CHAPTER VI

LACTIC ACID BACTERIA AS BIOCONTROL AGENT AGAINST PATHOGENIC FUNGI *Fusarium solani*-CS IN CHILLI PLANTS

6.1 INTRODUCTION

Recently, report of certain strains of LAB isolates showed ability to reduce fungal growth and improve the plants growth. *Lb. plantarum* LAB-C5 from durian and *Lb. pentousis* LAB-G7 from ginger showed strong suppression spreading of fungi *C. gloesporioides* and *C. capsici* on chilli fruits as well enhancing the plant growth and increasing the yield of chilli fruits (El-Mabrouk, 2014). Narasimha et al. (2012) observed that the LAB I and LAB II applied to seed and soil enhanced the growth of tomato plants in the field trials. LABs are reported to produce indole acetic acid (IAA) in the presence of different concentrations of tryptophan and highest amount of IAA was produced by KLF01 (Anupama et al., 2014). These researchers also noted that LAB strains KLF01, KLC02 and KPD03 applied to seeds significantly promoted root length, shoot length, root fresh weight and chlorophyll in pepper plants in greenhouse. Most of studies have confirmed reduced phytopathogenicity of infections in different crops after supplementing the soils with fungal or bacterial antagonists (Mukhopadhyay, 1987; Smith et al., 1990; Bashar & Rai 1994; Singh et al., 2002; Akrami et al., 2011; Ahmed, 2011). Similarly, in previous chapter it was observed that the cells and supernatant of LAB showed ability to improve

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the percentage germination and seedling systems of different variety of chilli seeds. Therefore, the isolates of LAB could be improved the chilli plant systems and productivit.

The control of plant diseases using antagonistic microorganisms can be an effective means of biocontrol to protect some commercial important crops (Compant et al., 2005; Cook & Baker, 1983; Vasseur et al., 1990). The large number plant diseases have been successfully controlled by using bacterial antagonists (Vidhyasekaran, 1997; Cook & Baker, 1983). Using microorganisms as biocontrol has been developed as an alternative to synthetic fungicide for treatment of infection by phytopathogenic fungi and significant success has been achieved to control both pre-harvest and post harvest diseases (Janisiewicz & Korsten, 2002). A variety of microbial antagonists has been reported to control several different pathogens on various fruits and vegetables (Fravel, 2005; Mari & Guizzardi, 1998). Some selected microorganisms such as lactic acid bacteria isolated from fresh fruits and vegetable showed inhibitory activities against phytopathogenic and spoilage bacteria and fungi (Trias et al., 2008a). El-Mabrouk et al. (2014) reported that application of CFS of *Lb. plantarum* LAB-C5 from durian and *L. pentousis* LAB-G7 from ginger to artificially infected chilli fruits with *C. gloesporioides* and *C. capsici* strongly suppress the spreading of anthracnose diseases of both fungi on chilli fruit.

The fungicides used to control phytopathogenic fungi are often applied extensively and residues are noticed on harvested produce causing hazards to environment, humans and animals (Harris et al., 2001); Pandy, 2003; Kumar et al., 2007).

Similarly, antimicrobial chemicals such as benzimidazoles, aromatic hydrocarbons and sterol biosynthesis inhibitors are often used in the control of plant diseases in agriculture. However, there is a series of problems against the effective use of these chemicals in areas where the fungi have developed resistance Brent & Hollomon (1998). Most studies have confirmed reduced phytopathogenicity of infections in different crops after supplementing the soils with fungal or bacterial antagonists (Mukhopadhyay, 1987; Smith et al., 1990; Bashar & Rai, 1994; Singh et al., 2002; Akrami et al., 2011; Ahmed, 2011). However, the regular use of fungicides can potentially pose a risk to the environment, particularly if residues persist in the soil or migrate off-site and enter waterways (e.g. due to spray drift, run-off) (Kibria et al., 2010; Komarek et al., 2010). If this occurs it could lead to adverse impacts to the health of terrestrial and aquatic ecosystems. For instance, concerns have been raised over the long term use of copper-based fungicides, which can result in an accumulation of copper in the soil (Wightwick et al., 2008; Komarek et al., 2010). This in turn can have adverse effects on soil organisms (e. g. earthworms, microorganisms) and potentially pose a risk to the long-term fertility of the soil (Wightwick et al., 2008; Komarek et al., 2010).

The demand for chillies in the world is increasing every year and good qualities which is free from pathogens and infection of diseased appearance or fungal toxins are prerequisites for import and export (Chandra et al., 2009). The isolates LAB are which can be found in any environment rich mainly in carbohydrates, such as plants, fermented foods and the mucosal surfaces of humans, terrestrial and marine animals (Aureli et al., 2011 & Barinov et al., 2011). Similarly, some plants harbor microbes inside the plant

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tissues and referred as endophytes. Endophytes have been defined as bacteria that are able to colonize living plant tissue without harming the plant or gaining benefit other than securing residency; the isolates are identified as *B. megaterium, Lb. casei, B. subtilis, B. cereus,* and *Lb. acidophilus* showed ability to produce indole acetic acid (IAA) and called as phyto-hormone producers or plant growth regulators (Mohite, 2013). Bacteria that colonize the rhizosphere and plant roots, and enhance plant growth by any mechanism are referred to as PGPR. Thus, these PGPR can exhibit a variety of characteristics responsible for influencing plant growth. The common traits include production of plant growth regulators (like auxins, gibberellins, and ethylene), siderophores, HCN and antibiotics (Arshad et al., 1992).

In previous chapter the data indicated that LAB isolated from soil (15 isolates), fermented food (7 isolates) and (3 ATCC culture) have ability to inhibit the *Fusarium* spp. and some of them used to enhance the development of chilli seedling systems. It is uncertain whether the antifungal activity is the result of an *in vitro* or *in vivo* effect. Therefore, the purpose of this study was to ascertain the antifungal activity of LAB cells in chilli plants infected with *Fusarium* species. The objectives of this study were (i) to evaluate the effect of cells of LAB-SS1 and LAB-FF11 in soil to promote chilli plants; (ii) to determine the pathogenicity of *F. solani*-CS in soil to effect the chilli plant growth. (iii) to determine the plants growth and productivity of chilli plants in present of LAB cells and pathogenic fungi *F. solani*-CS in soil. (iv) to detect the endophytic nature of LAB-MSS1, LAB-FF11 pathogenic fungi *F. solani*-CS which were used to inoculate during development of chilli plants.

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6.2 MATERIALS AND METHODS

6.2.1 Sample of chilli plants

About 200 of one month old chilli plants were purchased from Asia Nursery Sungai Buluh, Selangor, Malaysia and used for the experiments as in Figure 23 (A & B). The plants were transplanted in soil plastic bag size 16 × 16 cm and divided into six groups; each group contained more than 20 plants. The soil used was labeled as "Tanah Baik" with no fertilizer & sandy soil (ratio (3:1) purchased from Asia Nursery Sungai Buluh, Selangor, Malaysia and used for the experiments.

FIGURE 23: Chilli plants of one month old survived at Asia Nursery Sungai Buluh, Selangor, Malaysia and used for the experiments

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Notes:- Transferring treated plants into plastic bag filled with soil; (A) one month old plants ready for treatments and (B) transplanted plants after treated soil with cells of LAB and cells of *Fusarium solani*-CS

6.2.2 Preparation of LAB cells

Isolate LAB-MSS1 and LAB-FF11 cells were serially diluted concentration obtained (1 × $10^{7/ml.}$) were inoculated into 100 mL MRS broth and incubated for 24 h at 37°C using the method described by Hamed et at. (2011) with modification. The 24 h old cells of LAB culture (1.5% v/v) was added to 1000 mL of sterilized peptone water (OXOID CM0009). Then, 15 mL of the mixture was distributed to the centre of soil in the plastic bag size (16×16) cm and this process was repeated alteration of week, same quantity of the mixture was poured inside the root region until flower start.

6.2.3 Preparation of phytopathogenic fungi

Phytopathogenic fungi *Fusarium solani*-CS were grown in PDA was incubated at 28°C for 7 d and addeding sterilized distilled water (10 to 20 mL) was poured onto the plates, the fungal surface was gently scraped to loosen the spores and the spore suspensions was collected. The spore suspension at concentration obtained serial dilution of $1x10^5$/mL was prepared before each experiment using the method (El-Mabrouke, 2014) with modification. The spore suspension (1.5% v/v) was added to 1000 mL of sterilized peptone water (OXOID CM0009). Then 15 mL of the suspended mixture was distributed to the centre of soil in the plastic bag size (16×16) cm at once time during transplanting of chilli plants.

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6.3 *In Vivo* study

6.3.1 Growing chilli plants in soil treated with LAB and *Fusarium* species

The field experiment was conducted during October to January 2015 in Malaysia and treatments set up as shown in Table 36. The plants physiology and chilli plant architecture systems (length of shoot root and height of plants) were measured after 25 d (start branching), 45 d (before start flowering) and 65 d (before start fruiting). Plants that were not measured were left to bear fruits and the characteristics of the chilli fruits were recorded.

TABLE 36: Chilli plants divided in six groups survive in soil treated with LAB and *Fusarium* species

Plants Groups	Soil Treatments
I	Water (Control)
II	LAB MSS1
III	LAB MSS1 + *F. solani*-CS
IV	LAB-FF11
V	LAB-FF11+ *F. solani*-CS
VI	*F. solani*-CS

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6.3.2 Determination of fresh and dry weight of chilli plants

Fresh and dry weight of plant systems were observed after 25 d (start branching), 45 d (before start flowering) and 65 d (before start fruiting). Chili plants were taken out from field and total weight of whole plants, shoots and roots were determined separately using weighing scale (Saritorius AX4202). Chili plants shoots and roots were dried at 40°C in drying oven (BINDER) 24 h for 25 d old plants, 48 h for 45 d old plants and 72 h for 65 days old plants and the dry weight was determined triplicate.

6.3.3 Measurement of fruit length

A total of three chilli fruits of full maturity stage where the fruits started green into red for each treatment were plucked and measurement made at the shoulder region to apex region and length of chilli measuring tape in cm as shown (Figure 24).

FIGURE 24: Measurement of fruit length from shoulder to apex regions

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6.3.4 Measurement of fruits pericarp thickness of chilli fruits

The total of three fruits from each treatment was randomLy harvested fresh and the fruit was cut at the shoulder region, middle region and apex region with help of scalpel blade. The pericarp thickness of chilli fruit was measured using a Vernier caliper in mm and mean value from five chilli fruits per treatment were determined triplicate.

6.3.5 Determination of fresh and dry weight of chilli fruits

Fresh and dry weight of chilli fruits was determined after the fruits turn all red. Chilli fruits (total of five samples) were harvested and the individual weight of the fresh fruits were determined of (Saritorius AG Germany CPA 224S). The fruits were then dried at 40°C in drying oven (BINDER) for 24 h. The weight of the dried chilli fruits was recorded and the percentage moisture content of chilli mass was determined triplicate.

6.4 Confirmation of entophytic LAB and *Fusarium* species in treated chilli plants

6.4.1 Confirmation of LAB-MSS1 and LAB-FF11 from different chilli plant parts

Isolation of entophytic LAB from different parts of chilli plants survived in soil treated with LAB. Treated LAB strains were re-isolated from inoculated Plants systems samples were surface sterilized plant stem, placenta, root stem, seeds and soil as shown in Figure 25 (A, B, C & D) adopting procedure by Wanchai et al. (2007). The surface sterilized

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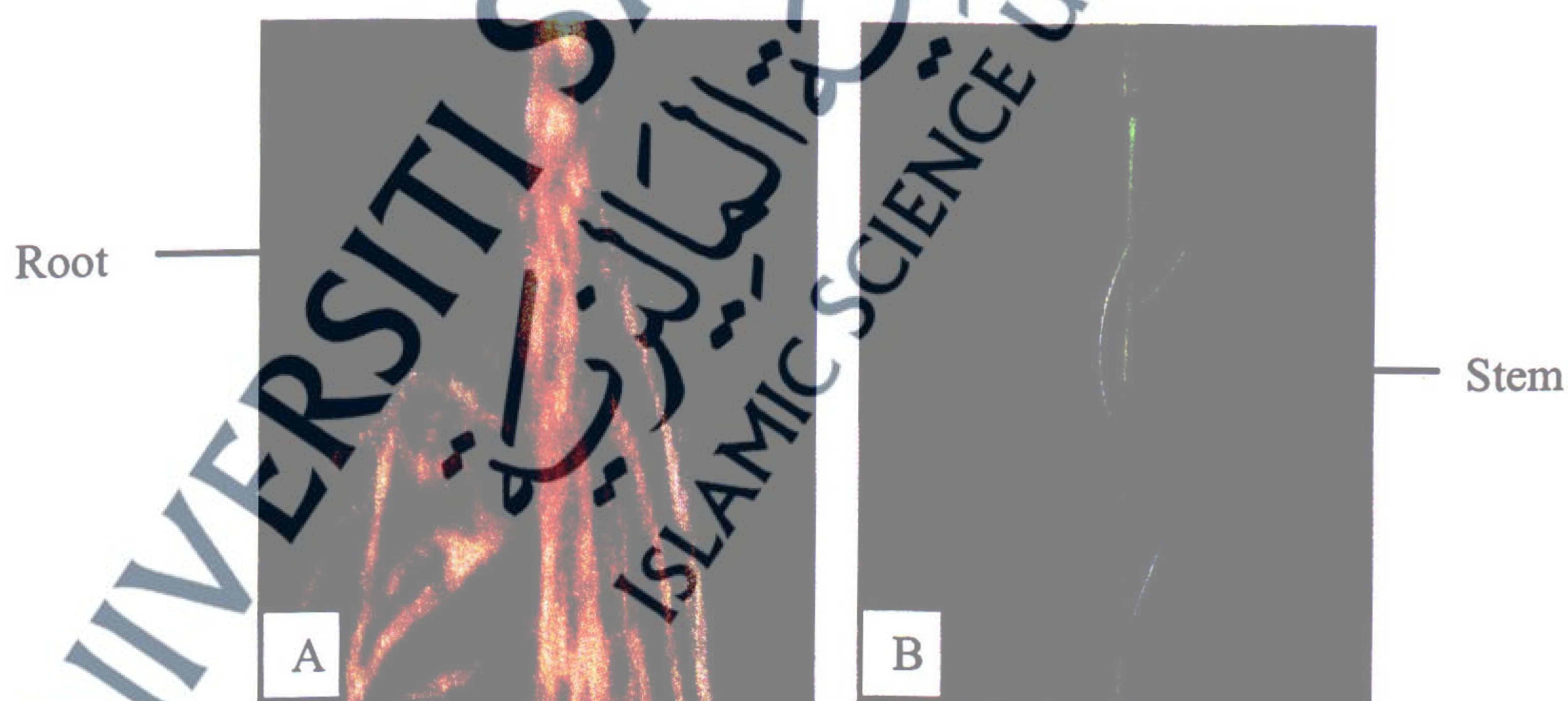
sterilized plants systems samples were transferred on De Man Rogosa and Sharpe Agar (MRSB-Oxoid, CM0359) and incubator at 37°C for 48. After the appearance white creamy colony were transferred on modified De Man Rogosa and Sharpe agar (MRSA-OXOID CM0361) with 0.7% CaCO3 and incubated under anaerobic conditions at 37°C for 24 h. Colonies showing clear zones about them were selected and streaked on MRS agar plates to obtain pure colonies. These colonies were tested for catalase activity with 4% H_2O_2. All isolates were checked for catalase negative and Gram positive reactions. Similarly, isolation of LAB from soil inoculated during plant growth adopting the method of (Matthias et al., 2012) as described in chapter III section 3.2.1. The identification was done comparing with characterization of previously isolated LAB-MSS1 and LAB-FF11 as mentioned in chapter III (Table 5 & Figure 2).

