CHAPTER V

IN VITRO STUDY OF LACTIC ACID BACTERIA ON GERMINATION OF CHILLI SEEDS INFECTED WITH *Fusarium* SPECIES

5. 1 INTRODUCTION

About 90% of the world food crops including chilli pepper are propagated by seeds (Maude, 1996). Seeds are the passive carriers of some important seed borne diseases caused by microorganisms such as fungi, bacteria, viruses and nematodes that can be carried with, on or in seed. Seeds infected with microorganisms would typically result in considerable yield losses. Fungi of the genera *Fusarium, Penicillium* and *Aspergillus* are commonly known to produce toxic substances (Singh et al., 1991). Anaso et al. (1981) reported that toxic metabolites of *F. equiseti* and *Drechslera rostrota* retarded root growth of wheat. Reduction in percentage seeds germination of soybean was observed when seeds were soaked in filtrates of fungi *Fusarium* species (Phomopsis & Lee, 1988). Similarly, the filtrates culture of pathogen *F. oxysporum udum* isolated from infected roots of different varieties of Pigeonpea (*Cojanus caion* L. Mills p.) inhibited the seed germination of variety ICP-2376 by 10% and the effects were seen on seedling systems; there was reduction in length of roots, shoots and seedling growth (Jalander and Gachonde, 2011). Seed borne fungi isolated from the chilli seeds in Karnataka, India, were *A. flavus, R. stolonifer, F. moniliformae, C. capsici* and *A. niger* (Asalmol et al., 2001). *Fusarium* species are associated with fruit rot of vegetable fruits in Malaysia and plant the pathogenic fungi isolated from chilli seeds were *F. moniliformae, F. solani* (Mart.) Sacc., *F. equesiti* (Gorda) Sacc., *F. oxysporum* Schl. Emend Sny and *Alternaria alternata* (Fr.) (Keissler, 1989). Fungicidal substances can be produced by many microorganisms mainly strains of *Enterobacter*, *Bacillus, Streptomyces* and *Pseudomonas* (Li & Rinaldi, 1999; Kaleli et al., 2006; Etcheverry et al., 2009). Most of bacterial metabolites belong to polyketide that are highly modular proteins, or chimeric proteins and behave as antibiotics (Levenfors et al., 2004).

Lactic acid bacteria also produced compounds that have antifungal activity against phytofungi in addition to having a broad spectrum antimicrobial activity against pathogenic bacteria (Lavermicocca et al., 2000; Schnrer and Magnusson, 2005; Wang et al., 2007; Rajase karan et al., 2009; El-Mabrouk et al., 2012). Chilli seeds obtained in Malaysia were treated with LAB-CFS of *Lb. plantarum* LAB-C5 from durian and *Lb. pentousis* LAB-G7 from ginger and showed enhanced seed germination, plumule length, shoot length, root length and inhibited the pathogenicity of *C. gloesporioides* of the infected chili seeds (El-Mabrouk et al., 2012). Additionally, *Lb. paracasei* subsp. *tolerans* (LAB I) and *Lb. paracasei* sub sp. *paracasei* (LAB II) were isolated from soil and dairy products, respectively, and showed an improvement in tomato seed germination and plants systems (fresh weight, shoot length, root length); confirming the efficacy of LAB as probable plant growth promoting bacteria (PGPB) (Narasimha et al., 2012). The present study was conducted with the following objectives (i) to evaluate the percentage germination of different varieties chilli seeds treated LAB-CFS isolates; (ii) to determine the pathogenicity of *Fusarium* species on percentage germination on different varieties chilli seeds; (iii) the determine of seed germination percentage of *Fusarium* species infected chilli seeds and application of LAB-CFS applied on alternate d; (iv) to observe the germination of chilli seeds infected with pathogenic fungi *Fusarium* species sowed in soil treated with LAB cells and (v) to detect the germination of chilli seed treated with LAB-CFS grown in soil infected with pathogenic fungi *Fusarium* species.

5.2 MATERIAL AND METHODS

5.2.1 *In vitro* assay in Petri Dish

The percentage of germination of three variety chilli seed was determined on petri dish and LAB-CFS was treated at different time application on chilli seed (Cili Kulai) was infected with *Fusarium* specie detail procedure as mentioned below.

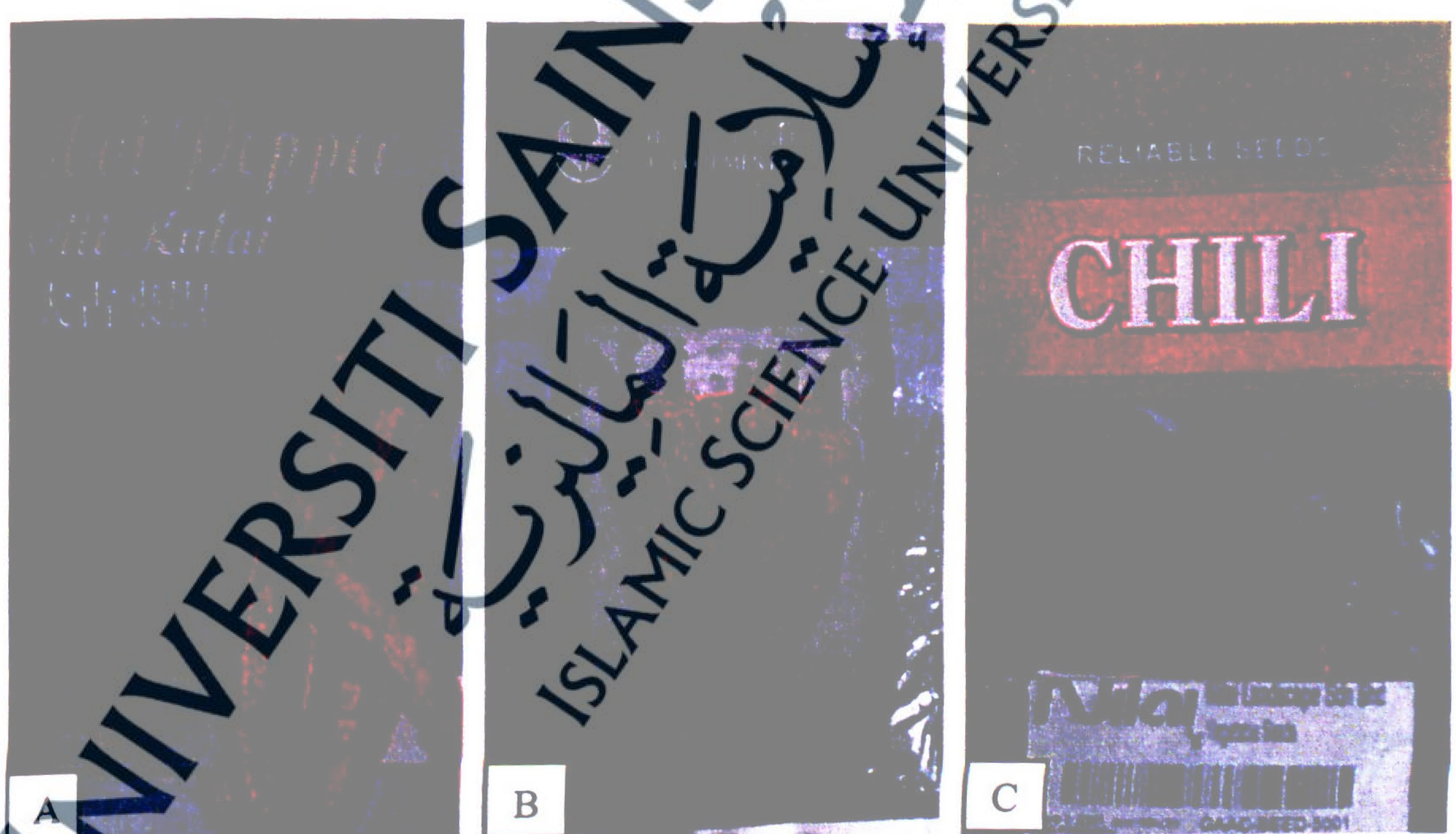
5.2.2 Preparation of LAB cells free supernatant

