* to fully understand its potential and optimize its application for sustainable agriculture.

REFERENCES

1. Agamy, R., M. Hashem and S. Alamri . 2013. Efffec of soil amendment with yeasts as bio-fertilizers on the growth and productivity of sugar beet. African Journal of. Agricultural Research. 8:46-56.
2. Amprayna, K., M.T. Rosea, M. Kecskés, L. Pereg, H.T. Nguyend, and I.R. Kennedy. 2012. Plant growth promoting characteristics of soil yeast (*Candida tropicalis* HY) and its effectiveness for promoting rice growth. Applied Soil Ecology. 61:295-299.
3. Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. *Polyphenoloxidase* in Beta vulgaris. Plant Physiology. 24:1-15.
4. Al-jubouri, K. D. H., I. J. Abdul Rasool, A. M.H. H. Al-Khafaji, A. J. Abdulsada, F. Y. Baktash, W. H. Hasoon, and Z. J. Al-Mousawi. 2025. Unraveling prolonged irrigation intervals and some sustainable treatments on potato starch composition, growth, and productivity in iraq. Iraqi Journal of Agricultural Sciences, 56(1), 321-329.
<https://doi.org/10.36103/s9q1w418>
5. Al-Khafaji, A. M.H. H., K. D. H. Al-jubouri, F. Y. Baktash, I. J. Abdul Rasool, and Z. J. Al-Mousawi. 2024. Amelioration potato plant performance under drought conditions in Iraq by using titanium dioxide, and biodegrading, biodegradable treatments. Iraqi Journal of Agricultural Sciences, 55(6), 1885-1893.
<https://doi.org/10.36103/03fway21>
6. Aldolaimy, O. M. S., H. A. Abdul- Ratha, and B. K. Abduljabar. 2024. Effect of bio-organic and mineral fertilization, on the growth and yield of cauliflower (Brassica oleraceae var.botrytis). Iraqi Journal of Agricultural Sciences –55(5):1667-1675.
<https://doi.org/10.36103/pt592r56>

7. Al-Silmawy, N. A. J. K., and H. A. Abdul-Ratha. 2023. Effect of biofertilizer, vermicompost and phosphate fertilizer on growth and yield of cauliflower (*Brassica oleraceae* Var. botrytis). Iraqi Journal of Agricultural Sciences. 54(2): 505- 515. <https://doi.org/10.36103/ijas.v54i2.1726>
8. Baqir, H. A., M.F.H. AL-hassan, and J. W. Mahmood. 2024. Role of bio health extract on wheat growth according to Zadoks decimal scale. Res. Crop. 25 (4): 547-552. DOI: 10.31830/2348-7542.2024.ROC-1130
9. Bajracharya, D. 1999. Experiments in plant physiology: A laboratory manual. New Delhi, India: Norasa Publishing House.
10. Bononi, L., J.B. Chiaramonte, C.C. Pansa, M.A. Moitinho and I.S. Melo. 2020. Phosphorus-solubilizing *Trichoderma* spp. from Amazon soils improve soybean plant growth. Scientific Reports. 10.
11. Brisson V.L, J. Richardy, S. M. Kosina, T. R. Northen, J. P. Vogel, and A. C. M. Gaudin 2022. Phosphate availability modulates root exudate composition and rhizosphere microbial community in a teosinte and a modern maize cultivar. Phytobiomes Journal. 6:83-94.
12. Chen, Y.P., P.D. Rekha, A.B. Arun, F.T. Shen, W.A. Lai, and C.C. Young. 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Applied Soil Ecology. 34:33-41.
13. Cong, P.T., T.D. Dung, T.M. Hien, N.T. Hien, A.T.M.A. Choudhury, M.L. Kecskés and I.R. Kennedy. 2009. Inoculant plant growth-promoting microorganisms enhance utilisation of urea-N and grain yield of paddy rice in southern Vietnam. Eur. Soil Biology and Biochemistry. (45), 52-61.
14. Cruz, R.C., S.M.C. Werneck, C.S. Oliveira, P.C. Santos, B.M. Soares, D.A. Santos, and P.S. Cisalpino. 2013. Influence of different media, incubation times, and temperatures for determining the MICs of seven antifungal agents against *Paracoccidioides brasiliensis* by microdilution. Journal of Clinical Microbiology. 51:436-443.
15. Dheyab S., N., A M.H. H. Al-Khafaji, I. J. Abdul Rasool, K. D. H. Al-jubouri, F. Y. Baktash, Z. J. Al-Mousawi, and D. A. Hanoon. 2025. Reducing water consumption and improving soil, root quality of potato via environmentally sustainable treatments. Iraqi Journal of Agricultural Sciences, 55(special):1-9. <https://doi.org/10.36103/przef771>
16. Francis, B., C.T. Aravindakumar, P.B. Brewer, and S. Simon. 2023. Plant nutrient stress adaptation: A prospect for fertilizer limited agriculture. Environmental and Experimental Botany. 213:105431.
17. Gomaa, A., S. Moawad, I. Ebadah and H. Salim. 2005. Application of bio-organic farming and its influence on certain pests infestation, growth and productivity of potato plants. Journal of Applied Sciences Research. 1:205-211.
18. Haridy, M.S., G.K.A. El-Baki, E.N. Fawzy. 2022. Potentiality of some yeast species for promotion of growth and productivity of soybean plants (*Glycine max* L.). Journal of Advanced Biomedical and Pharmaceutical Sciences. 5:64-78.
19. Kecskés, M.L., M.T. Rose, C.T.T. Kim, O.N. Kim, E. Michel, B. Lauby, M. Rakotondrainibe, A.V. Casteriano, A. Palágyi, G. Krishnen and I.R. Kennedy. 2008. Identification and quality control of BioGro inoculant biofertiliser strains. In: Kennedy, I.R., A.T.M.A. Choudhury, M.L. Kecskés, M.T. Rose, (Eds.), Proceedings of a Project (SMCN/2002/073) Workshop Held in Hanoi Vietnam. ACIAR Proceedings (130). Canberra, pp. 117-125.
20. Khunpilueg, P., A. Chotiyarnwong, P. Chotiyarnwong, V. Tepjun, J. Phoomthaisong, N. Wanasai, A. Kasiwiat, and A. Maliphun. 2012. A new aroma vegetable soybean “Chiang Mai 84-2”. Chiang Mai Field Crops Research Center (CMFCRC).
21. Kim, I.S. 2021. Current perspectives on the beneficial effects of soybean isoflavones and their metabolites for humans. Antioxidants. 10:1064.
22. Lacaz, C.S. 1994. *Paracoccidioides brasiliensis*: morphology, evolutionary cycle; maintenance during saprophytic life; biology, virulence, taxonomy. CRC Press. 13-25.
23. Mardamootoo, T., C.C.D. Preez and J.H. Barnard. 2021. Agricultural phosphorus