FIGURE 25: Isolation of endophytic LAB and fungi from different parts of treated plants and soil



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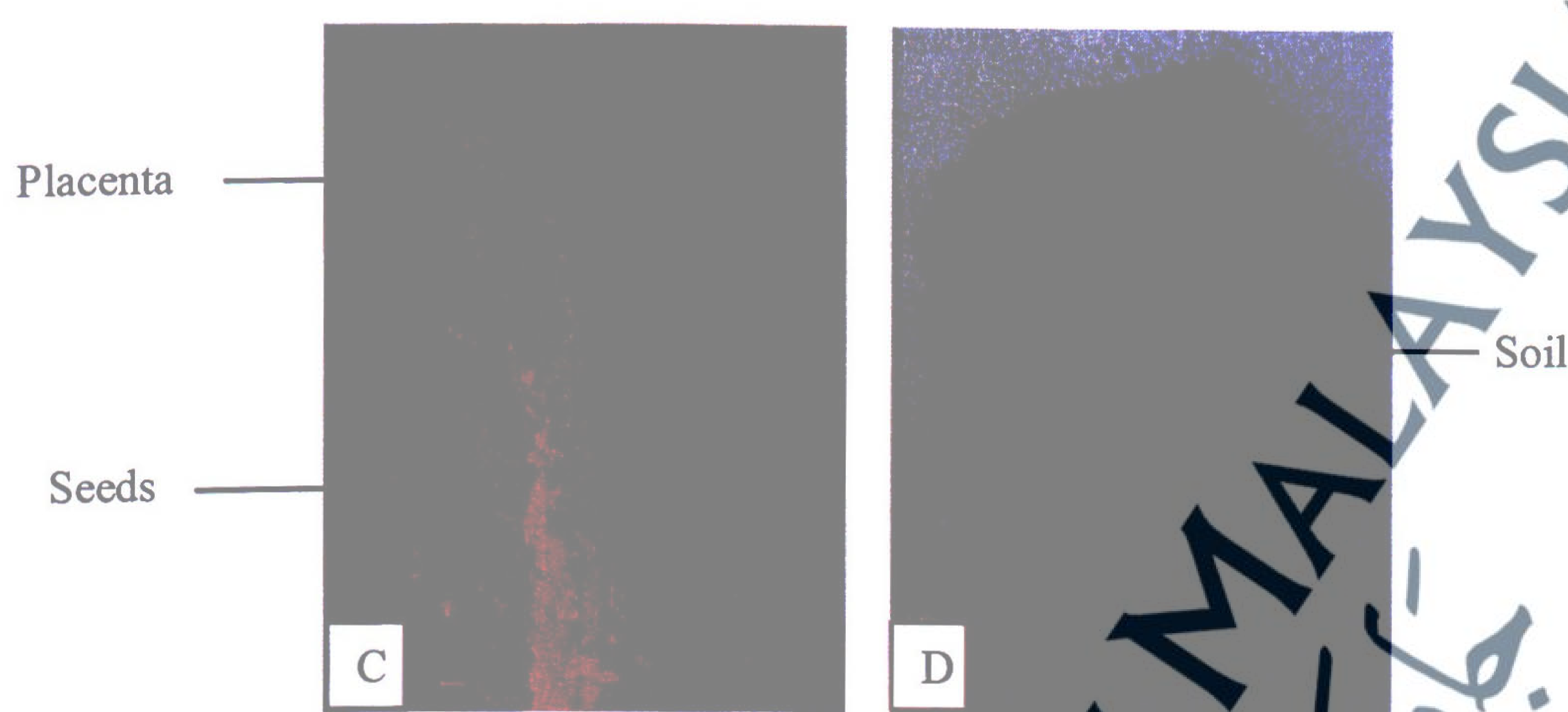

Notes:- Samples showing different parts of chilli selected for LAB and fungi isolation namely: (A) main root of inner part (B) main stem inner part (C) placenta and seeds and (D) treated soil after approximately 90 day

6.4.2 Confirmation of *F. solani*-CS from different chilli plant parts

Phytopathogenic *F. solani*-CS was confirmed that from different part of plants grown in soil infected fungi, plants systems samples stem, placenta, root stem, seeds and soil displayed same as in Figures 25 were collected from fungal treatment plant and transferred on was done following the method of Jha (1995). Chilli samples were washed with distilled water and dry with tissue paper, followed by surface-sterilized in 1% sodium hypochlorite for 2 min, then aseptically placed on potato dextrose agar (PDA Oxoid, CM0139) using sterilized forceps. Re-isolation of phyto-pathogen *Fusarium* sp. from soil inoculated during plant growth adopting the method of Latiffah et al. (2010) with modification. The soil samples were taken from rhizosphere of chilli plant which soil was infected during the transplantation time. The soil samples were air-dried at room temperature and serially diluted obtained concentration (1×10^5) fractions spreading with

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help of glass rod on PDA. The plates were incubated at 28°C for 5-7 d. After incubation, colonies of different shape and colors were sub-cultured on PDA incubated at room temperature at 28°C for 5 to 7 d, and pure culture of each colony type was maintained on PDA and stored at 4°C. The identification was done refer to characteristic compared to previously isolated fungi *Fusarium* sp. as described in chapter III (Figure 4 & Table 6).

6.5 Data analysis

Mean ± standard deviation obtained from each analysis was analyzed using One-way analysis of variance (ANOVA) and the mean significant was done by the Tukey test at ($P<0.05$). The statistical analyses were performed using Minitab 16 software.

6.6 RESULTS

6.6.1 Effect of cells of LAB and fungi on plant growth characteristics

Changes in plant growth characteristics after treatment with LAB alone or LAB and fungi together were observed at three different stages of growth: after 25 d (started branching), 45 d (before flowering) and after 65 d (before fruiting). Significant changes ($P<0.05$) were observed in plant growth treated with the two LAB compared to other treatments during the growing period of 25 d, 45 d and 65 d after transplanting.

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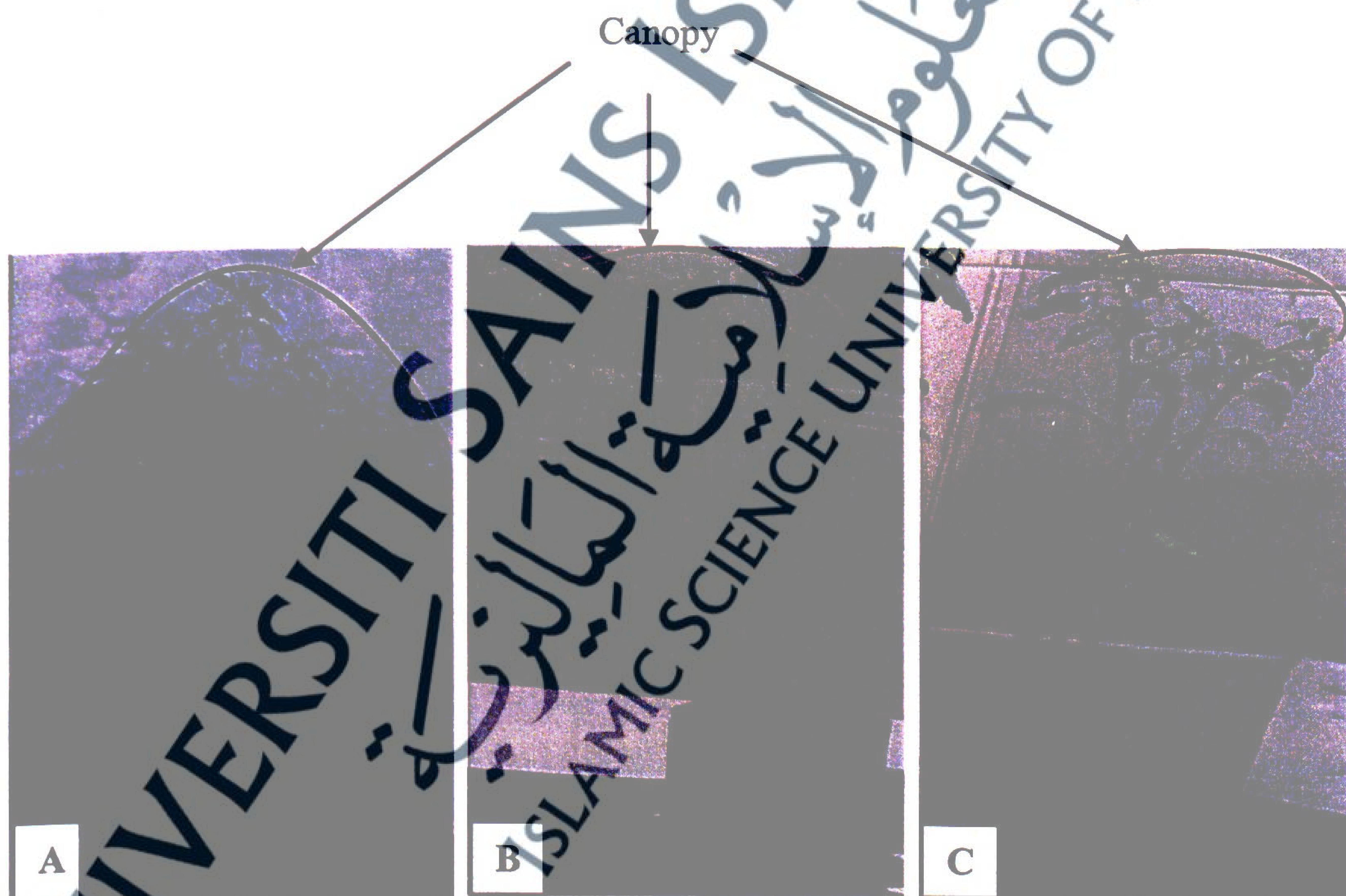
6.6.1.1 Effect of different treatments on plant canopy and plant height

It was observed that the canopy width of chilli plants was significantly ($P<0.05$) affected by days after transplanting. The canopy of chilli plants receiving both cells of LAB or cells of LAB and fungi treatments namely, group II, III, V and VI were significantly different after 45 d transplanting with canopy width ranges between 59.33 cm to 66.16 cm (Figure 26A).

FIGURE 26: Plant showing canopy area after 65 d transplanting



Notes:- (A) chilli plant showing broadest canopy from treatment group II (B) chilli plant showing narrow area canopy receiving from fungi infected (negative control) group VI (C) chilli plant group I (positive control) also has narrow area of canopy

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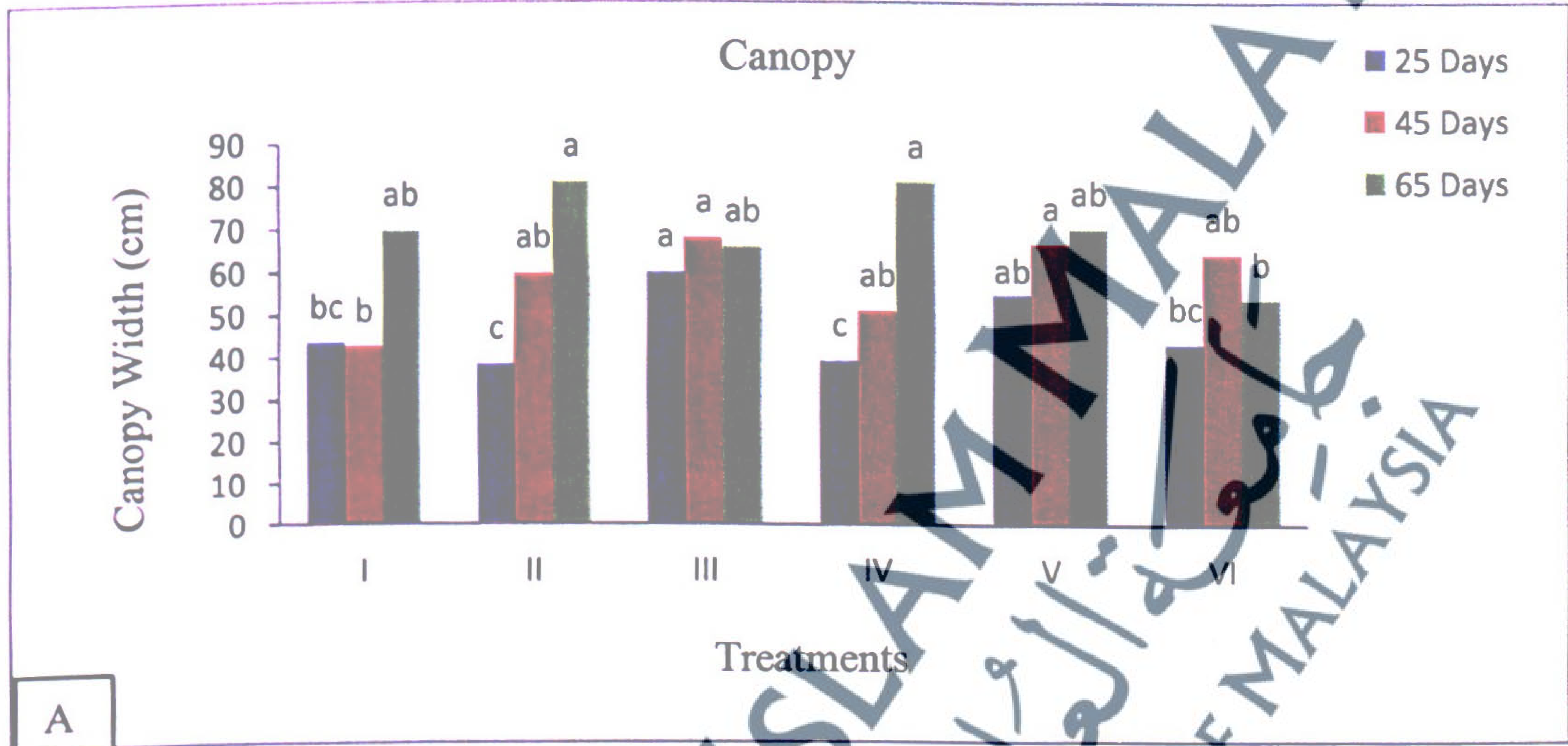
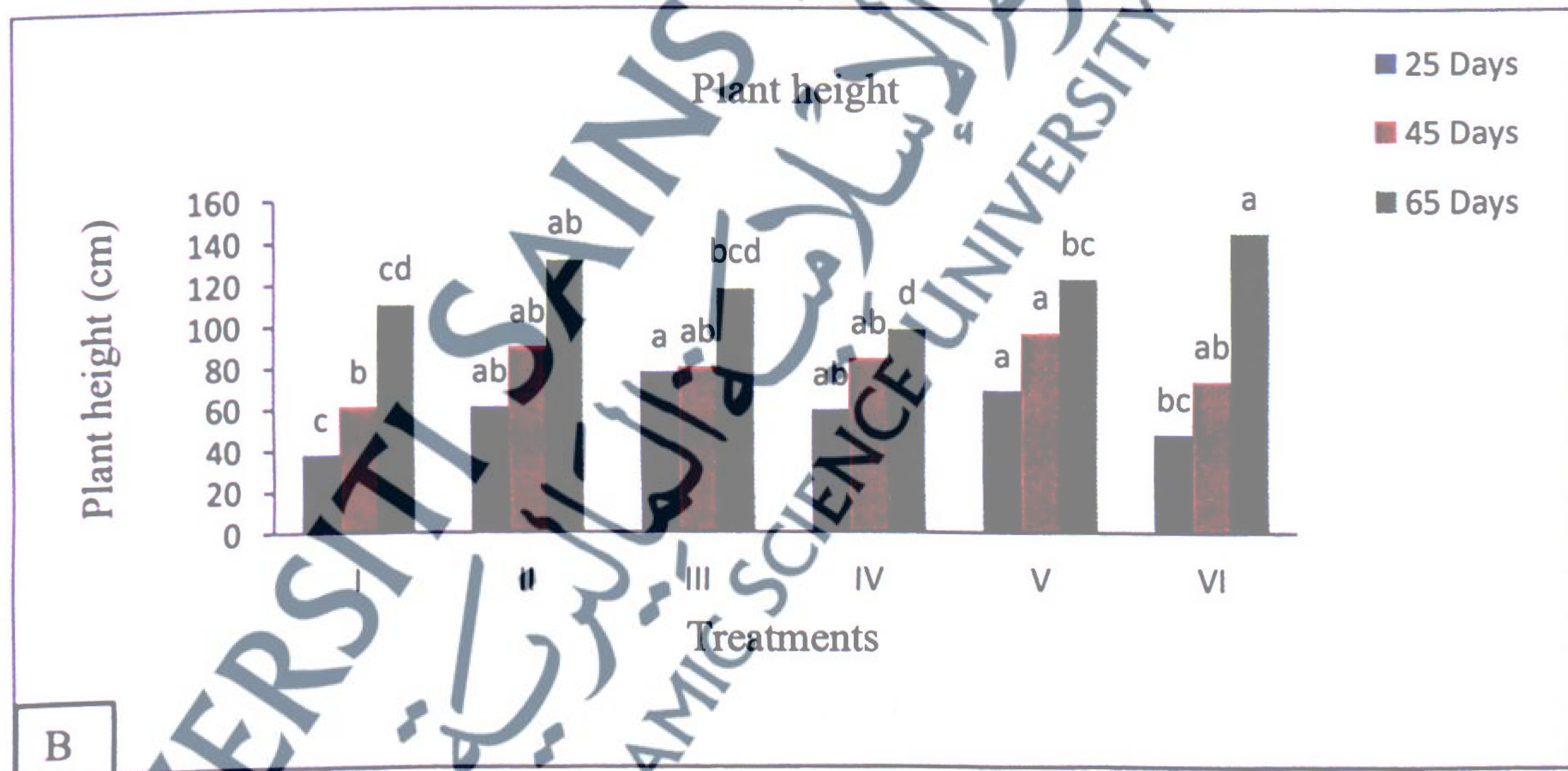
FIGURE 27: Effect of different treatments on plant canopy and plant height after 25, 45 and 65 d transplanting