The LAB isolates were inoculated into MRS broth (Oxoid) and incubated for 24 h at 37°C in aerobic shaker incubator (SASTEC). The LAB-CFS was prepared by centrifuging the broth 11500 × g rpm for 10 min at 4°C (Centrifuge Combi-514R, Korea). The supernatant of each LAB isolates was filtrated using sterile filtered 0.45 µm-pore-size Millipore filter (Schleicher and Schuell, Dass el, Germany).

5.2.3 Preparation of chilli seeds

Three variety of chilli seeds namely, Cili Kulai, Red spicy-0639) and chilli pepper (GAAC-SEED-5001) were purchased from Nilai, Negeri Sembilan, Malaysia. The types of chilli seeds were used for treatments packets added in Figure 15 (A, B & C). The chilli seeds were prepared following the method described by Khare (1996) with modification. The seeds were washed with running tap water and surface sterilized with 1 % sodium hypochlorite solution for 1-2 min and then washed with sterile distilled water 2-3 times. Then seeds were air-dried in laminar flow cabinet to remove the moisture after that surface sterilised chillies seeds were used for further.

FIGURE 15: Three different verities of chilli seeds packets used for treatments



Notes:- Chilli seeds used in the experiment CHAPTER 5. (A) Cili Kulai, (B) Red spicy-0639) and (C) (GAAC-SEED-5001)

5.2.4 Preparation of *Fusarium* Culture

Fusarium spp. were grown on PDA (Oxoid) and incubated at room temperature at 28^{o}C for 5 day using the method described by Hamed et at. (2011) with modification. 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 were collected. The spore suspension at concentration of 1×10^{5} spores/ml was obtained using serial dilution and then fungal spores were homogenized in a sterilized blender for few minutes and used for treatments on chilli seeds and soil

5.2.5 Effect of LAB and fungi *Fusarium* species on germination of chilli seeds

The surface sterilized chilli seeds were soaked in the LAB-CFS for 1 h using procedure Hamed et at. (2011) with modification. Then, seeds were air-dried in laminar flow cabinet until to remove the moisture and placed with the aid of sterilized forceps in petri dishes. A sterilized dried brown paper was aseptically placed inside the Petri dish. Each Petri dish contained ten chilli seeds. Another set of chili seeds were soaked in fungal spore suspension for 1 h, air dried, and placed in the petri dish as previously described above. The chilli seeds soaked in sterilized distilled water was used as control. All treated chilli seeds in petri dishes were incubated at room temperature in dark cabinet for two weeks to allow seed germination. Seeds that germinated were counted and the percentage germination was calculated using the equation shown below. The experiments were done in triplicate.

$$[GS (\%)] = [NGS / TNTS] \times 100$$

Where,

GS (%) = Germination percentage of seeds

NGS = Total number of germinated seeds

TNTS = Total number of treated seeds

5.2.6 Determination of seed germination percentage of *Fusarium* infected chilli seeds and application of LAB-CFS applied on alternate day

The sterilized chilli seeds that were infected with the fungi and placed in the petri dishes as described above. A drop of LAB-CFS (10 µL) was inoculated on each seed at day 0, 3, 5 and 7. The seeded petri dishes were placed in dark cabinet at room temperature for two weeks to allow seed germination. The number of seeds germinated was counted and seed germination was calculated using the equation described in section 5.2.5. The experiment was done in duplicate.

5.3 *In vitro* assay in plastic tray

5.3.1 Effect of LAB cells treatment on chilli seeds germination

In this experiment hot chilli pepper seeds of Cili Kulai were used and surface sterilized as described in section 5.2.3 method using Hameed, 2012 with modification. The eyes of the plastic tray were filled with 25 g potting soil labeled as Tanah Baik with no fertilizer in

Figure 16 (A & B). A sterilized seed was sowed in each eye. Each eye of the plastic tray was inoculated with 5 mL of overnight LAB culture poured in the center of the eye of the plastic tray. The cell concentration of each LAB isolate was determined using Nanophotomerter-P330-IMPLEN at $OD_{600}{}^{o}{}_{A}$ with cell count/mL of each LAB/mL was as follows: LAB-MSS1 (39×10^{8}), LAB-MSS5 (64×10^{8}) IDLAB6 (60.8×10^{8}), IDLAB7 (41.9×10^{8}) and LAB-FF11 (60.5×10^{8}). The seeded trays were sprayed with water, covered with dark colored plastic bags and kept at room temperature at 30°C for two weeks to allow seed germination. The number of seed germinated, seedling shoots and roots lengths were measured. The treatments were done in triplicate.

FIGURE 16: Soil in packet was used to chilli plants growth, Tanah Baik soil with

Notes:- No fertilizer was used for chilli seeds germination in the experiments (A) soil in packed and (B) soil without packed

5.3.2 Effect of LAB cells treatment on germination of fungi infected seeds

The hot chilli pepper seeds of Cili Kulai were surface sterilized as described in 5.2.3 above. The seeds were inoculated with spore suspension of *Fusarium* species as described in section 5.2.4. The fungi infected seeds were sowed in the eyes of plastic trays. Each tray eye was filled with 25 g potting soil (labeled as Tanah Baik with no fertilizer). Initially, 5 mL overnight LAB culture cells were inoculated in the center of soil filled in the plastic tray eye. The seeded trays were sprayed with water, covered with dark colored plastic bags, placed in dark cabinet at room temperature at 30^{o}C for two weeks to allow seed germination. The number of seed germinated was counted and the percentage germination was calculated using the equation shown in section 5.2.5, seedling shoots and roots length was measured with general scale in (mm). The treatments were done in triplicate.