management for environmental protection: a review. Journal of Geoscience and Environment Protection. 9:48-81.

24. Mekki, B.B and A.G. Ahmed. 2005. Growth, yield and seed quality of soybean (*Glycine max* L.) as affected by organic, biofertilizer and yeast application. Research Journal of Agriculture and Biological Sciences. (1), 320324.

25. Millipore. 2018. Culture media for industrial microbiology. Darmstadt, Germany. Available online: www.sigmaaldrich.com. (Accessed on March 4, 2024).

26. Nair, R.M., V.N. Boddepalli, M.R. Yan, V. Kumar, B. Gill, R.S. Pan, C. Wang, G.L. Hartman, R.S. Souza and P. Somta. 2023. Global status of vegetable soybean. Plants. 12:609.

27. Otlewska, A., M. Migliore, K. Dybka-Stepien, A. Manfredini, K. Struszczyk-Swita, R. Napoli, A. Białkowska, L. Canfora, and F. Pinzari. 2020. When salt meddles between plant, soil, and microorganisms. Frontiers in Plant Science. 11:1-23.

28. Pradhan, S.N., A. Patra and T. Behera. 2020. Phosphorus: an ultimate limiting soil nutrient. Food and Scientific Reports. 1:31-42.

29. Puppala, K.R., T. Naik, A. Shaik, S. Dastager, R.V. Kumar, J. Khire and M. Dharne. 2018. Evaluation of *Candida tropicalis* (NCIM 3321) extracellular phytase having plant growth promoting potential and process development

30. Puri, A., K.P. Padda, and C.P. Chanway. 2020. In vitro and in vivo analyses of plant growth promoting potential of bacteria naturally associated with spruce trees growing on nutrient-poor soils. Applied Soil Ecology. 149:103538.

31. Rafiquen, M., T. Sultan, I. Ortas, H.J. Chaudhary. 2017. Enhancement of maize plant growth with inoculation of phosphate-solubilizing bacteria and biochar amendment in soil. Soil Science and Plant Nutrition. 63:460-469.

32. Shahid, M.Q., M.F. Saleem, H.Z. Khan, and S.A. Anjum. 2009. Performance of soybean (*Glycine max* L.) under different phosphorus levels and inoculation. Pakistan Journal of Agricultural. 46.

33. Shome, S., A. Barman, Z.M. Solaiman. 2022. Rhizobium and phosphate solubilizing bacteria influence the soil nutrient availability, growth, yield, and quality of soybean. Agriculture. 12:1-18.

34. Singh, D.P., V. Singh, V.K. Gupta, R. Shukla, R. Prabha, B.K. Sarma, and J.S. Patel. 2020. Microbial inoculation in rice regulates antioxidative reactions and defense related genes to mitigate drought stress. Scientific Reports. 10:4818.