Notes:- All data was analysed by one-way ANOVA (unstacked) with respect to separately 25 d, 45 d and 65 d and mean with the different superscript indicate significant differences between samples ($P<0.05$). Length of plant systems (A) canopy width (B) total plant height of treatments group plant; Group I: Chilli plants treated with water (positive control); Group II: chilli planted in soil treated with cells of LAB-MSS1; Group III: roots soaked in cells of LAB-MSS1 for 1h followed by planting in soil drenched with fungi *F. solani* CS; Group IV: chilli planted in soil treated with cells of LAB-FF11 for 1h planted in soil; Group V: roots soaked in cells of LAB-FF11 for 1h followed by planting in soil drenched with fungi *F. solani* CS, and Group VI: chilli planted in soil drenched with *F. solani*-CS (negative control

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However, the canopy width for chilli plants in group I, II and IV was significantly (P<0.05) increased from 69.50 to 80.66 cm after 65 d of transplanting (Figure 26B). The canopy width of group II and IV were 80.83 and 80.66 cm, respectively. In contrast chilli plants of group I (positive control) and group VI (negative control) were observed that to have a lower value of 69.50 cm and 53.00 cm, respectively (Figure 27B & C). Generally, the plant height of chilli plants increased significantly (P≤0.05) after 45 days transplanting except group I (Figure 27A). However, the plant height of chilli plants in group IV was lower (98.00) after 65 days compared to other treatments. The chilli plant group III showed the highest plant height value of 77.33 cm compared to other plant groups after 25 d of transplanting in Figure 27B.

6.6.1.2 Effect of different treatments on length of shoot and root

The shoot length of chilli plant was significantly (P<0.05) affected by treatments. It was observed that the chilli plant shoot length of group II, III, IV and V increased significantly (P<0.05) after 25 d transplanting (Figure 29 A). The shoot length showed value of 46.00 cm to 50.66 cm with ranges between 59.33 cm to 66.16 cm compared to shoot length of group I (positive control) and group VI (negative control) (Figure 29 B). Similarly, the different treatments affected the root length of chilli plants, especially group III that was observed to produce longer root length (27.66 cm) after 25 d transplanting compared to other treatments group of chilli plants (Figure 28).

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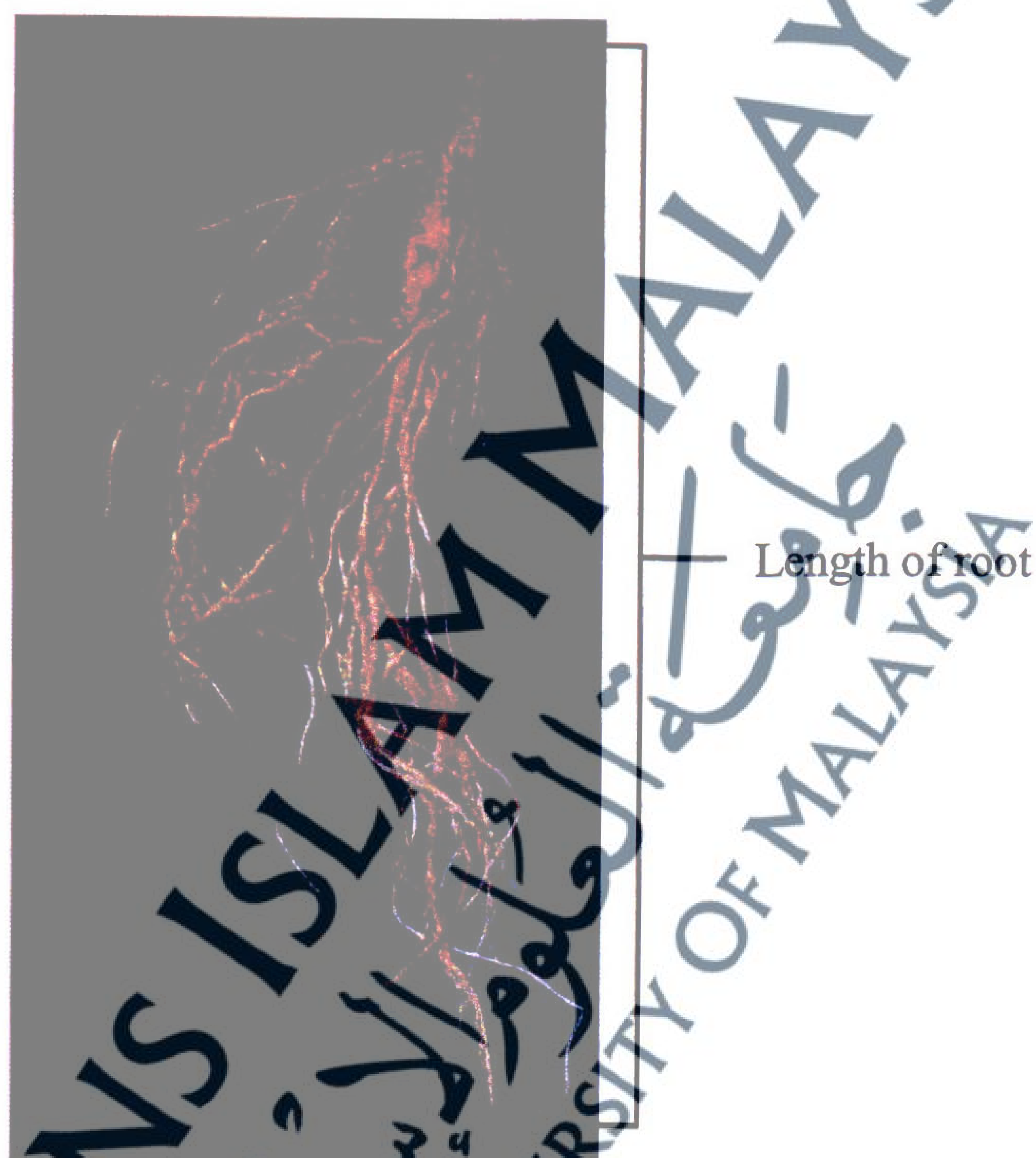
FIGURE 28: Chilli plant group III showed length of shoot after 25 d of transplanting



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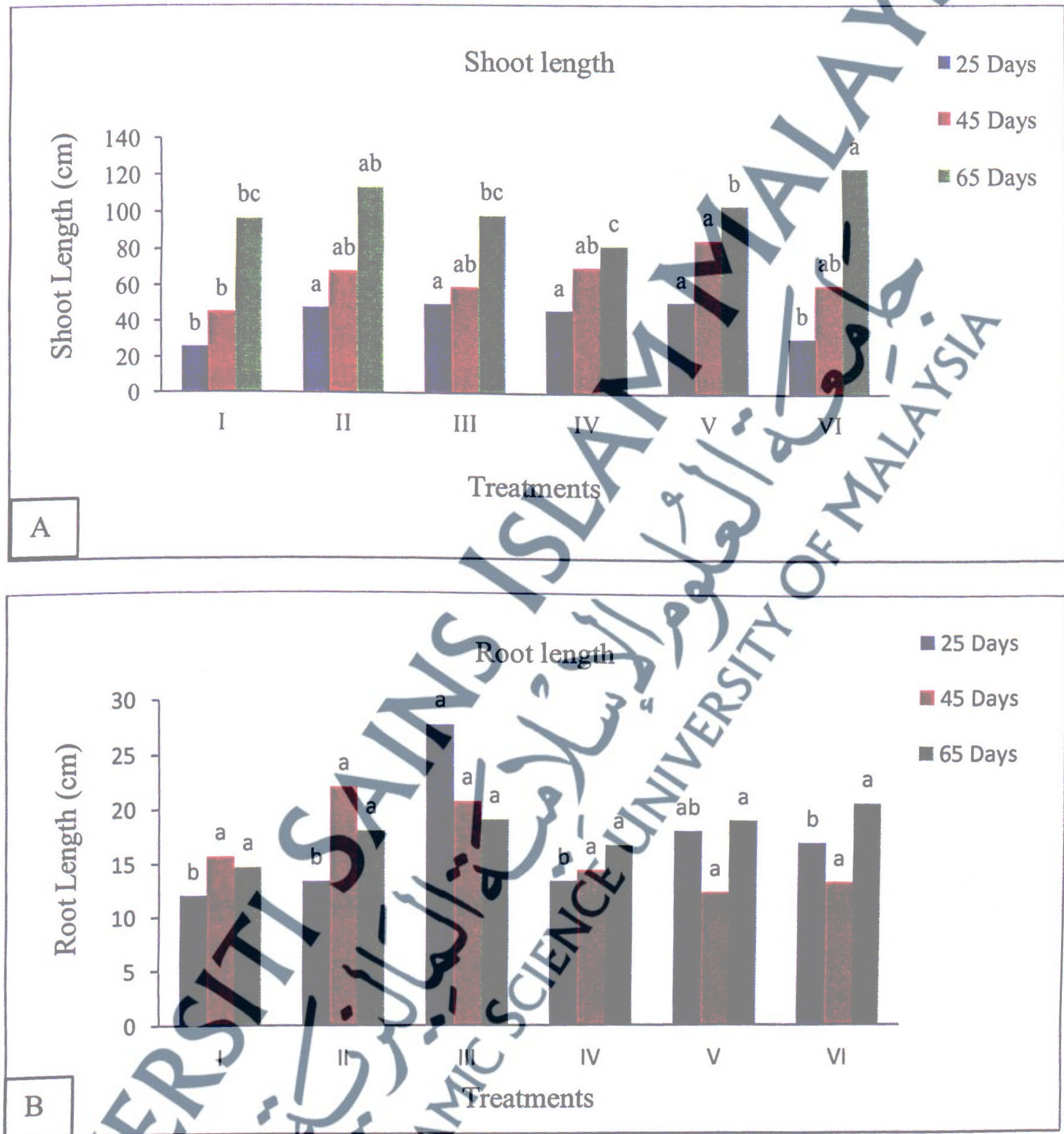
However, after 25 d of transplanting there was no observable increase in shoot length of group I and VI with value of 25.66 cm and 30.66 cm. In contrast, the root length of chilli plants increased significantly (P<0.05) after 45 days transplanting for group I, II and III and the observed values were 15.66 cm, 22.00 cm and 20.66 cm, respectively in Figure 29B compared to other groups respectively. However length of shoot and root were recorded significantly (P<0.05) longer in plant group VI and the longer of shoot value of 123.00 cm after 65 days transplanting respectively (Figure 30).

FIGURE 29: Effect of different treatments on plant shoot and root after 25, 45 and 65 d transplanting



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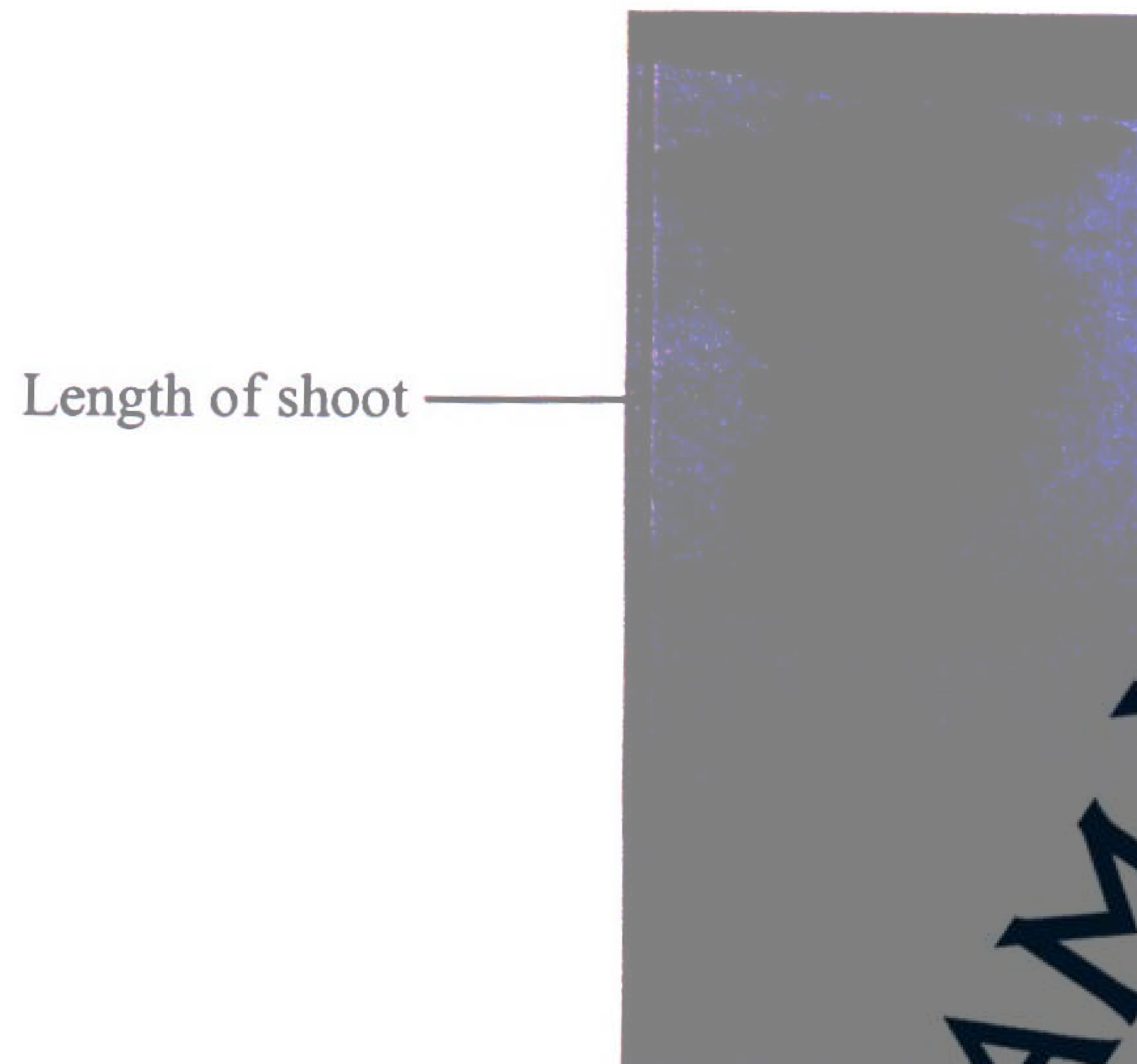
Notes:- All data was analysed by one-way ANOVA (unstacked) with respect to separately 25 d, 45 d and 65 d and mean with the different superscript indicate significant differences between samples ($P<0.05$). Length of chilli plant systems (A) length of shoot (B) root of treatments group plant; Group I: Chilli plants treated with water (positive control); Group II: chilli planted in soil treated with cells of LAB-MSS1; Group III: roots soaked in cells of LAB-MSS1 for 1h followed by planting in soil drenched with fungi *F. solani*-CS; Group IV: chilli planted in soil treated with cells of LAB-FF11 for 1h planted in soil; Group V: roots soaked in cells of LAB-FF11 for 1h followed by planting in soil drenched with fungi *F. solani*-CS, and Group VI: chilli planted in soil drenched with *F. solani*-CS (negative control)