5.3.3 Effect of LAB CFS on chilli seeds germination

The hot chilli pepper seeds of Cili Kulai were surface sterilized as described in section 5.2.3. The seeds were soaked with LAB-CFS for 1 h, air-dried in laminar air flow cabinet then sowed in tray eye filled with 25 g potting soil (labeled as Tanah Baik with no fertilizer) at one seed per tray eye. The seeded trays were sprayed with water, covered with dark colored plastic bags and kept at room temperature for two weeks to allow seed germination. The number of seed germinated was counted and the percentage germination was calculated using the equation shown in section 5.2.5, seedling shoots and roots length was measured with general scale in (mm) after two week incubation at room temperature 28°C. The treatments were done in triplicate.

5.3.4 Effect LAB-CFS treatment on seed germination sowed in soil infected with *Fusarium* species

The hot chilli pepper seeds of Cili Kulai were surface sterilized as described in section 5.2.3. The seeds were soaked in LAB-CFS of LAB for 1 h, air dried in laminar cabinet and sowed in tray eye filled with 25 mg potting soil (labeled as Tanah Baik with no fertilizer). The soil of each tray eye was inoculated in the center with 5 mL of seven day old fungal spore suspension. Culture cells of seven days old *Fusarium* spp. in the center of soil filled tray eye. The seeded trays were sprayed with water, covered with dark colored plastic bags and placed in dark cabinet at room temperature at 30°C for two weeks to allow seed germination. The number of seed germinated was counted and the percentage germination was calculated using the equation shown in section 5.2.5, seedling shoots and roots length was measured with general scale in (mm). The treatments were done in triplicate.

5.4 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.

5.5 RESULTS

5.5.1 Germination of chilli seeds treated with CFS-LAB and *Fusarium* species

Chilli seeds namely, Cili Kulai, Red spicy-0639 and GAAC-SEED-5005 that was without LAB (control) and treated with LAB-CFS showed significantly difference ($P<0.05$) good germination in the range of 82.6 to 93.3% compared to chili seeds infected with *Fusarium* species in Table 29 after incubation.

TABLE 29: Percentage germination of chilli seeds treated with LAB-CFS and *Fusarium* species after two week incubation

Treatments	Germination (%)		
	Cili Kulai	Red spicy-0639	GAAC-SEED-5001
Control	93.3^{a}	83.3^{ab}	82.6^{a}
LAB-CFS treatment			
LAB-MSS1	90.0^{ab}	93.3^{a}	83.3^{a}
LAB-MSS5	86.6^{abc}	83.6^{ab}	86.7^{a}
IDLAB6	90.0^{ab}	83.6^{ab}	83.3^{a}
IDLAB7	86.6^{abc}	83.6^{ab}	83.3^{a}
LAB-FF11	93.3^{a}	93.3^{a}	90.0^{a}
Fusarium Infected chilli			
Fungi-CL	63.3^{d}	63.3^{c}	70.0^{ab}
Fungi-CS	53.3^{d}	53.3^{c}	60.0^{b}
Fungi-FC	70.0^{bcd}	70.0^{bc}	70.0^{ab}
Fungi-LR	66.6^{cd}	60.0^{c}	66.6^{ab}

Notes:- Means with the same letter in the same column are not significant different ($P>0.05$) and Means with the different letter in the same column are significantly different ($P<0.05$); LAB isolates: LAB-MSS1 = *Lb. plantarum*1, LAB-MSS5 = *P. pentosaceus*1, IDLAB6 = *Lb. acidophilus* ATCC314, IDLAB7 = *Lb. plantarum* ATCC8014, LAB-FF11 = *Lb. Plantarum*1; Fungi *Fusarium* spp: CL = *F. oxysporum* f. sp. *lycopersici*, CS = *F. solani*, FC = *F. acuminatum* and LR = *F. proliferatum*

The fungi infected seeds infected showed reduced percentage of seed germination significantly different (P<0.05) in the range of 53.3 to 70%. Fungi *F. solani* -CS significantly reduced seed germination of Cili Kulai and Red spicy-0639 to 53.3 % after two week incubation. All the seeds obtained from different chilli cultivar seemed to be slightly affected by fungi-FC which showed 70% germination compared to seeds infected with other *Fusarium* species. Interestingly, the fungi *Fusarium* species did not only suppressed the germination of chilli seeds and was also observed that *Fusarium* species were effected to early germination because germination of chilli seeds were appeared late compare to grown chilli seeds in presence of LAB-CFS and cells of LAB isolates as mentioned in below Figure 17 (A, B & C) after six d incubation.

FIGURE 17: Seed germination experiment after six d incubation

Notes:- Seeds placed on brown paper for seed germination study. (A) Seeds without LAB and Fungi (control) shoot and root not appeared; (B) Seeds inoculated with fungi *F. acuminatum*-FC also showed slow growth, and (C); seeds inoculated with LAB-FF11 showing good shoot and root earlier than A and B after six days of incubation

It was observed that control seeds from selected Chilli Kulai (seeds without LAB-CFS and fungi *F. oxysporum* f. sp. *lycopersici*-CL) showed 93% germination. The germination of seeds infected with *F. oxysporum* f. sp. *lycopersici*-CL was significantly