35. Somdee, T., U. Mahaweerawat, J. Wibulutai, N. Dungkokruad, and S. Yungyuen. 2017. Polyphenol contents, antioxidant and anticancer activity (MCF-7) of soybean products in Thailand. Chiang Mai Journal of Science. 44:176-183.

36. Song, M., X. Wang, H. Xu, X. Zhou⁴ and C. Mu. 2023. Effect of *Trichoderma viride* on insoluble phosphorus absorption ability and growth of *Melilotus ofcinalis*. Scientific Reports. 13:12345.

37. Statistix10. 2013. Analytical software user's manual. Tallahassee, Florida. Available online: <https://www.statistix.com/>. (Accessed on March 2, 2024).

38. Tartoff, K.D., and C.A. Hobbs. 1987. Improved media for growing plasmid and cosmid clones. Bethesda Research Laboratories. 9:12.

39. Uzma, F., C.D. Mohan, A. Hashem, N.M. Konappa, S. Rangappa, P.V. Kamath, B.P. Singh, V. Mudili, V.K. Gupta, C.N. Siddaiah, S. Chowdappa, A.A. Alqarawi, and E.F.A. Allah. 2018. Endophytic fungi alternative sources of cytotoxic compounds: a review. Frontiers in Pharmacology. 9:309.

40. Vassileva, M., G.D.O. Mendes, M.A. Deriu, G.D. Benedetto, E. Flor-Peregrin, S. Mocali, V. Martos, and N. Vassilev. 2020. Fungi, P-solubilization and plant nutrition. Microorganisms. 10:1716.

41. Verma, P., A.N. Yadav, V. Kumar, D.P. Singh, and A.K. Saxena. 2017. Role of phosphate-solubilizing fungi in improving plant nutrient uptake and crop quality. Department of Microbiology, Akal College of Basic Sciences, Eternal University, Sirmour, India.

42. Wahid, F., M. Sharif, S. Steinkellner, A. Khan, M. Marwat, and S. Khan. 2016.

Inoculation of arbuscular mycorrhizal fungi and phosphate solubilizing bacteria in the presence of rock phosphate improves phosphorus uptake and growth of maize. *Pakistan Journal of Botany*. 48:739-747.

43. Wang, H., J. Guo, X. Chen, and H. He. 2023. The metabolomics changes in Luria-Bertani broth medium under different sterilization methods and their effects on *Bacillus* growth. *Metabolites*. 13:958.

44. Xu, X.L., X.L. Mao, L.V. Zwieten, N.K. Niazi, K. Lu, and N.S. Bolan. 2020. Wet-drying cycles during a rice-wheat crop rotation rapidly (im) mobilize recalcitrant soil phosphorus. *Journal of Soils and Sediments*. 20:3921-3930.

45. Zahid, M., M.K. Abbasi, S. Hameed and N. Rahim. 2015. Isolation and identification of indigenous plant growth promoting rhizobacteria from Himalayan region of Kashmir and their effect on improving growth and nutrient contents of maize (*Zea mays* L.). *Frontiers in Microbiology*. 6:1-15.

46. Zhenghai Zhang, Ning Liu, Cai Shao, Hai Sun, Zhengbo Liu, Yiming Guan, Lianju Wu, Linlin Zhang, Xiaoxi Pan, Yayu Zhang, Bing Zhang. 2020. Arbuscular mycorrhizal fungi biofertilizer improves American ginseng (*Panax quinquefolius* L.) growth under the continuous cropping regime, *Geoderma*, 363,114155,

<https://doi.org/10.1016/j.geoderma.2019.114155>

47. Zhu, J., J., Wu, Z. Shi, et al. 2022. Taxonomic response of bacterial and fungal populations to biofertilizers applied to soil or substrate in greenhouse-grown cucumber. *Sci Rep* 12, 18522.

<https://doi.org/10.1038/s41598-022-22673-4>

48. Zhengyang, Liu, Wang, Y., Hao, X. et al. 2023. Biodiversity of the beneficial soil-borne fungi steered by *Trichoderma*-amended biofertilizers stimulates plant production. *npj Biofilms Microbiomes* 9, 46 .

<https://doi.org/10.1038/s41522-023-00416-1>

49. Xue Han, Yulong Li, Haiyang Li, Gang Han, Jiao Xi, Yutao Liu, Yanjiang Zhang, Quanhong Xue, Qiao Guo, and Hangxian Lai. 2022. Actinobacterial biofertilizer improves the yields of different plants and alters the assembly processes of rhizosphere microbial communities. *Applied Soil Ecology*, 171, 104345.

<https://doi.org/10.1016/j.apsoil.2021.104345>

50. Žaklina Karaklajić-Stajić, Arijana Pešaković, Slobodan Milenković, and Olga Mitrović. 2013. Biofertilizer affecting yield related characteristics of strawberry (*Fragaria×ananassa* Duch.) and soil micro-organisms. *Scientia Horticulturae*, 150:238-243,

<https://doi.org/10.1016/j.scienta.2012.11.016>