FIGURE 30: Chilli plant group VI showed length of shoot after 65 d of transplanting



6.6.1.3 Effect of different treatments on fresh weight of plant, shoot and root

The weight of chilli plant was observed to increase significantly (P<0.05) after transplanting. The plants in group II, III, IV and V showed significantly higher in fresh weight with values ranged from 35.15 to 48.40 g compared to positive (group I) and negative (group VI) controls; the fresh weight of group I and VI plants were 28.97 g and 24.45 g, respectively (Figure 31A). However, no major changes was observed in the fresh shoot weight for all groups during 25 d of growth period except for fresh shoot weight of group III plants with value of 42.92 g/ plant. In contrast, pronounced changes in fresh shoot weight were observed after 45 days transplanting. It was observed that major changes in group V plants had greater fresh shoot value of 137.40 g/plants (Figure 31B).

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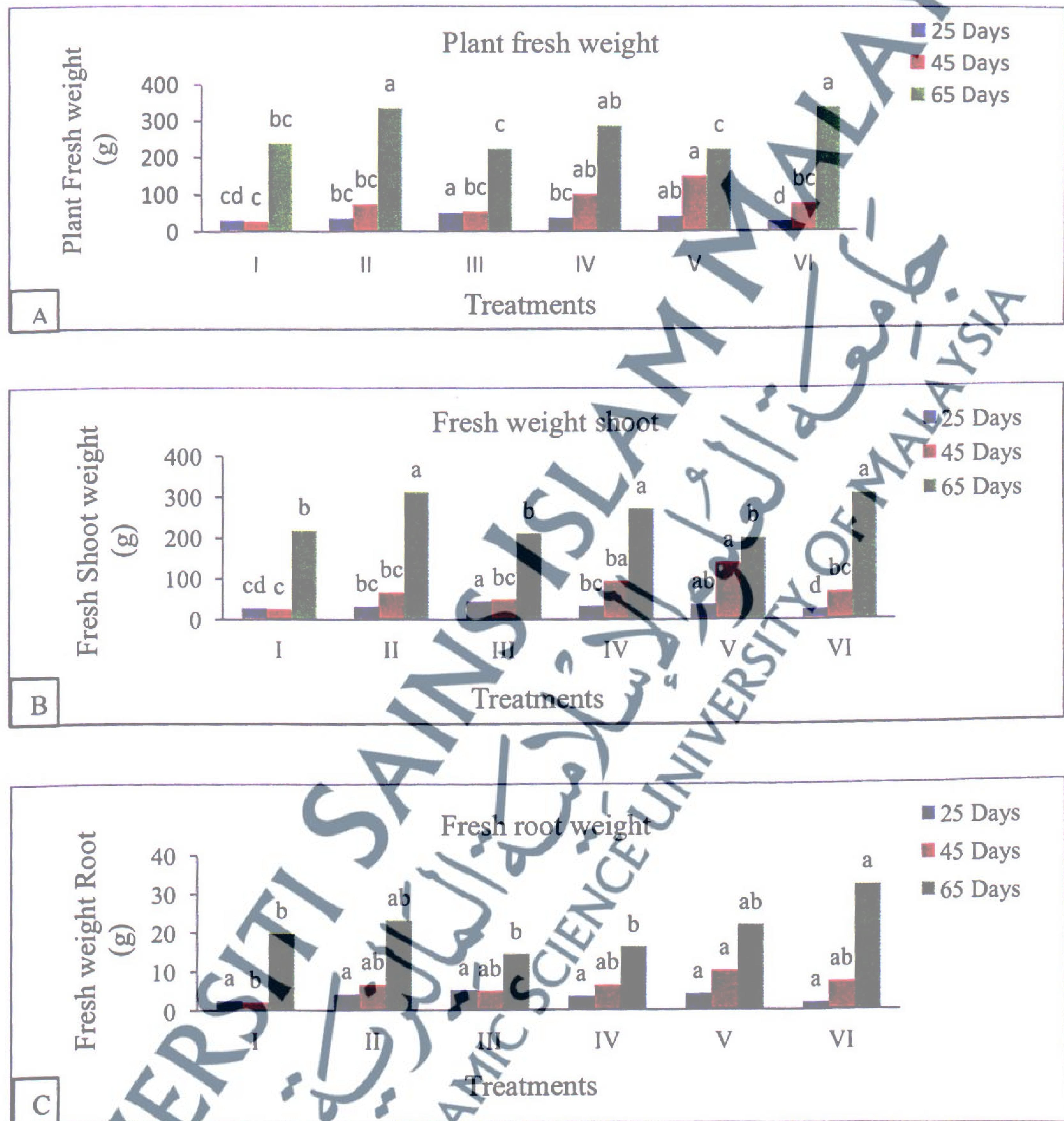
FIGURE 31: Effect of different treatments on weight of fresh plant parts after 25, 45 and 65 d transplanting



Notes:- All data was analysed by one-way ANOVA (unstacked) with respect to separately 25 d, 45 d and 65 d and mean with the different superscript indicate significant differences between samples (P<0.05). Fresh weight of chilli plant systems: (A) fresh weight of plant (B) fresh weight of shoot, (C) fresh weight of root of treatments; group plant; Group I: Chilli plants treated with water (positive control); Group II: chilli planted in soil treated with cells of LAB-MSS1; Group III: roots soaked in cells of LAB-MSS1 for 1h followed by planting in soil drenched with fungi *F. solani* CS; Group IV: chilli planted in soil treated with cells of LAB-FF11 for 1h planted in soil; Group V: roots soaked in cells of LAB-FF11 for 1h followed by planting in soil drenched with fungi *F. solani* CS, and Group VI: chilli planted in soil drenched with *F. solani*-CS (negative control)

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Further significant changes (P<0.05) was recorded for fresh weight plant, shoot and root for plants in group II and VI after 65 days transplanting compared to other group of chilli plant. The greater value of 32.12 g was recorded in plant group VI which survived in soil infected *F. solani*-CS (Figure 31C).

6.6.1.4 Effect of different treatments on dry weight of plant, shoot and root

The dry weight of chilli plant was observed to slightly to increase significantly (P<0.05) after transplanting. There was significant difference (P<0.05) in dry weight of group III compared to plants from other group after 25 d transplanting (6.89 g/plant) and after 45 d transplanting 6.14 g/plant (Figure 32A). The dry weight of plants from other groups was in the range of 2.84 g to 5.17 g/plant. The dry weight of plant increased significantly (P<0.05) for all plants after 65 d of transplanting, especially plant of group VI that received infected with the fungi. The recorded dry weight of plants in group VI was 77.32 g/plant higher than dry weight of other plant group range 60.72g to 41.58 g/plant. Similarly, plants of group V that were treated with LAB and the fungi produced shoots with significantly (P<0.05) higher dry weight values (Figure 32B). However, after 45 d of transplanting the dry weight of shoots from group V plants was 18.80 g/plant, while the dry weight of shoots from other plants were range 1.80 g to 13.23 g/plant. However, the major changes was observed in the dry shoot weight for all groups during 65 d of growth period but interestingly for dry shoot weight of group II plants showed with heavier value of 310.93 g/ plant compared to other treatments groups (Figure 32B).

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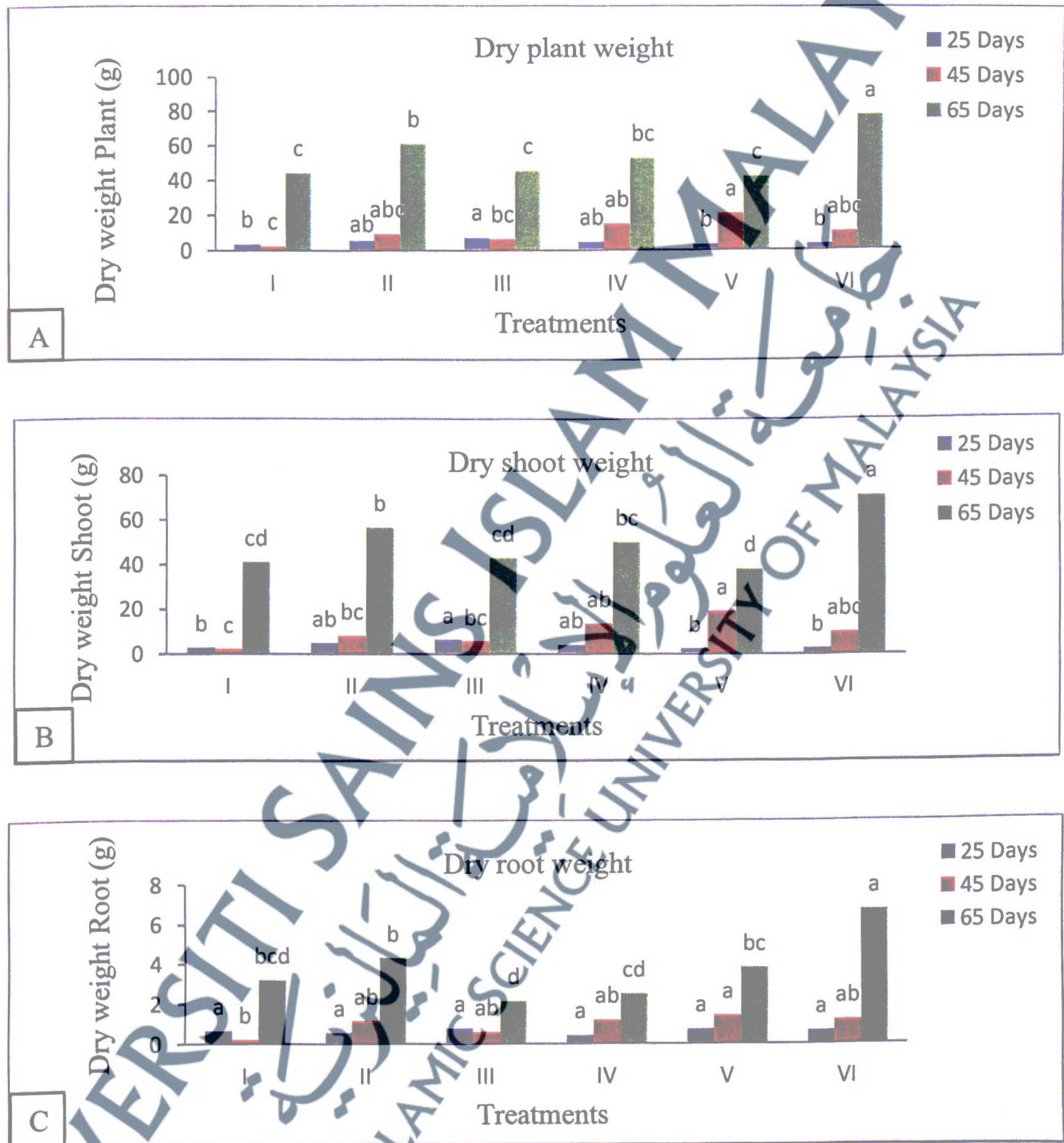
FIGURE 32: Effect of different treatments on weight of dry plant parts after 25, 45 and 65 d transplanting



Notes:- All data was analysed by one-way ANOVA (unstacked) with respect to separately 25 d, 45 d and 65 d and mean with the different superscript indicate significant differences between samples ($P<0.05$).Fresh weight of chilli plant systems: (A) dry weight of plant (B) dry weight of shoot, (C) dry weight of root of treatments; Group I: Chilli plants treated with water (positive control); Group II: chilli planted in soil treated with cells of LAB-MSS1; Group III: roots soaked in cells of LAB-MSS1 for 1h followed by planting in soil drenched with fungi *F. solani* CS; Group IV: chilli planted in soil treated with cells of LAB-FF11 for 1h planted in soil; Group V: roots soaked in cells of LAB-FF11 for 1h followed by planting in soil drenched with fungi *F. solani*-CS, and Group VI: chilli planted in soil drenched with *F. solani*-CS (negative control)

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Further no significant changes (P<0.05) were recorded for dry weight of root in all groups after 25 d transplanting (Figure 32C). However, significant changes (P<0.05) was recorded for dry weight of root in all group were showed value from ranges 0.55 g/plant root to 1.37 g/plant root after 45 d transplanting. In case of dry root weight of treatment chilli plant group VI was observed that highest after 65 d transplanting showed value of 6.74 g/plant. It was important that Value of dry plant weight, dry shoot weight and dry root weight after 65 d transplanting were increased in plant group II and VI especially Figure 32 (A, B & C).

6.6.2 Effect of LAB and fungi on chilli fruit characteristics

Chilli fruits reached marketable maturity after 90 d of transplanting. One of the signs of *Fusarium* infection in chilli plants is failure of the chilli fruits to reach maturity stage and resulting in fruit drops (Figure 33). Therefore, in this experiment the number of chillies both green and red formed in plants of each group was recorded as an indicator of successful biocontrol of LAB against phyto-pathogen *F. solani*-CS. However, fruits productivity were noticed fever in plant group I and group VI, thus, the pathogenicity of fungi *Fusarium solani*-CS and nutritional effect both were observed between fruiting to repning of fruits. Incase of productivity of chilli fruits were noticed to increase in plant group II, III, IV and V, because, these groups plants were survived in soil treated LAB cells.