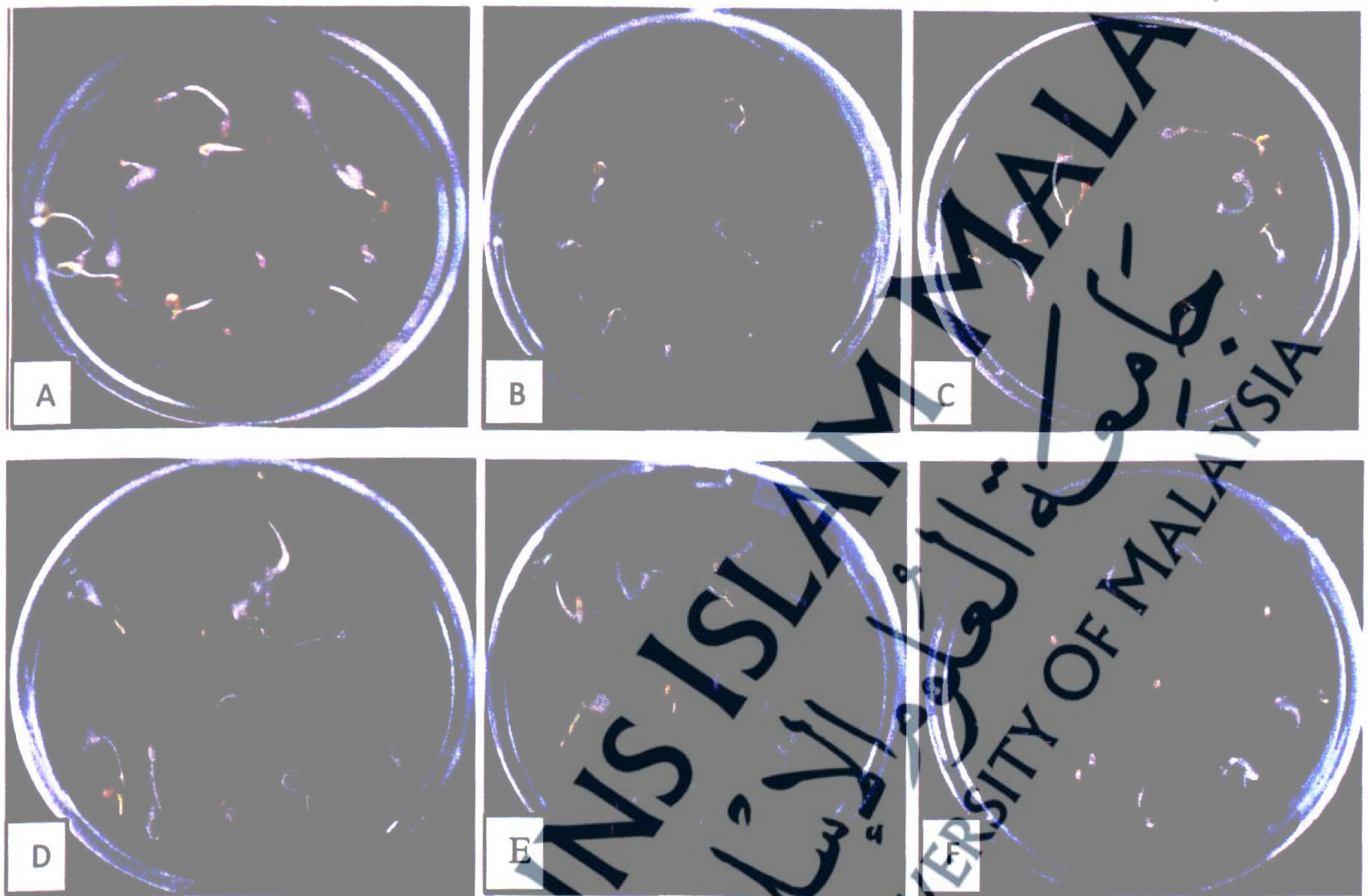
(P<0.05) reduced to 85% even though it was treated with LAB-CFS (Table 30) and as shown by the reduction in percentage seed germination when LAB-CFS was applied on the third, fifth and seventh day after fungal infection. Application of LAB-CFS after the seeds were infected with *F. oxysporum* f. sp. *lycopersici*- CL did not improve significantly the germination percentage. However, application of LAB-CFS of IDLAB-7, LAB-MSS1 and LAB-FF11 seems to enhance the germination of the *F. oxysporum* f. sp. *lycopersici*- CL infected seeds to 80, 75 and 70%, respectively, than seeds the germination of fungal infected seeds 65% after two week incubation at room temperature 28°C in dark cabinet as in Figures 18).

TABLE 30: Percentage Germination of chilli Seed treated with LAB-CFS and *F. oxysporum* f. sp. *lycopersici*-CL

LAB-CFS	Germination (%)			
	Fungi infection (d)			
	0	3	5	7
Control	93.0^{a}	93.0^{a}	93.0^{a}	93.0^{a}
LAB-MSS1+CL	98.0^{a}	80.0^{a}	75.0^{ab}	75.0^{ab}
LAB-MSS5+CL	85.0^{ab}	85.0^{a}	75.0^{ab}	60.0^{b}
LDLAB6+CL	85.0^{ab}	75.0^{ab}	65.0^{c}	65.0^{c}
IDLAB7+CL	90.0^{a}	75.0^{ab}	70.0^{ab}	80.0^{a}
LAB-FF11+CL	85.0^{ab}	85.0^{a}	65.0^{c}	70.0^{ab}
Fungi-CL	65.0^{b}	65.0^{c}	65.0^{c}	65.0^{c}

Notes:- Means with the same letter in the same column are not significant different (P>0.05) and Means with the different letter in the same column are significantly different (P<0.05);LAB-CFS was applied to the seeds at 0, 3, 5 and 7 day during 2 week germination period; LAB isolates: LAB-MSS1 = *Lb. plantarum*1, LAB-MSS5 = *P. pentosaceus*1, IDLAB6 = *Lb. acidophilus* ATCC314, IDLAB7 = *Lb. plantarum* ATCC8014, LAB-FF11 = *Lb. plantarum*1; Fungi *Fusarium* sp: CL = *F. oxysporum* f. sp. *lycopersici*

FIGURE 18: Chilli Kulai seeds treated with LAB-CFS showing germination after two week incubation under dark cabinet



Notes:- Seeds placed on brown paper for seed germination study. (A) Control showing weak shoots and roots (B) Seeds with LAB-MSS1, (C) LAB-MSS5, (D) IDLAB6, (E) IDLAB7 and (F) LAB-FF11chilli seeds inoculated were good germination after two week of incubation and showing good shoot and root.

Similarly, germination of chilli seeds infected with fungi *F. solani*-CS was significantly lower ($P<0.05$) with 55% germination compared to control (93%) or treated with CFS of LAB isolates. Treatment of chili seeds with LAB CFS at day 0, 3, 5 and 7 d enhanced the germination rate of for Fungi *F. solani* -CS infected seeds. It was observed that Fungi *F. solani*-CS was more easily inhibited by the CFS of LAB-MSS1 and IDLAB7 with percent seed germination of 85% after 7 d, and was higher than Fungi *F. solani* -CS infected seeds in Table 31.

TABLE 31: Germination percentage of infected *F. solani*-CS chilli seeds and treated with LAB-CFS

LAB-CFS	Germination (%)			
	Fungi infection (d)			
	0	3	5	7
Control	93.0[a]	93.0[a]	93.0[a]	93.0[a]
LAB-MSS1+CS	85.0[a]	90.0[a]	80.0[ab]	85.0[a]
LAB-MSS5+CS	95.0[a]	95.0[a]	75.0[ab]	75.0[ab]
LDLAB6+CS	90.0[a]	90.0[a]	90.0[a]	75.0[ab]
IDLAB7+CS	85.0[a]	90.0[a]	70.0[ab]	85.0[a]
LAB-FF11+CS	95.0[a]	95.0[a]	90.0[a]	75.0[ab]
Fungi-CS	55.0[b]	55.0[b]	55.0[b]	55.0[b]

Notes:- Means with the same letter in the same column are not significant different (P>0.05) and Means with the different letter in the same column are significantly different (P<0.05); LAB-CFS was applied to the seeds at 0, 3, 5 and 7 day during 2 week germination period; LAB isolates: LAB-MSS1 = *Lb. plantarum*1, LAB-MSS5 = *P. pentosaceus*1, IDLAB6 = *Lb. acidophilus* ATCC314, IDLAB7 = *Lb. plantarum* ATCC8014, LAB-FF11 = *Lb. Plantarum*1; Fungi *Fusarium* sp: CS = *F. solani*

Similar results were obtained for other LAB-CFS was applied at 0, 3, 5 and 7 day during 2 week on chili seeds infected with fungi *F. acuminatum* -FC as in Table 32) better percentage germination was recorded 98% with LAB-CFS of LAB-MSS1; eventhough chilli seeds were infected with fungi *F. acuminatum*–FC. Where as, percentage germination of chilli seeds infected with fungi *F. proliferatum*–LR improved 95% when treated with LAB-CFS of LAB-FF11 and LAB-MSS5 in Table 33 after two week incubation

TABLE 32: Germination percentage of infected *F. acuminatum*-FC chilli seeds and treated with LAB-CFS

LAB-CFS	Germination (%)			
	Fungi infection (d)			
	0	3	5	7
LAB-MSS1+FC	98.0^{a}	85.0^{a}	85.0^{a}	75.0^{a}
LAB-MSS5+FC	85.0^{a}	85.0^{a}	80.0^{a}	65.0^{a}
LDLAB6+FC	90.0^{a}	75.0^{a}	70.0^{a}	75.0^{a}
IDLAB7+FC	95.0^{a}	80.0^{a}	85.0^{a}	65.0^{a}
LAB-FF11+FC	90.0^{a}	85.0^{a}	85.0^{a}	75.0^{a}
Fungi-FC	65.0^{b}	65.0^{a}	65.0^{a}	65.0^{a}

Notes:- Means with the same letter in the same column are not significant different (P>0.05) and Means with the different letter in the same column are significantly different (P<0.05); LAB-CFS was applied to the seeds at 0, 3, 5 and 7 day during 2 week germination period. LAB isolates: LAB-MSS1 = *L. plantarum*1, LAB-MSS5 = *P. pentosaceus*1, IDLAB6 = *L. acidophilus* ATCC314, IDLAB7 = *Lb. plantarum* ATCC8014, LAB-FF11 = *L. Plantarum*1; Fungi *Fusarium* sp: FC = *F. acuminatum*

TABLE 33: Germination percentage of infected *F. proliferatum*-LR chilli seeds and treated with LAB-CFS

LAB-CFS	Germination (%)			
	Fungi infection (d)			
	0	3	5	7
LAB-MSS1	85^{ab}	85.0^{a}	85.0^{a}	65.0^{ab}
LAB-MSS5	95.0^{a}	80.0^{ab}	80.0^{a}	70.0^{ab}
LDLAB6	85.0^{ab}	85.0^{a}	70.0^{a}	85.0^{a}
IDLAB7	85.0^{ab}	65.0^{ab}	85.0^{a}	65.0^{ab}
LAB-FF11	95.0^{a}	85.0^{a}	85.0^{a}	85.0^{a}
Fungi-LR	60.0^{b}	60.0^{b}	65.0^{a}	60.0^{b}

Notes:- Means with the same letter in the same column are not significant different (P>0.05) and Means with the different letter in the same column are significantly different (P<0.05);LAB-CFS was applied to the seeds at 0, 3, 5 and 7 day during 2 week germination period. LAB-MSS1 = *L. plantarum*1, LAB-MSS5 = *P. pentosaceus*1, IDLAB6 = *L. acidophilus* ATTCC314, IDLAB7 = *L. plantarum* ATCC8014, LAB-FF11 = *L. Plantarum*1; Fungi *Fusarium* sp: LR = *F. proliferatum*

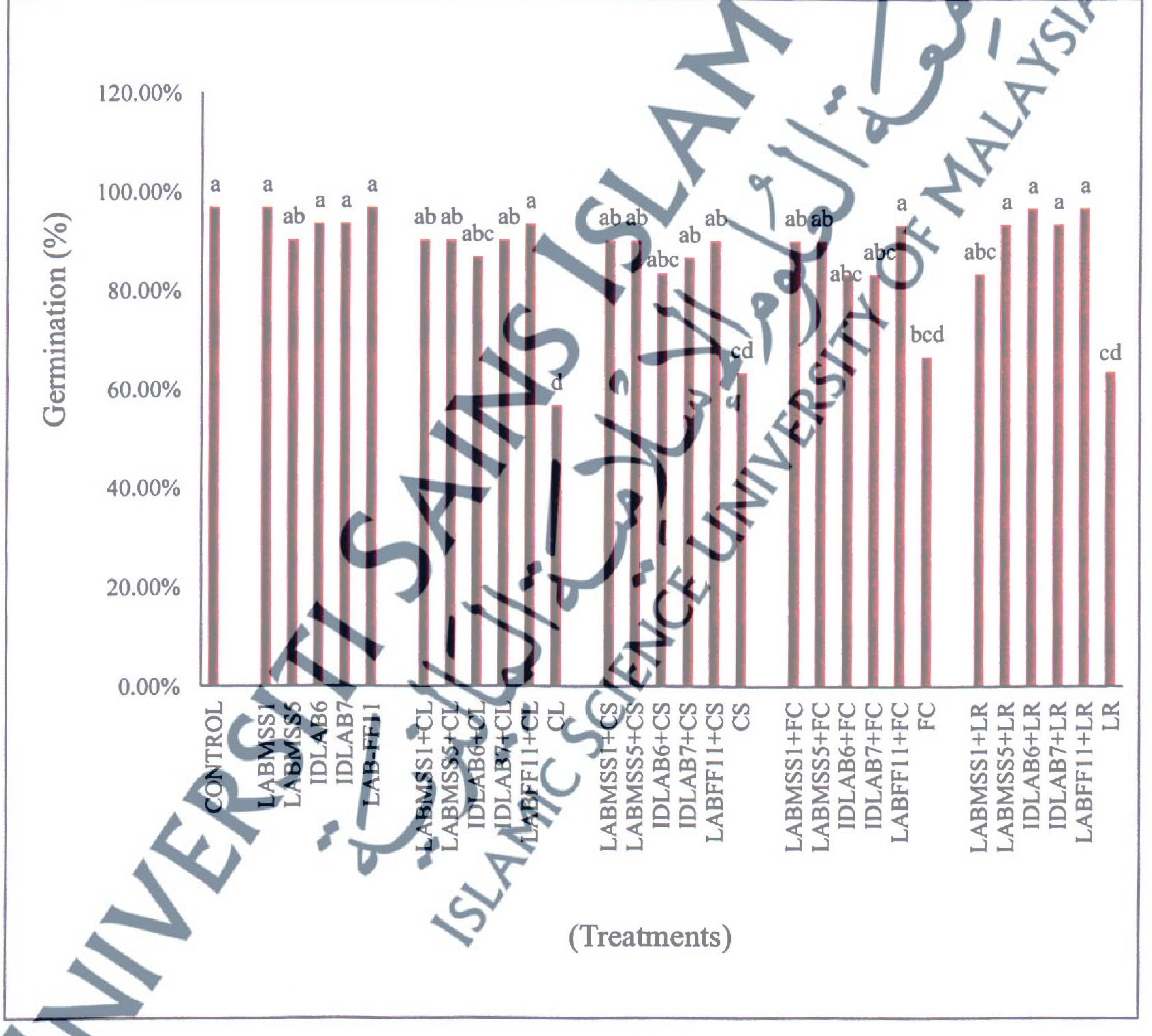
5.5.2 Percentage seed germination of chilli Kulai infected with *Fusarium* species sowed in soil treated with LAB cells