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FIGURE 33: Some *F.solani*-CS infection in chilli plants showing failure of the fruits to reach maturity stage and resulting in fruit drops

Notes:- Surviving plant in soil infected with *F. solani*-CS caused wilting disease before turned from green into red

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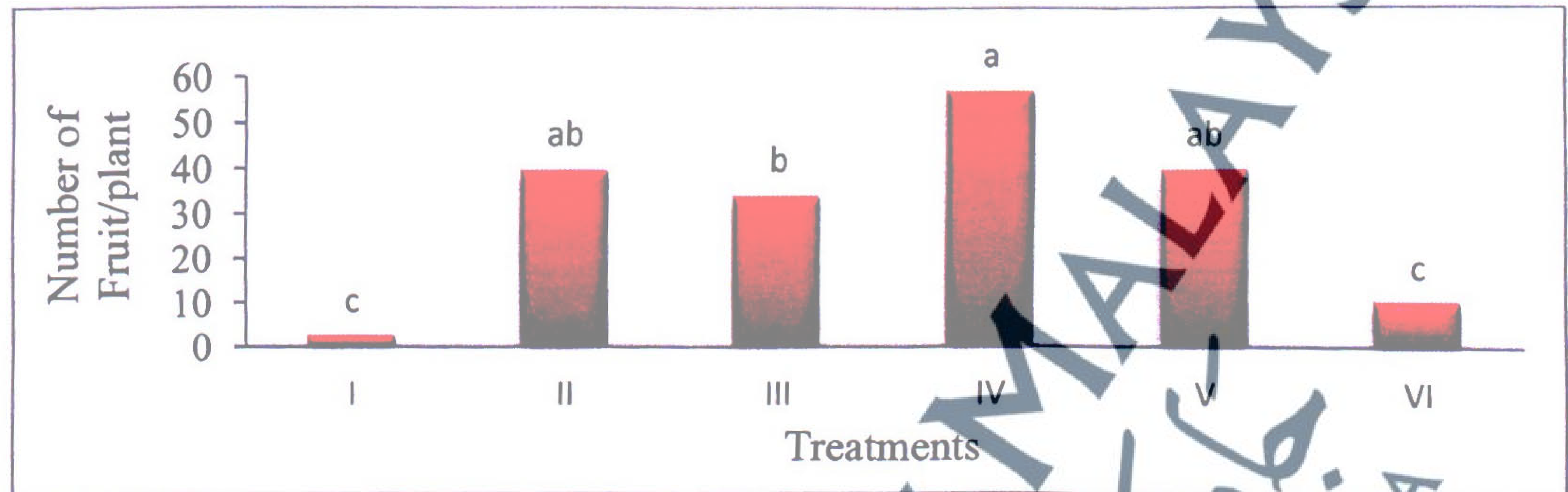
6.6.2.1 Fruit and seed productivity of chilli plants given different treatments

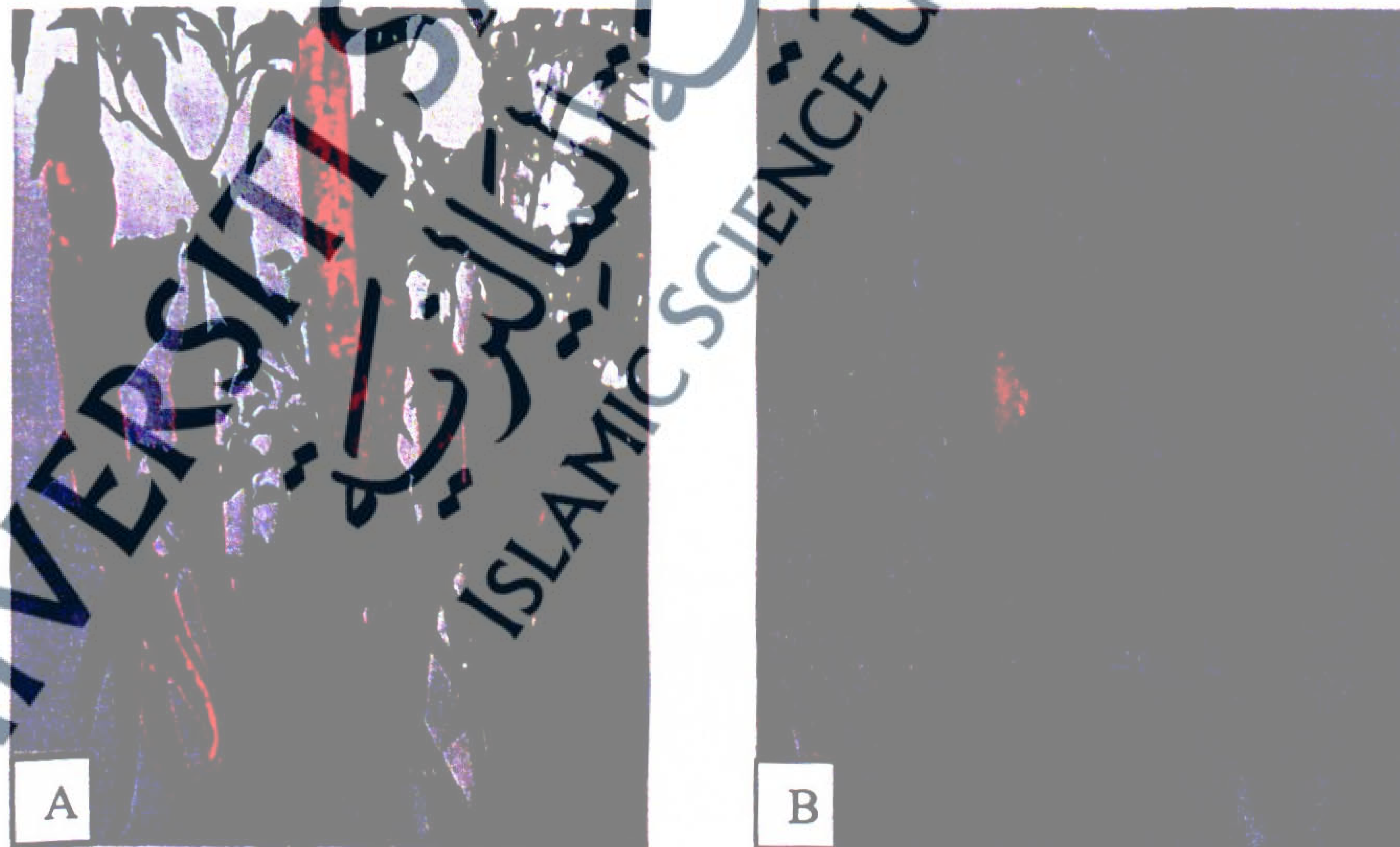
Plants given the LAB treatments and LAB with fungi produced significantly ($P<0.05$) more fruits (both red and green matured fruits) than plants from group-I (positive) and plant group-VI (negative) controls in Figure 30. Approximately 56.33 fruits /plant was collected from plant group IV, significantly ($P<0.05$) more fruits than from plants of group II, III and V that produced 33.33 to 39.00 fruits per plant after 90 days transplanting in Figure 34A. In contrast the plants from group-I (positive control) and group-VI (negative controls) produced few fruits about 2.00 and 9.33 fruits per plant, respectively (Figure 34B). The group I plants did not able to survived further and produce fruits only three small fruits were received before turning in redness.

Figure 34: Effect of different treatments on number of fruits per plant after 90 d transplanting



Notes:- All data was analysed by one-way ANOVA (unstacked) and mean with the different superscript indicate significant differences between samples (P<0.05). Treatments of plants Group I: Chilli plants treated with water (positive control); Group II: chilli planted in soil treated with cells of LAB-MSS1; Group III: roots soaked in cells of LAB-MSS1 for 1h followed by planting in soil drenched with fungi *F. solani* CS; Group IV: chilli planted in soil treated with cells of LAB-FF11 for 1h planted in soil; Group V: roots soaked in cells of LAB-FF11 for 1h followed by planting in soil drenched with fungi *F. solani*-CS, and Group VI: chilli planted in soil drenched with *F. solani*-CS (negative control)

FIGURE 35: Chilli fruits on plants receiving LAB-FF11 and infected with fungi *Fusarium solani*-CS



Notes:- (A) Chilli fruits on plants receiving LAB-FF11 showed to increase productivity and (B) infected with fungi *Fusarium* solani-CS showed decrease the productivity

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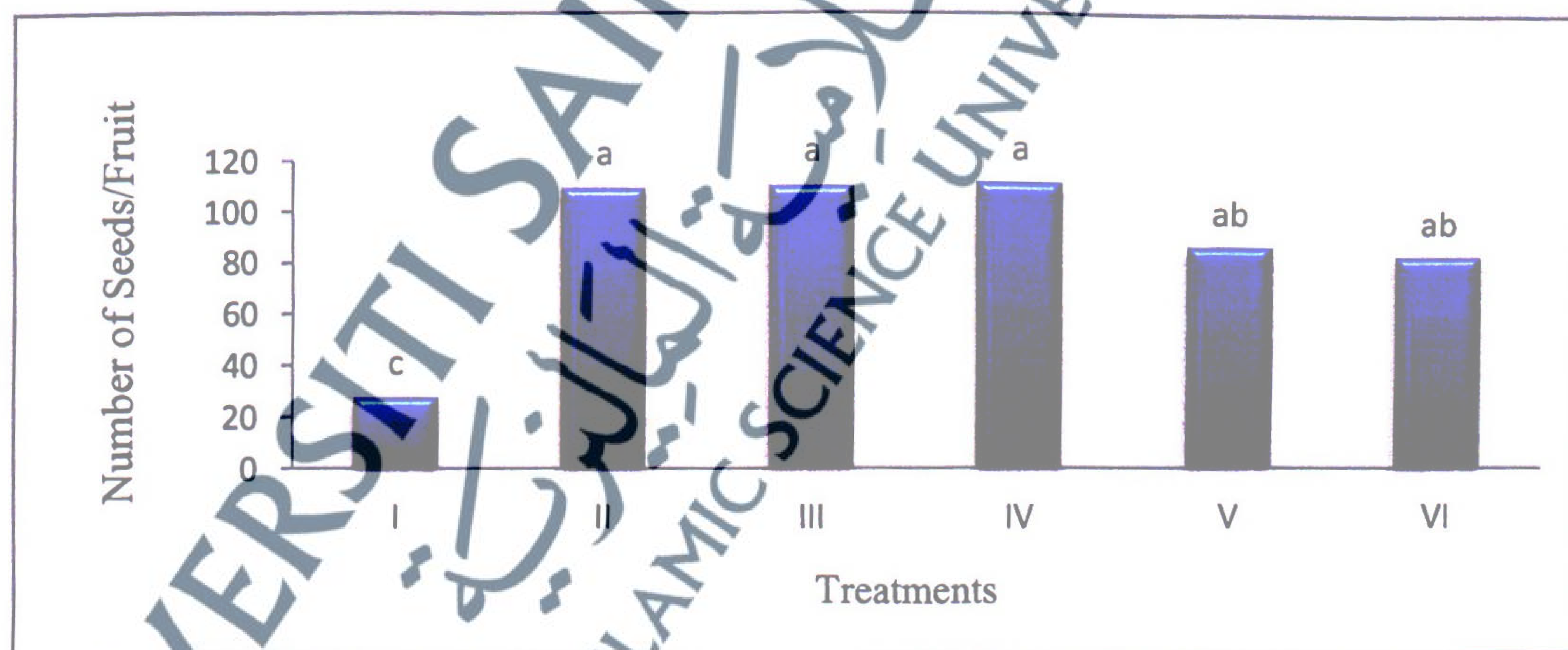
The number of seeds per fruit produced per plant was significantly (P<0.05) affected by the different treatments. It seems that more seeds were produced in fruits from plants that were treated with LAB even in the presence of the fungi (Figure 36) after 90 days mature fruits. This was evidence by the number of seeds in the range of 86.20 to 111.20 seeds/fruit from chilli plants of group II, III, IV and V. The maximum number of seeds per fruit 111.20 from plants group IV treatment with LAB-FF11. Less number of seeds was observed produced by plants that received no treatment (group I, positive control) and plants infected with the fungi (group VI, negative control); the number of seeds recorded was 26.40 and 82.60 seeds per fruit, respectively.

FIGURE 36: Effect of different treatments on number of seeds per plant after 90 d transplanting



Notes:- All data was analysed by one-way ANOVA (unstacked) and mean with the different superscript indicate significant differences between samples (P<0.05). Treatments of plants Group I: Chilli plants treated with water (positive control); Group II: chilli planted in soil treated with cells of LAB-MSS1; Group III: roots soaked in cells of LAB-MSS1 for 1h followed by planting in soil drenched with fungi *F. solani* CS; Group IV: chilli planted in soil treated with cells of LAB-FF11 for 1h planted in soil; Group V: roots soaked in cells of LAB-FF11 for 1h followed by planting in soil drenched with fungi *F. solani* CS, and Group VI: chilli planted in soil drenched with *F.* solani-CS (negative control).

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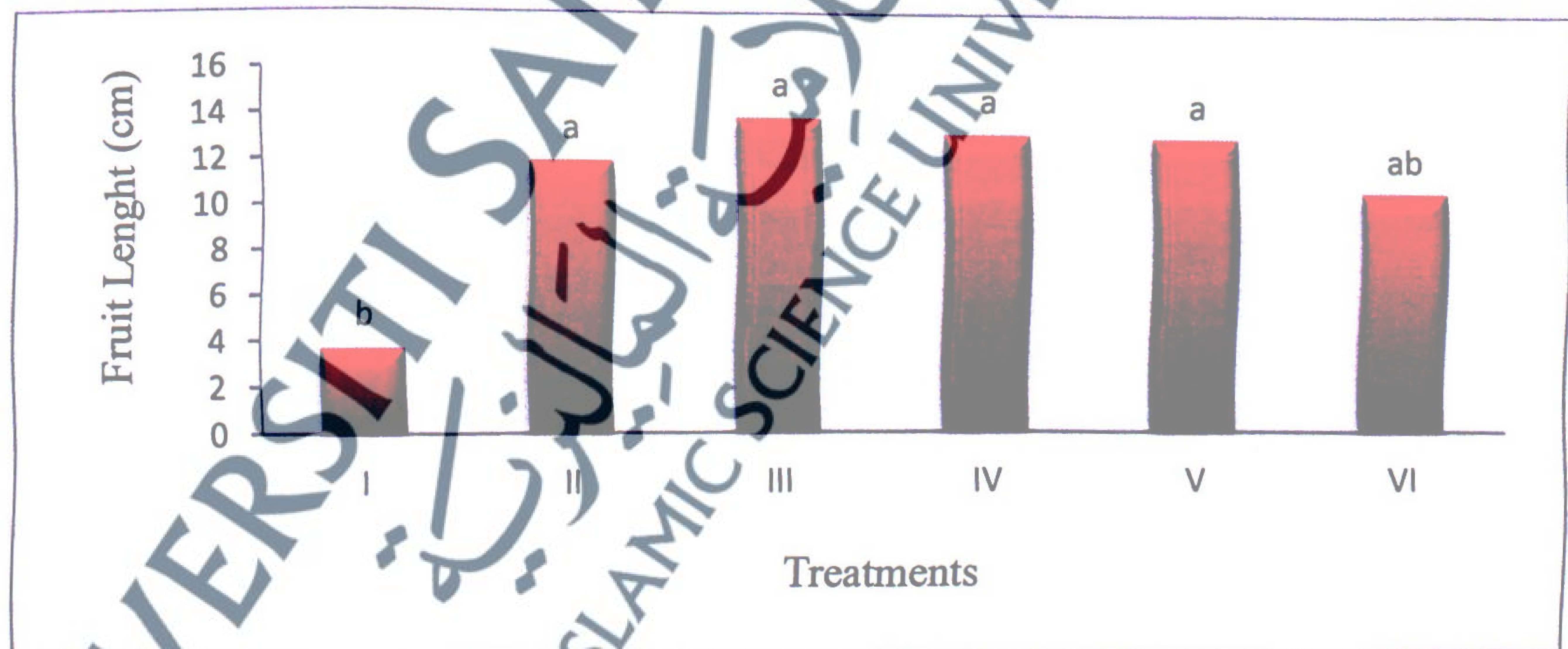
6.6.2.2 Effect LAB and fungi on fruit length and pericarp thickness

Application of LAB to the soil significantly (P<0.05) affected the fruit length, circumference and pericarp thickness (Figure 37). The fruits were longer in length with an average of 13.66 cm/fruit for plants from group III, 11.72cm/fruit from plants of group II, 12.86 cm/fruit from plants of group V and to 13.02 cm/fruit from plants of group IV maturity Figure 38 (A, B, C & D) then plants from group I (3.54 cm/fruit) and this fruits dropped before turning green to red. However, plants infected with the fungi alone also produced relatively long fruits (10.50 cm/fruit) but the some plants fruits were water soaked , some plants fruits were turned not in to red properly and therefore theses did not acceptable for consumers in Figure 38 E and Figure 39) after 90 days transplanting.