The percentage germination of Chili Kulai seeds that were infected with *Fusarium* spp. was reduced between 56 and 66.0% compare to control (96.0%) when grown in soil seeds infected with fungi *F. acuminatum* -FC and Fungi *F. solani* -CS grown in soil without LAB cells inoculation showed 63% germination while germination of seeds infected with *F. oxysporum* f. sp. *lycopersici*- CL and *F. proliferatum* -LR was 56.0% and 66.0%, respectively compare to control as shown in (Figure 19) before sowing the seeds significantly ($P<0.05$) improved the percentage germination of all the fungi infected seeds by 95 to 96%. The antifungal activity of LAB cells seems to be related to the LAB and the pathogenic fungi. The percentage germination of *F. oxysporum* f. sp. *lycopersici*- CL infected chilli seeds were improved to more than 96.0% when the soil was treated with LAB-FF11 cells. Similarly, treatment of soil with LAB-FF11 increased percentage germination of fungi *F. proliferatum* -LR and fungi *F. solani* -CS infected chilli seeds by 96.9 and 96%, respectively. IDLAB6 cell inoculation to the potting soil of also improved the percentage germination of fungi-LR infected chilli seeds by 96.6%. A slight reduction in percentage germination (95%) was observed for chili seeds infected with Fungi *F. solani* -CS when the soils were treated with cells LAB-MSS5, IDLAB6, and IDLAB7.

In contrast, inoculating the potting soil with cells of IDLAB6 and IDLAB7 allowed lower percent germination (80 to 83%) of seeds infected with *F. oxysporum* f. sp.

lycopersici- CL, fungi *F. solani* -CS and fungi *F. acuminatum* -FC. Similarly, cells of LAB-MSS1 reduced the percentage germination (83%) of fungi *F. proliferatum* -LR infected chilli seeds compare to control.

FIGURE 19: Percentage seed germination of chilli Kulai infected with *Fusarium* species sowed in soil treated with LAB cells



Notes:- Means with the same letters are not significant different (P>0.05) and Means with the different letters are significantly different (P<0.05); LAB isolates: LAB-MSS1 = *Lb. plantarum*1, LAB-MSS5 = *P. pentosaceus*1, IDLAB6 = *Lb. acidophilus* ATCC314, IDLAB7 = *Lb. plantarum* ATCC8014, LAB-FF11 = *L. Plantarum*1; Fungi *Fusarium* spp: CL = *F. oxysporum* f. sp. *lycopersici*, CS = *F. solani*, FC = *F. acuminatum* and LR = *F. proliferatum*

5.5.3 Percentage seed germination of chilli Kulai soaked in LAB-CFS and sowed in soil infected with *Fusarium* species

Seeds sown in soil infected with *Fusarium* spp. significantly ($P<0.05$) reduced the percentage germination of chili seeds between 56 to 66.6% compared with the control which showed 97% germination as mentioned in (Figure 20). However, treatment of chilli seeds with LAB-CFS before sowing in soil.

FIGURE 20: Percentage germination of chilli seeds treated with LAB-CFS and sowed in soil infected with *Fusarium* species

Notes:- Means with the same letters are not significant different ($P>0.05$) and Means with the different letters are significantly different ($P<0.05$); LAB-MSS1 = *L. plantarum*1, LAB-MSS5 = *P. pentosaceus*1, IDLAB6 = *Lb. acidophilus* ATCC314, IDLAB7 = *Lb. plantarum* ATCC8014, LAB-FF11 = *Lb. Plantarum*1; Fungi *Fusarium* spp: CL = *F. oxysporum* f. sp. *lycopersici*, CS = *F. solani*, FC = *F. acuminatum* and LR = *F. proliferatum*