Figure 37: Effect of different treatments on fruit length of chilli



Notes:- All data was analysed by one-way ANOVA (unstacked) and mean with the different superscript indicate significant differences between samples (P<0.05). Fruit collected Treatments plants Group I: Chilli plants treated with water (positive control); Group II: chilli planted in soil treated with cells of LAB-MSS1; Group III: roots soaked in cells of LAB-MSS1 for 1h followed by planting in soil drenched with fungi *F. solani* CS; Group IV: chilli planted in soil treated with cells of LAB-FF11 for 1h planted in soil; Group V: roots soaked in cells of LAB-FF11 for 1h followed by planting in soil drenched with fungi *F. solani* CS, and Group VI: chilli planted in soil drenched with *F. solani*-CS (negative control).

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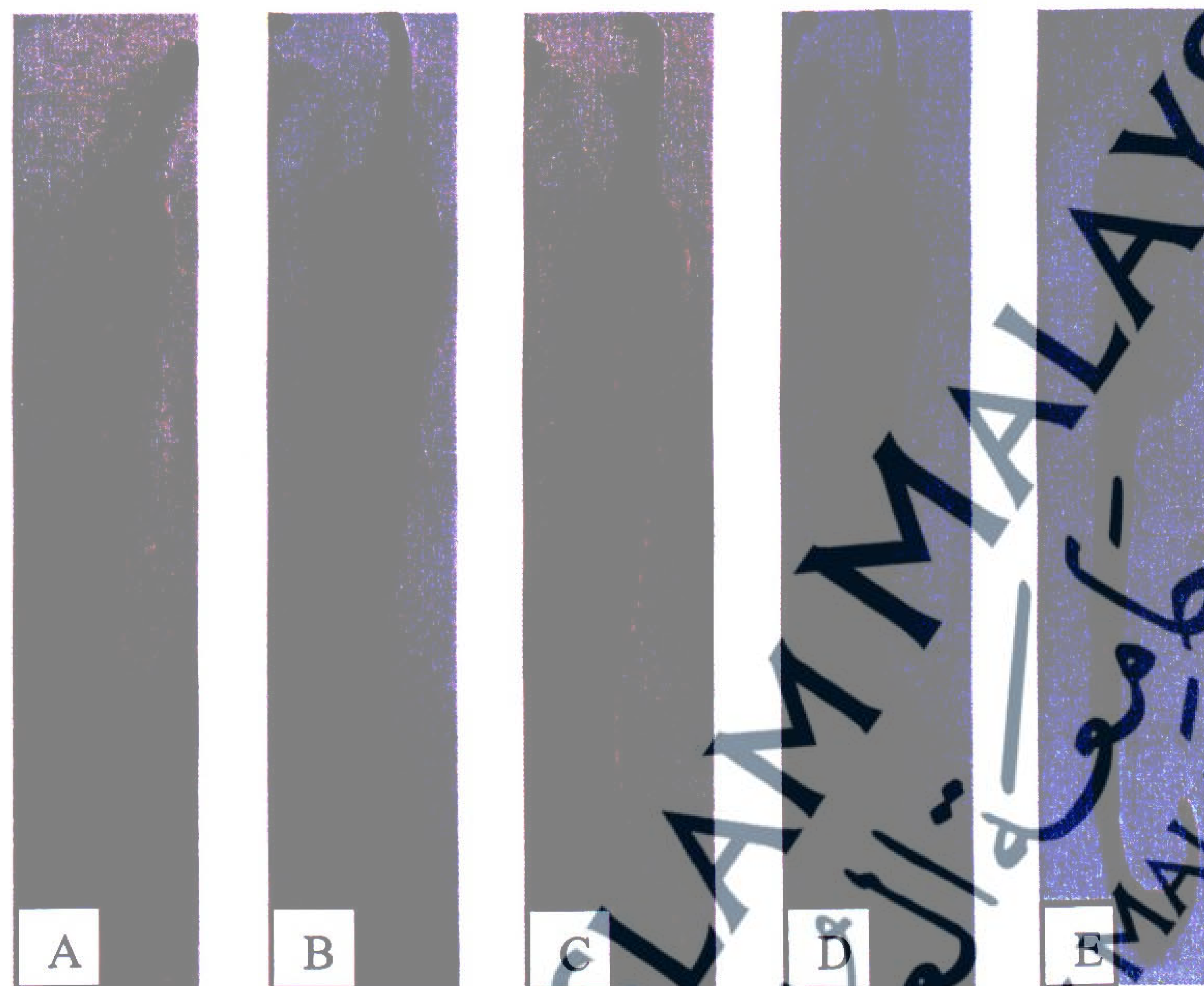
FIGURE 38: Appearance of chilli fruits from plants groups treated with LAB and fungi



Notes:- Fruits received from treatments of plants (A) Group II: chilli planted in soil treated with LAB-MSS1; Group III: roots soaked in cells of LAB-MSS1 for 1h followed by planting in soil drenched with fungi *F. solani* CS; Group IV: chilli planted in soil treated with cells of LAB-FF11 for 1h planted in soil; Group V: roots soaked in cells of LAB-FF11 for 1h followed by planting in soil drenched with fungi *F. solani*-CS, and Group VI: chilli planted in soil drenched with *F. solani*-CS (negative control).

FIGURE 39: The fruits showing water soaked received plants group VI

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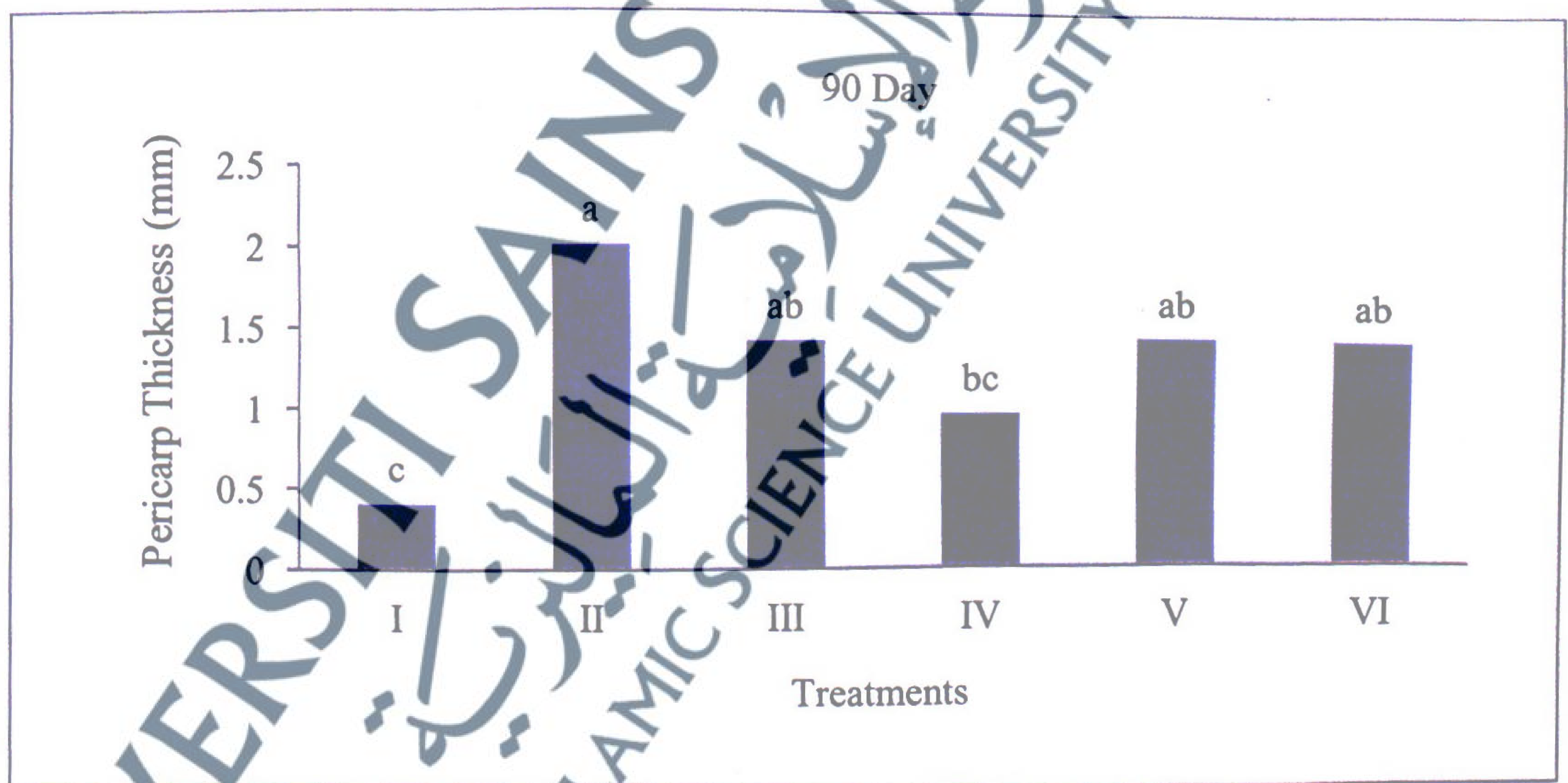
Similarly, the pericarp thickness of chilli fruits was significantly (P<0.05) affected by the treatments Figures 40. A significantly (P<0.05) thicker pericarp of 2.00 mm in Figure 41 was recorded from fruits obtained from plants of group II that was treated with LAB-MSS1 compared to the fruit pericarp thickness of negative and positive controls group plants (Figure 41A). Interestingly, the fruit pericarp from group II plants was also thicker than the pericarp of fruits from plant of group I, III, IV V and VI; the thickness of fruit pericarp ranged between 0.40 mm to 1.41 mm. However, the plants from group VI also produced fairly thick pericarp of 1.40 mm which was received from fungi infected plants (Figure 41B) after transplanting.

FIGURE 40: Pericarp thickness of fruit receiving from different plant group



Notes:- All data was analysed by one-way ANOVA (unstacked) and mean with the different superscript indicate significant differences between samples (P<0.05). Treatments of plants Group I: Chilli plants treated with water (positive control); Group II: chilli planted in soil treated with cells of LAB-MSS1; Group III: roots soaked in cells of LAB-MSS1 for 1h followed by planting in soil drenched with fungi *F. solani* CS; Group IV: chilli planted in soil treated with cells of LAB-FF11 for 1h planted in soil; Group V: roots soaked in cells of LAB-FF11 for 1h followed by planting in soil drenched with fungi *F. solani* CS, and Group VI: chilli planted in soil drenched with *F. solani*-CS (negative control).

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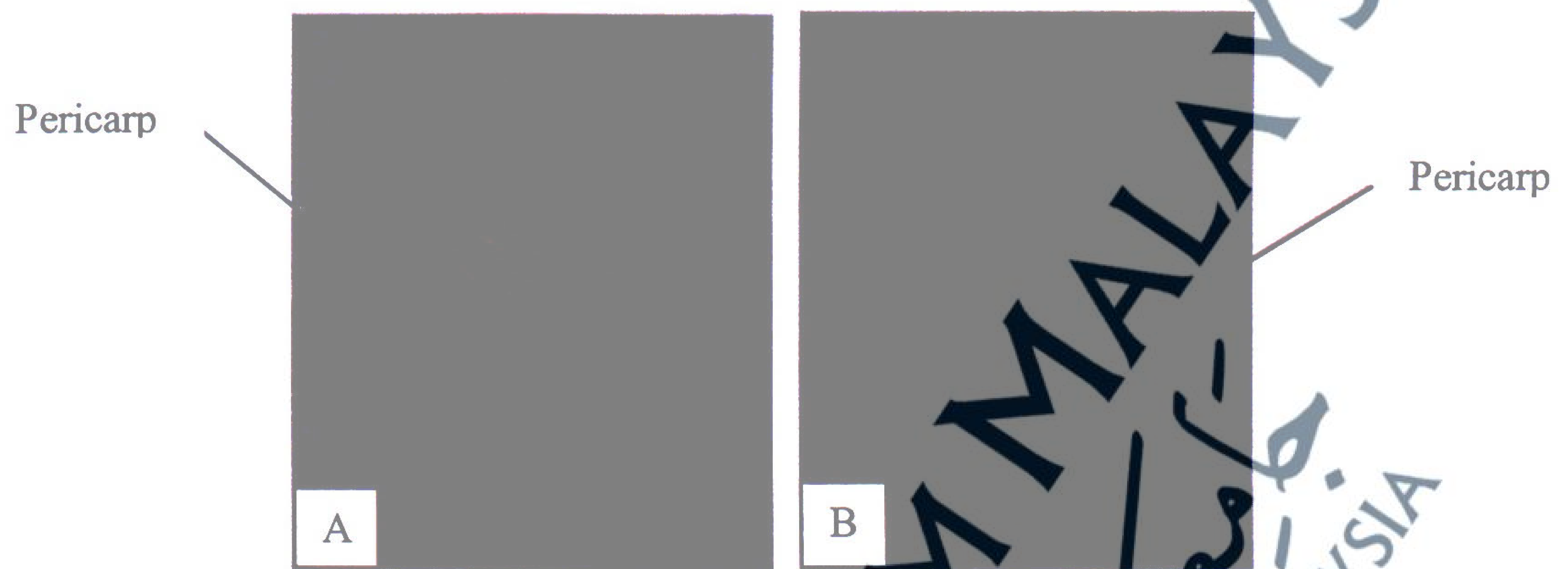
FIGURE 41: Pericarp thickness of chilli fruits



Notes:- Receiving fruits; (A) plant group II showing thicker pericarp with fresh placenta and seeds (B) group VI (negative control) showing spoilage blackness placenta and seeds before turning into redness

6.6.2.3 Effect of LAB and fungi on weight of fresh and dry fruit of chilli

The fresh and dry weight of fruits was significantly ($P<0.05$) affected by treatments given to the chilli plants. It was observed that the weight of fresh chilli fruits from group II, III, IV and VI was significantly ($P<0.05$) higher than fruits from Group I. The fresh weight of fruits from Group II, III, IV, V and VI were between 9.51 to 16.80g/fruit compared to fresh weight of fruit group I (positive control) with value of 3.43g/fruit in Figure 42A. It was observed that fruits treated with fungi alone produce heavier weight (16.80 g/fruit) compared to other fruits, but the fruit was water-soaked and of unacceptable quality. However, the dry weight of chilli fruits was not affected ($P>0.05$) by the different treatments. The dry weight of fruits from all the plants groups from was in the range of 0.25 g/fruit to 1.64 g/fruit in Figure 42B after maturity.

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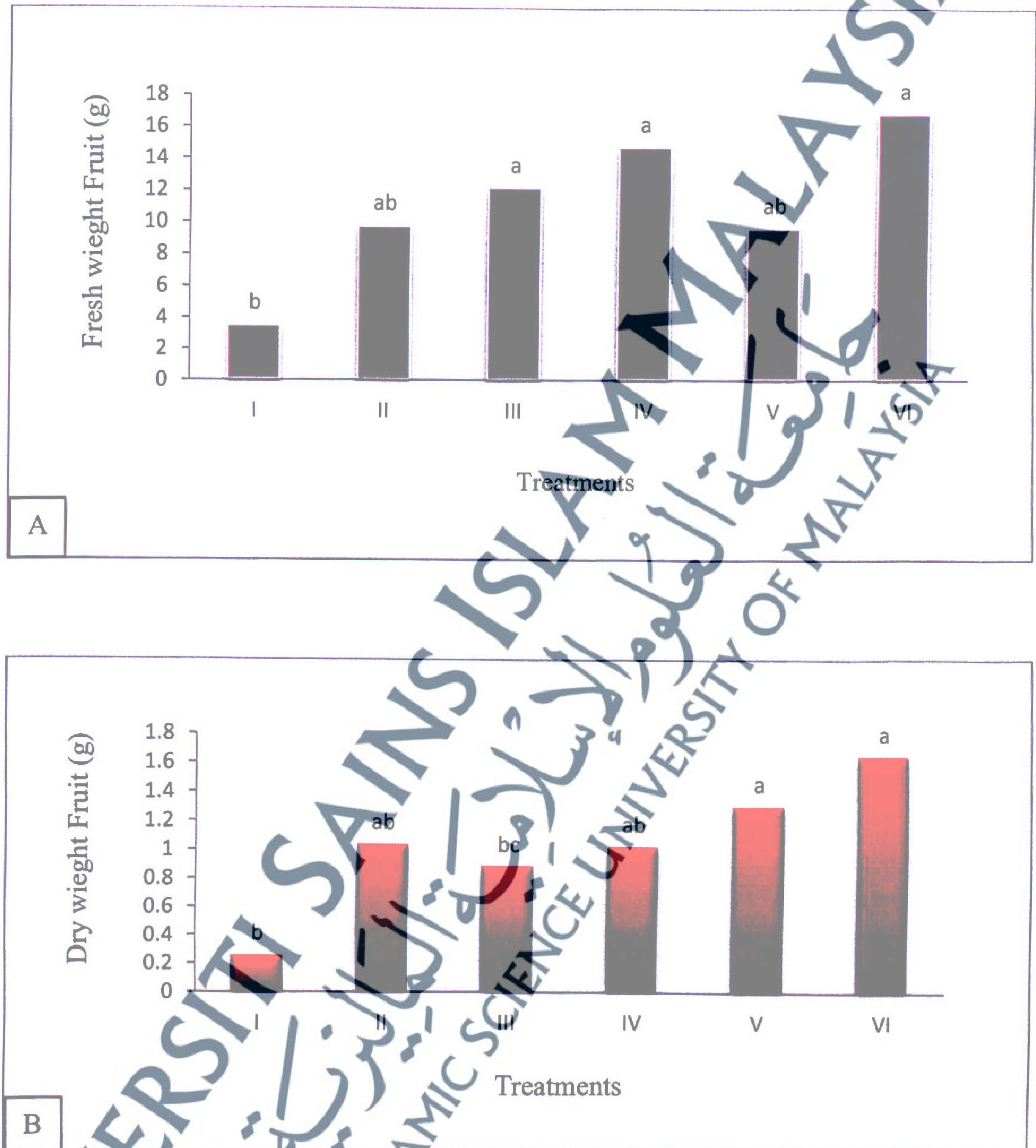
FIGURE 42: Effect of different treatments on fresh and dry weight chilli fruits



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Notes:- All data was analysed by one-way ANOVA (unstacked) and mean with the different superscript indicate significant differences between samples ($P<0.05$). (A) fresh weight of fruit and (B) dry weight of fruit; Treatments of plants Group I: Chilli plants treated with water (positive control); Group II: chilli planted in soil treated with cells of LAB-MSS1; Group III: roots soaked in cells of LAB-MSS1 for 1h followed by planting in soil drenched with fungi *F. solani* CS; Group IV: chilli planted in soil treated with cells of LAB-FF11 for 1h planted in soil; Group V: roots soaked in cells of LAB-FF11 for 1h followed by planting in soil drenched with fungi *F. solani* CS, and Group VI: chilli planted in soil drenched with *F. solani*-CS (negative control).

6.7 Confirmation of LAB and fungi treated plant parts and soil

6.7.1 Confirmation of both LABs isolates

Isolates LAB-MSS1 and LAB-FF11 were soaked and drenched in soil during developing time of chilli plant and were confirmed in plant stem, placenta, root stem, seeds and soil. These isolates showed clear zone on the modified MRS agar with 0.7% $CaCO_3$ and were catalase negative when tested with 4% H_2O_2 Table 37 and Figure 43 A, B, C and A1, B1 C1 on the modified MRS agar with 0.7% $CaCO_3$. Microscopic observation showed that the LAB isolates were rod shaped. The re-isolated LAB microscopic observation showed similar characterization and morphology of colony in Figure 44 (A & B) compared with as previously described in Chapter III, Section 4.9.1. The re-isolated LAB from different plant parts showed endophytic bacterial nature which has the ability to enter easily into plants by using nutritional ways.

TABLE 37: Confirmation of LAB in inoculated chilli plant parts and soil

Sources	Isolates					
	LAB-MSS1			LAB-FF11		
	Catalase tests	Gram's stain test	Shapes	Catalase tests	Gram's stain test	Shapes
Stems cells	-	+	Rod	-	+	Rod
Roots	-	+	Rod	-	+	Rod
Placenta	-	+	Rod	-	+	Rod
Seeds	-	+	Rod	-	+	Rod
Soil	-	+	Rod	-	+	Rod

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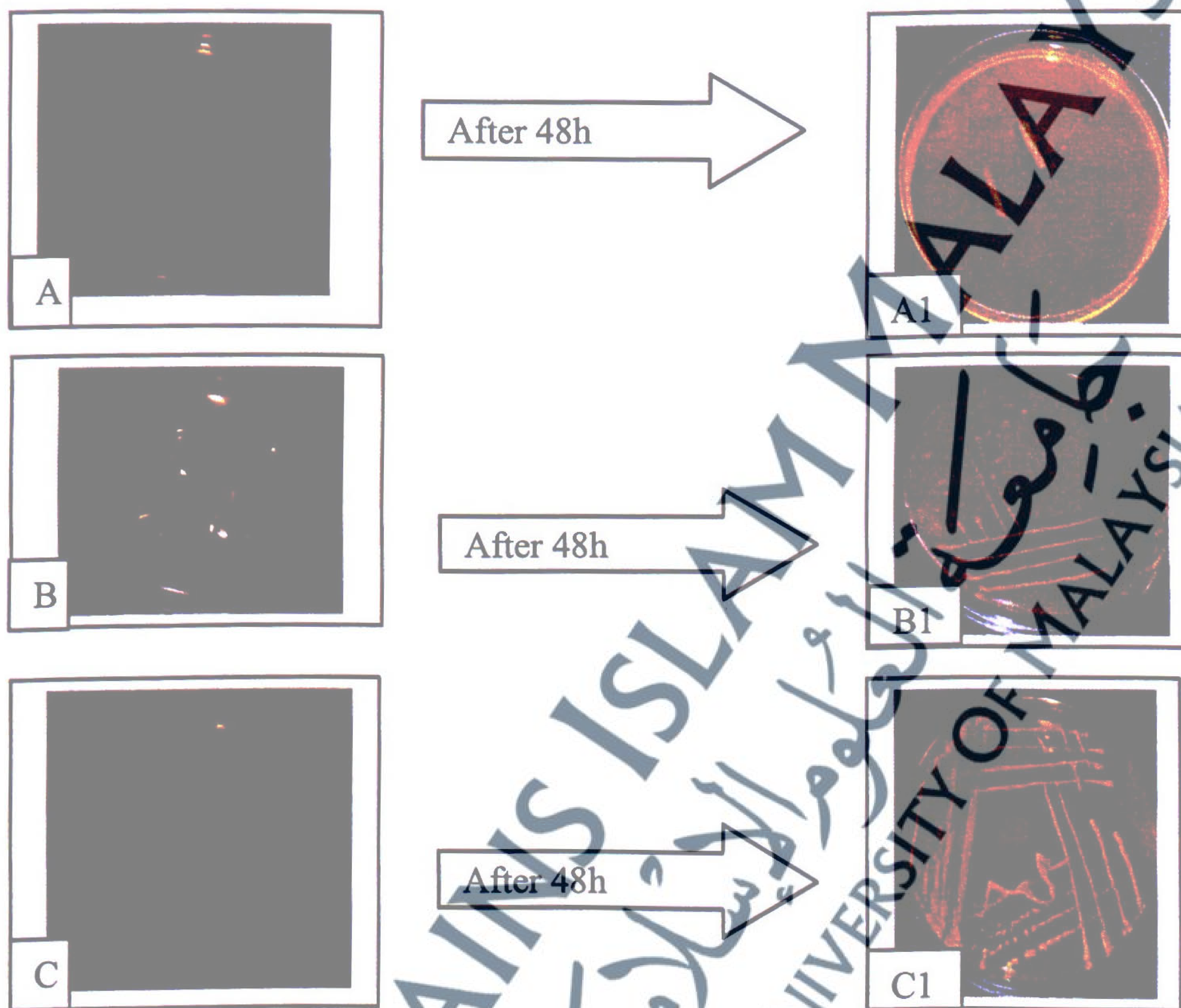
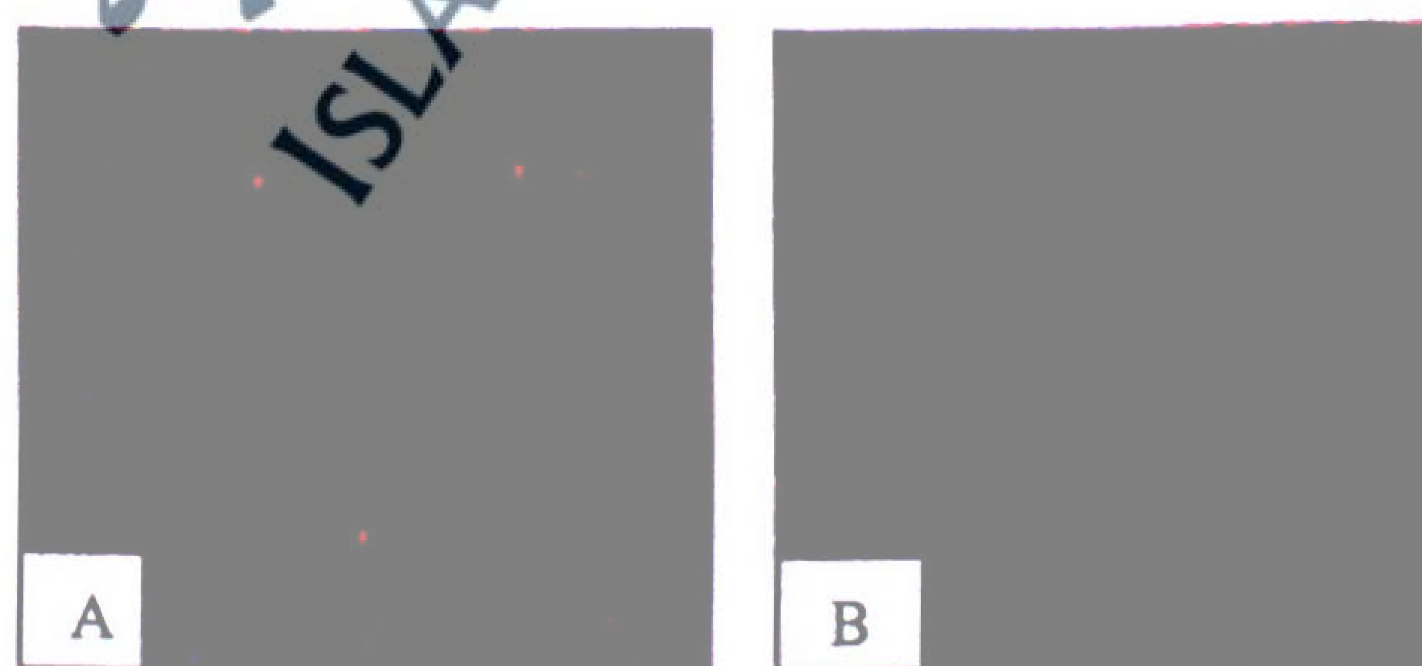
FIGURE 43: Confirmation of endophytic LAB from inoculated plants



Notes:- (A) = control and A1 after incubation 72 h at 37°C showed no growth, (B) = LAB-MSS1 and (C) = LAB-FF11 from stem showed white colony growth confirmed as the endophytic nature; B1 and C1 were showed pure colony LAB-MSS1 and LAB-FF11on MRSA with $CaCO_3$ after 24h incubation at 37°C

FIGURE 44: Isolation of lactic acid bacteria (LAB) isolated from treated plants showing the shapes and size under the microscopic observation



Notes:- Microscopic morphology (A) = LAB-MSS1 and (B) = LAB-FF11were showed rod shape

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6.7.2 Confirmation of fungi *F. solani*-CS isolate

Phytopathogenic fungi *F. solani*-CS was confirmed that which were inoculated in soil during plants transplanting time and isolated different plant parts (stem, placenta, root stem and seeds). Identification of the fungal re-isolates followed the description previously described in Chapter III, Section 4.9.2. Fungal isolated from inoculated soil and plants parts were clearly endophytic in nature and entered into plants through the root vessels Table 38). The re-isolated *F. solani*-CS microscopic observation showed similar characterization and morphology of colony compared with previously isolate in Figure Figure 45 A, A1, B and C after 95 day transplanting of chilli plant.