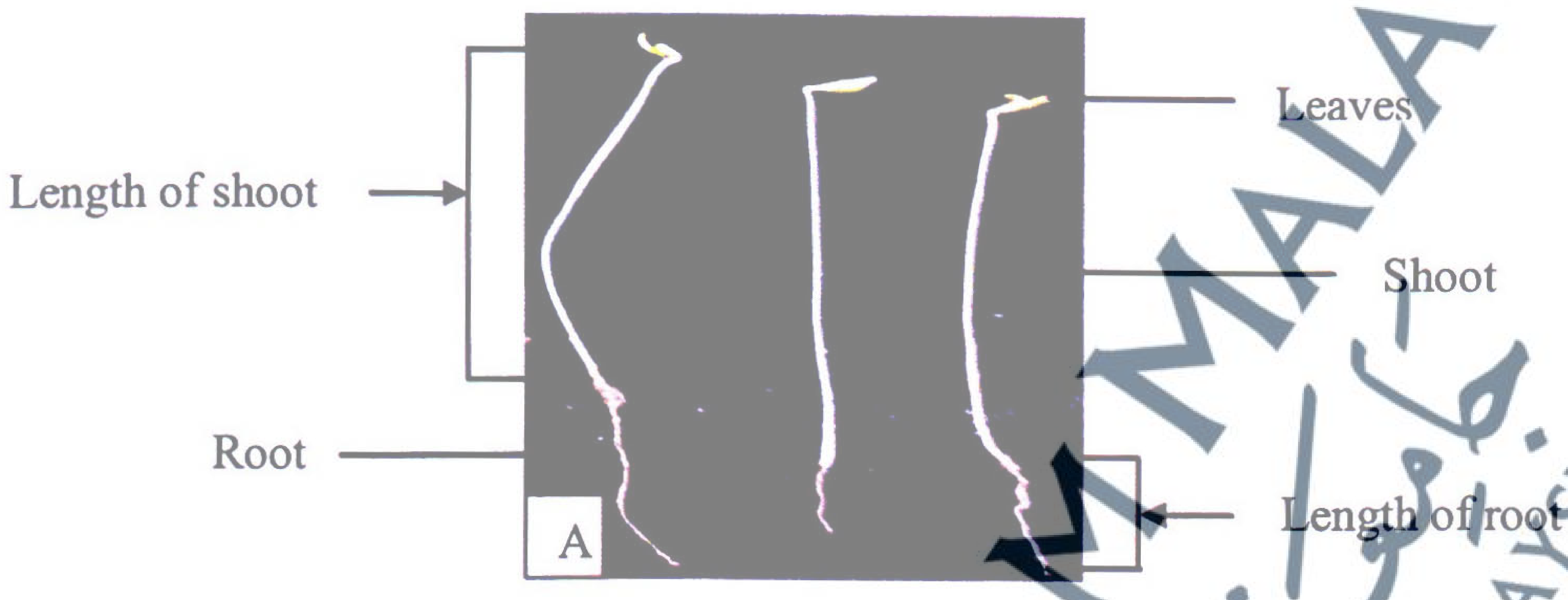
The *Fusarium* infected soil significantly (P<0.05) improved percentage germination from 80.0 to 97.0% particularly for seeds treated with LAB-CFS -LAB FF11 (98.0%). This antifungal effect of LAB-CFS depends on the species of LAB and fungi similar to that observed when applying LAB cells to soil. It was observed that seeds treated with LAB-CFS of IDLAB6, LAB-MSS1, and LAB-MSS5 enhanced seed germination to 83.0%, 90.0%, and 96%, respectively in soil infected with Fungi *F. solani* -CS. It was observed that CFS of LAB-MSS5 was affective against fungi *F. acuminatum*-FC, LAB-CFS-IDLAB6 was affective against fungi *F. proliferatum* -LR while LAB-CFS-IDLAB7 and LABFF11 was affective against fungi *F. oxysporum* f. sp. *lycopersici*- CL and allowed good seed germination in *Fusarium* infected soil.

5.5.4 Seedling heights shoots and roots of *Fusarium* species infected chilli seeds grown in soil treated with LAB Cells

5.5.4.1 Seedling height

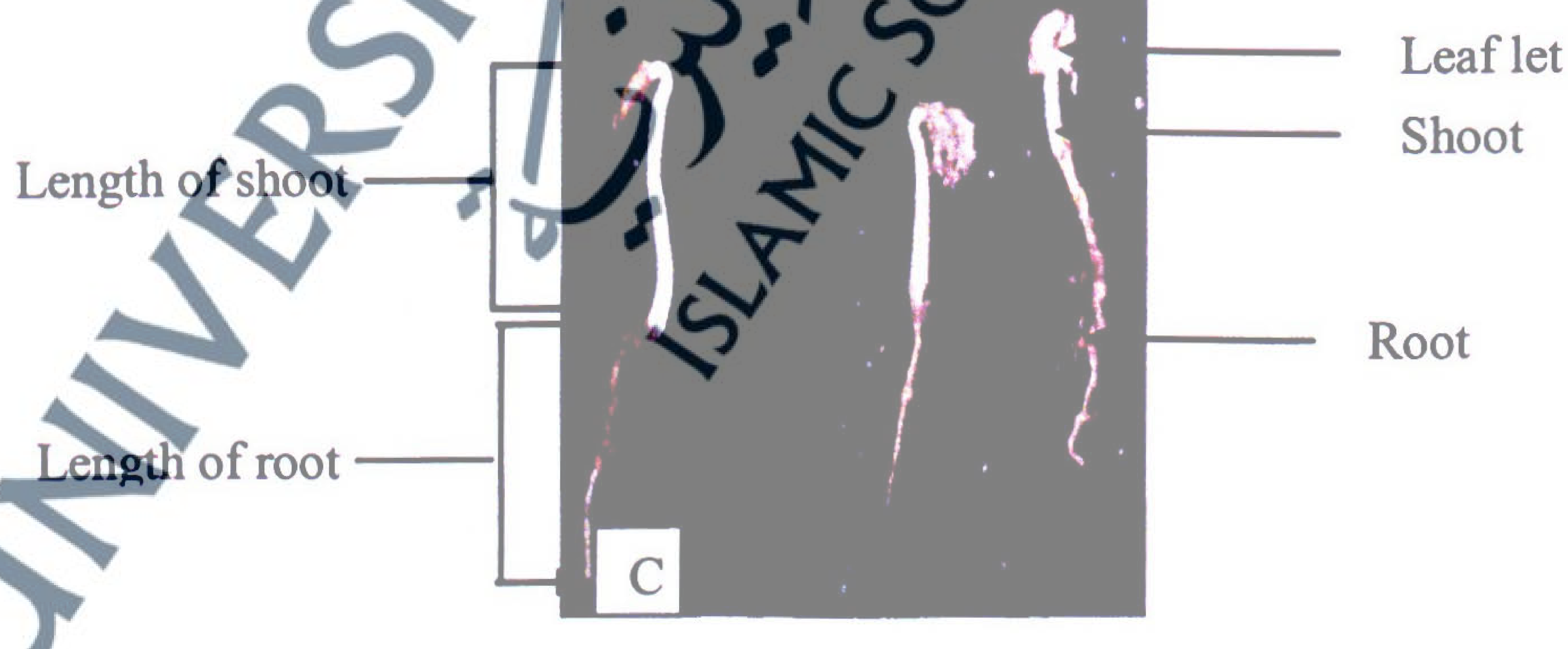
It was observed that fungi infected seeds in sowed soils treated with LAB cells significantly (P<0.05) increased the seedling height. The increase in seedling height was variable depending on the fungi infecting the seeds and the LAB cells inoculated to the soil. The seedling height of fungi *F. acuminatum*-FC infected chilli seeds was increased from 7.56±1.36 cm in soil without LAB-MSS1 cells to 12.33±2.84 cm in soil with LAB-MSS1 cells as in below Figure 21 (A, B & C). Furthermore, other LAB cells and fungi *Fusarium* species on Length of chilli seedlings systems of seeds as described below (Table 34) after two week incubation in soil.