TABLE 38: Confirmation of *F. solani*-CS in infected chilli plant parts and soil

Sources	Fungal with *F. solani*-CS
Stems cells	Positive
Roots	Positive
Placenta	Positive
Seeds	Positive
Soil	Positive

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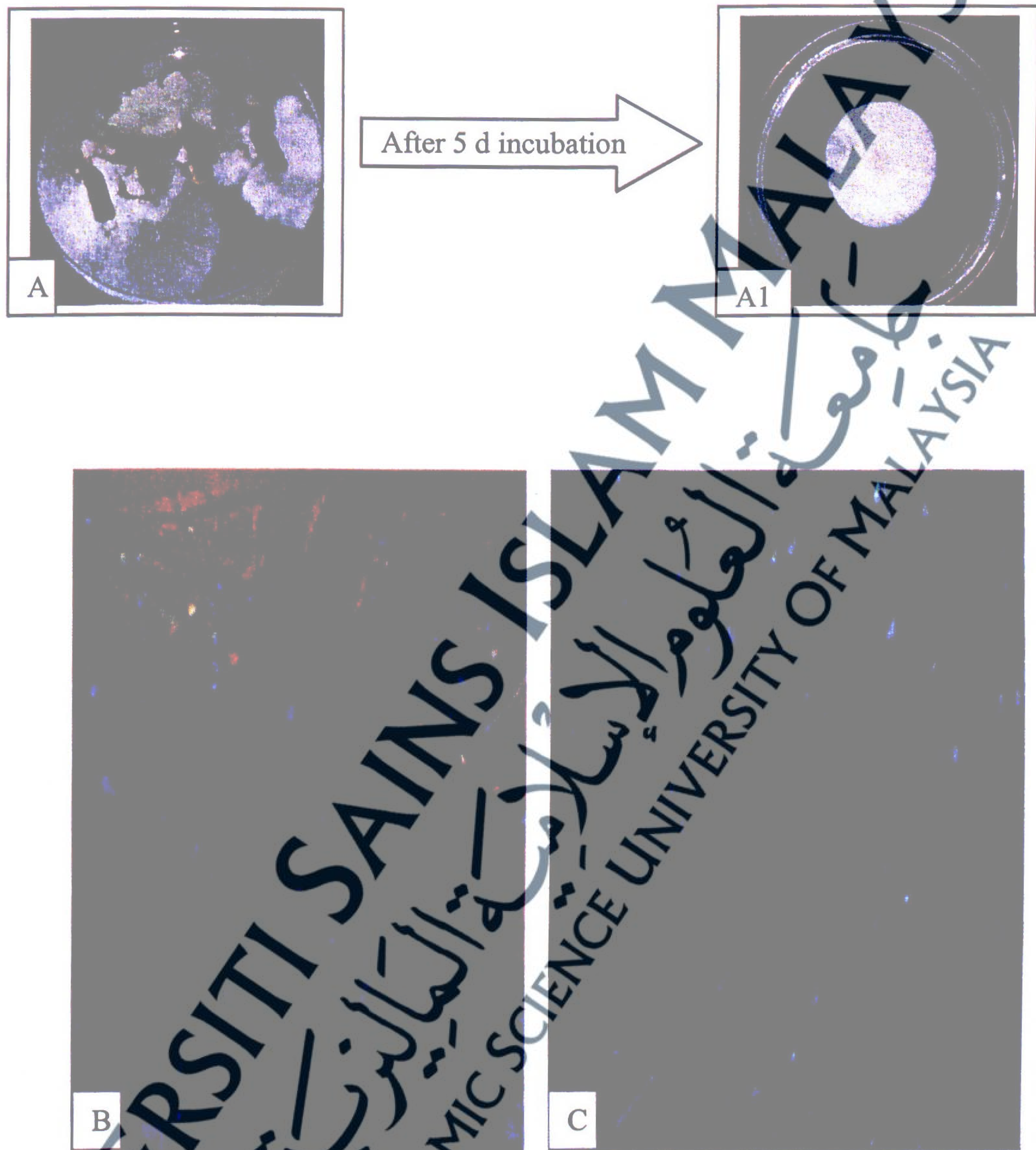
FIGURE 45: Confirmation of *Fusarium solani*-CS from treated chilli



Notes:- A= *F. solani*-CS from stem showed mycelia growth confirmed the endophytic nature and B1 showed pure colony of *F. solani*-CS on PDA after 5 d incubation at 28°C, B= areal mycelia and C= mycelia with spores micro scopic observation

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6.8 DISCUSSION

6.8.1 Effect of different treatments on plant canopy and plant height

Interaction between plants and bacteria is known to have positive effects on plant growth. Microorganisms such as *Bacillus* spp. and *Streptomyces* sp. isolated from rhizospheric soil promoted plant growth of chilli plants by enhancing plant-height and canopy-width after 135 d transplanting (Moumita et al., 2011). It was observed that growth of chilli plants treated with *Lb. plantarum* LAB-C5 and *Lb. pentousis* LAB-G7 was enhanced El-Mabrouk (2014). LAB isolates (KLF01, KLC02 and KPD03) were used to enhance the plants growth systems or plant growth parts significantly (P<0.05) increased canopy and plant height of chilli plant in greenhouse, however, these isolates were not identified. Similar observation was noted in this study chilli plants treated with LAB-MSS1 and LAB-FF11 also increased the growth of plants (Figures 27A). However, chilli plant group II a greater canopy area (80.83 cm) per plant (Figure 26A) after 65 days transplanting and the chilli plant group III showed the highest plant height value of 77.33 cm compared to other plant groups after 25 d of transplanting. This results were recorded similar to Anupama (2014) reported that LAB is one of the alternative method to choose to avoid hazardous compounds and identified as biocontrol agents such as LAB isolates (KLF01, KLC02 and KPD03) were used to enhance the plants growth systems or plant growth parts significantly (P<0.05) increased canopy and plant height of chilli plant in greenhouse. Similarly, other microorganisms such as *Bacillus* species and *Streptomyces* sp. isolated from rhizospheric soil were identified as plant growth promoting

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rhizobacteria that have ability to enhance the plant-height canopy-width of chilli plants after transplanting (Moumita et al., 2011).

6.8.2 Effect of different treatments on length of shoot and root

The larger root system is important for healthy and robust growth of a plant. Through the root system nutrients and water are absorbed and transported to different parts of the plants. Growth of plant is also indicated by the increase in length of shoot and plant branching. The factors affecting plant growth can be the direct or indirect mechanisms that involve phosphorus, nitrogen fixation and solubilisation production as well as the production of phyto-hormones such as auxins, gibberellins, cytokinins and lowering of ethylene concentration (Kloepper et al., 1989; Glick & Ibid, 1995; Glick et al., 1999). In this study it was observed that plant root and shoot increased significantly ($P \leq 0.05$) (Figure 28) the root length of chilli plants, especially group III that was observed to produce longer root length 27.66 cm per plant after 25 d transplanting (Figure 28). However, after 45 d of transplanting the shoot increased length value of 84.33 cm per plant group V in Figure 29A eventhough these plants survived soil infected with fungi *F. solani*-CS. Similar observation was reported by Hamed et al. (2011). These researchers recorded that tomato plants treated with *Lactobacillus* sp. (LAB-1), *Lb. acidophilus* sp. (LAB-2), *Lactobacillus* sp.4 (LAB-4), *Lactobacillus* sp. 5 (LAB-5) that were isolated from yoghurt and milk, and strain of *Lb. plantarum* NRRL B-4524 (LAB-3) collected from National Centre for Agricultural Utilization Research (USA) enhanced the tomato plant systems either the plant infected with LAB alone or LAB and *F. oxysporum*.

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Growth of tomato seedlings was enhanced when the seeds and soils were treated with LAB I and LAB II in pot trials (Narasimha et al., 2012). LABs are good producers of bio-fertilizers and the presence of LAB in the soil improved soil fertility as reported by Prachyakij et al. (2008) and Duangporn et al. (2009). It was reported that *Lb. plantarum* strain isolated from fermentation of the wild fruits improved the availability of plant nutrients such as Cu, Zn and Fe (Prachyakij et al., 2008) and enhanced plant growth. It may be possible that the LAB used in this study (*Lb. plantarum* I) also have similar function as biofertiliser that could enhance chilli plant growth. Plant shoot and root elongation is influenced by phytohormones such as indole acetic acid (IAA), gibberellins and cytokinins. Recently, it was observed that LAB also produced indole acetic acid (IAA) in the presence of different concentrations of tryptophan and the highest amount of IAA was produced by KLF01 LAB isolate (Anupama et al., 2014), however, the identification of the LAB was not reported.

6.8.3 Effect of different treatments on fresh and dry weight of plant, shoot and root

The effect of LAB treatment and fungal infection of chilli plants was evaluated by the mass of whole plant, shoots and roots. It was observed that significant ($P<0.05$) increase in fresh and dry weight of plant, shoots and roots was recorded after the 45 d period of growth especially plants receiving treatment V and VI, then the dry weight remain constant until 65 d (Figure 28A). The plants in group V had dry weight for shoot (18.80g/plant) after 45 d than increased to 9.63g/plant that received from plant infected with the fungi group VI (Figure 29B). This observation was similar to that reported by

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El-Mabrouk (2014) who reported that *Lb. plantarum* LAB-C5 from durian and *Lb. pentousis* LAB-G7 from ginger increased the fresh and dry weight of chilli plants infected with fungi *C. gloesporioides* and *C. capsici*.

Similarly, Moumita et al. (2011) observed that *Streptomyces* sp. and *Bacillus* spp. also have ability to change the fresh and dry weight of chilli plant, shoot and root. Group VI plants were infected with the fungi alone. Eventhough higher values of the fresh and dry weight of plants, shoots and roots were recorded for plants in group VI 77.32 g, 70.57 g and 6.74 g/plant after 65 d respectively, some plants began to shrivel and no fruits were produced indicating wilting of the chilli plant (Figure 33).

Wilting is one of the main symptoms of *Fusarium* infection in chilli plants. Species of the genus *Fusarium* are known for causing serious plant diseases on many economically important plants including those in Malaysia (Liew et al., 1998; Darnetty et al., 2008; Nur et al., 2008; Siti et al., 2008). Similar wilting symptom was reported by Nurul et al. (2014) of the red chilli (*C. annuum*). *Fusarium* species found to cause wilting in plants that are *F. oxysporum*, *F. semitectum*, *F. solani*, *F. proliferatum, F. pseudocircinatum*, *F. sacchari*, *F. equiseti* and *F. verticillioides* in Malaysia. These phytopathogenic fungi have ability to infect rotting tissues and caused wilt disease especially chilli crops. These pathogenic fungi have ability to enter into the plant through xylem and later colonize the cortex of roots without causing any symptoms of disease (Appel & Gordon, 1994). *Fusarium* species has the ability to survive and live in plant tissue, as well in soil of infected plants (Edel et al., 1997). This was supported by the

finding of Hussain et al. (2013) who isolated *F. solani* from the roots of naturally fungi infected chilli plant showing wilt disease (Hussain et al., 2013).

6.8.4 Effect of LAB and fungi on chilli fruit characteristics

Fruits are the final products of plants; it should be fresh and free from any infective pathogenic microorganisms. It is desirable that yield of fruits is high without using any synthetic chemical and/or fertilizers. Chilli fruits both green and red are widely used in many dishes; however, the production of chilli is reduced by the occurrence of chilli plant diseases. The chilli wilt has been found to be the most frequently encountered disease problem (Skaggs et al., 2000). It was observed that the productivity of chilli plants was recorded less in both positive and negative controls group plants; the number of fruits was 9.33 per plant infected with *F. solani*-CS group VI (Figure 33B). In this study selected LABs were applied to soil as an attempt to improve the plant growth and productivity of chilli fruits thus avoiding the addition of any synthetic chemicals and fertilizers. The number of fruit increased significantly ($P \leq 0.05$) in plants treated with LAB or LAB and fungi; the number of fruits was number 56.33 fruits per plant (Figure 34), with high of number seeds 111.20/fruit from plant group IV. Plants from soil treated with LAB-MSS1 and fungi (group III) produced that were fruit longer 13.66 mm/fruit and the pericarp wider 2 mm/fruit from plant group II (Figure 40). Similarly, El-Mabrouk (2014) reported that application of *Lb. plantarum* LAB-C5 and *Lb. pentousis* LAB-G7 to the soil increased the productivity of chilli plants, eventhough the chilli plant were infected with fungi *Colletrotrichum* species. Moumita et al. (2011) also recorded that application of

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Bacillus spp. and *Streptomyces* sp. to the soil increased the number of fruits per plant, higher weight fruit to fruit length and number of seeds/fruit.

It was difficult to observe the symptoms of *F. solani*-CS pathogenicity in the chilli plants during the growing period because some of fungi infected plants were well developed. This effect was similar to that reported by Qureshi et al. (2004), Ehteshamul (2006) and Samrah et al. (2009). These researchers reported that chilli plant survived well even when infected with Fusarium spp. but the harvestable of chilli fruits were greatly reduced. The four main diseases that lead to wilting in chilli are *Fusarium* wilt, *Phytophthora* root rot, *Verticillium* wilt and *Rhizoctonia* root rot. Plants infected with these Phytopathogens would result in loss of productivity.

6.9 Confirmation on the presence of LAB and fungi in treated plant parts and soil

The isolates were identified as *B. megaterium, Lb. casei, B. subtilis, B. cereus* and *Lb. acidophilus* have been suggested to play a significant role as biofertiliser as well phytohormones promoter in plants (Mohite et al., 2013). It was reported that LAB could be endophytic in nature and it could easily enter from the soil to the plant tissue via the root system (El-Mabrouk, 2014). They confirmed the endophytic characteristics of *Lb. plantarum* LAB-C5 and *Lb. pentousis* LAB-G7 by isolating the bacteria from treated chilli plants. Similarly, this study detected the presence of LAB-MSS1 and LAB-FF11 from different parts of chilli plants (stem, placenta, root stem and seeds) and confirmed by gram staining, morphology and catalase test in Table 37).

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It was also confirmed the endophytic characteristics of pathogenic fungi *F. solani*-CS that was detected present in different parts of fungi infected plants (stem, placenta, root and seeds in Table 38 showed positive infection in chilli plant. The finding of this study agrees with Tayung et al. (2011) who isolated from healthy inner bark of *Taxusbaccata* and fungus was identified as *F. solani* based on morphological and molecular characterization. Earlier, Chakravarthi et al. (2008) and Li et al. (2005) suggested the endophytic nature of fungi *F. solani*.

Kloepper and Schroth (1981) observed that plant growth promoting rhizobacteria (PGPR) mediated plant growth promotion by the alteration of the whole microbial community in rhizosphere niche through the production of various substances. Bacteria in soil rhizosphere such as *Bacillus* species, *Pseudomonas* species, *Azotobacter* spp. and *Rhizobium* spp. are reported responsible to produce indole acetic acid (IAA), ammonia production (Joseph et al., 2007). Other researchers reported *Pseudomonas fluorescens* induced systemic resistance, antifungal activity in crops (Saravanakumar et al., 2007); *P. chlororaphis* showed inhibitory activity against pathogenic microorganisms (Liu et al. 2007); *Azotobacter chroococcum* produced gibberellins, kinetin, IAA (Verma et al. 2001), and *Lb. plantarum* strain produced plant nutrients such as Cu, Zn and Fe (Prachyakij et al., 2008) Generally, the plant growth promoting rhizobacteria (PGPR) promote plant growth directly by either facilitating resource acquisition (nitrogen, phosphorus and essential minerals) or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of biocontrol agents (Glick 2012). It may be possible the presence of LAB

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MSS and FF11 in the soil and in the plants as endophytic modulate plant growth regulator. However, this was studied in this experiment and therefore, needs to be further elucidated. Because both LAB and fungi were found that endophytic could be used as systematic biocontrol agent against fungi.

6.10 CONCLUSION

The findings of study indicate that LAB-MSS1 and LAB-FF11 when applied to soil plant enhanced the growth of chilli plant systems namely, plant height canopy shoot and root at three different stages of chilli growth systems even in the presence of phytopathogenic fungi *F. solani* CS. Treatment of soil with the two different sources both LAB cells showed ability to improve plant growth and chilli fruit yield productivity after harvesting, eventhough, chilli plant survived in soil infected with *F. solani*-CS. In contrast, chilli plant which was survived in soil infected with *F. solani*-CS did not improve to the plant systems and productivity of chilli fruit. Similarly, chilli plants which grown in soil without LAB cells and *F. solani*-CS used as control lost productivity of chilli fruit after 65 d transplanting and these chilli plant did not to improve well. The study also demonstrated the endophytic nature of the LAB MS1 and LAB FF11. Finally, it was summarized that both LAB-MSS1 and LAB-FF11 could be use as fungicides agents and as plant promoters in agriculture for future study. The fungi *F. solani*-CS was noticed more pathogenic on chilli plants and fungi could be pathogenic on other plants.

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