FIGURE 21: Length of chilli seedlings inoculated with LAB cells after two weeks incubation



Notes:- (A) germinated seeds inoculated with cells of LAB-MSS1 showing good shoot growth

Notes:- (B) germinated seeds did not inoculated without cells of LAB or fungi (control) showing good root elongation growth



Notes:- (C) germinated seeds infected with *F. acuminatum*-FC showing weak seedling shoot and root elongation growth and no leaf appeared

TABLE 34: Seedling heights of seeds infected with *Fusarium* species and sowed in soil inoculated with LAB cells

Treatments	Seedling Height (cm)	Seedling Shoot (cm)	Seedling Root (cm)
CONTROL	11.66 ± 0.76^{a}	8.83 ± 0.76^{ab}	2.83 ± 0.28^{ab}
Cells of LAB treatments			
LAB-MSS1	9.03 ± 0.50^{ab}	7.33 ± 0.76^{ab}	1.70 ± 0.26^{ab}
LAB-MSS5	9.23 ± 1.16^{ab}	7.53 ± 1.40^{ab}	1.70 ± 0.01^{ab}
IDLAB6	9.73 ± 0.68^{ab}	7.63 ± 1.20^{ab}	2.10 ± 0.55^{ab}
IDLAB7	9.90 ± 1.68^{ab}	8.83 ± 1.41^{ab}	2.06 ± 0.95^{ab}
LAB-FF11	10.36 ± 1.89^{ab}	7.50 ± 0.81^{ab}	2.90 ± 1.10^{ab}
Seeds infected with CL and sowed in Cells of LAB treated soil			
LAB-MSS1+CL	10.30 ± 1.57^{ab}	8.73 ± 1.41^{ab}	1.56 ± 0.51^{ab}
LAB-MSS5+CL	10.23 ± 1.06^{ab}	9.16 ± 1.04^{a}	1.06 ± 0.20^{ab}
IDLAB6+CL	9.90 ± 0.65^{ab}	8.16 ± 0.35^{ab}	1.73 ± 0.64^{ab}
IDLAB7+CL	9.13 ± 1.00^{ab}	8.60 ± 0.64^{ab}	1.06 ± 0.40^{ab}
LAB-FF11+CL	10.16 ± 0.57^{ab}	8.56 ± 0.11^{ab}	1.16 ± 0.69^{ab}
Fungi-CL	7.30 ± 1.65^{b}	6.43 ± 1.88^{ab}	0.86 ± 0.23^{ab}
Seeds infected with CS and sowed in Cells of LAB-treated soil			
LAB-MSS1+CS	9.33 ± 0.28^{ab}	8.30 ± 0.34^{ab}	1.03 ± 0.05^{ab}
LAB-MSS5+CS	9.43 ± 0.20^{ab}	8.03 ± 0.41^{ab}	1.23 ± 0.11^{ab}
IDLAB6+CS	9.06 ± 0.92^{ab}	8.23 ± 0.46^{ab}	1.16 ± 0.76^{ab}
IDLAB7+CS	11.66 ± 0.57^{a}	8.06 ± 0.40^{ab}	3.60 ± 0.94^{a}
LAB-FF11+CS	10.76 ± 0.25^{ab}	8.66 ± 0.76^{ab}	2.10 ± 1.01^{ab}
Fungi-CS	9.23 ± 0.20^{ab}	7.80 ± 0.76^{ab}	1.43 ± 0.05^{ab}
Seeds infected with FC and sowed in Cells of LAB treated soil			
LAB-MSS1+FC	12.33 ± 2.84^{a}	9.40 ± 0.52^{a}	2.93 ± 2.92^{ab}
LAB-MSS5+FC	10.36 ± 0.41^{ab}	8.90 ± 017^{ab}	1.46 ± 0.25^{ab}
IDLAB6+FC	10.63 ± 0.63^{ab}	8.90 ± 017^{ab}	1.73 ± 0.73^{ab}
IDLAB7+FC	9.46 ± 0.55^{ab}	7.50 ± 0.50^{ab}	1.96 ± 0.55^{ab}
LAB-FF11+FC	11.16 ± 1.15^{a}	9.30 ± 1.53^{a}	1.86 ± 0.55^{ab}
Fungi-FC	7.56 ± 1.36^{b}	6.00 ± 1.00^{b}	1.56 ± 0.60^{ab}

Continued			
Seeds infected with LR and sowed in			
Cells of LAB treated soil			
LAB-MSS1+LR	10.16±0.76[ab]	9.00±0.86[ab]	1.16±0.28[ab]
LAB-MSS5+LR	9.33±1.15[ab]	8.00±1.00[ab]	1.50±0.50[ab]
IDLAB6+LR	9.16±0.76[ab]	8.16±1.25[ab]	1.00±1.86[b]
IDLAB7+LR	9.16±0.28[ab]	7.66±0.57[ab]	1.50±0.50[ab]
LAB-FF11+LR	10.16±1.25[ab]	9.40±1.51[a]	0.76±0.25[ab]
Fungi-LR	8.90±0.79[ab]	8.30±1.12[ab]	0.60±0.36[b]

Notes:- Means with the same letter in the same column are not significant different (P>0.05) and Means with the different letter in the same column are significantly different (P<0.05); LAB-MSS1 = *Lb. plantarum*1, LAB-MSS5 = *P. pentosaceus*1, IDLAB6 = *Lb. acidophilus* ATCC314, IDLAB7 = *Lb. plantarum* ATCC8014, LAB-FF11 = *Lb. Plantarum*1; Fungi *Fusarium* spp: CL = *F. oxysporum* f. sp. *lycopersici*, CS = *F. solani*, FC= *F. acuminatum* and LR = *F. proliferatum*

Similarly, seedling height infected with fungi *F. oxysporum* f. sp. *lycopersici*- CL sowed in soil treated with LAB-MSS1 increased from 7.30±1.65 cm (in control) to 10.30±1.57 cm (with LAB-MSS1 cells treatment). The seedling height of fungi *F. proliferatum* -LR infected seed increased from 8.90±0.79 cm (in control) to 10.16±0.76 cm with LAB-MSS1 cells treatment. Cells of IDLAB7 increased the seedling height of chili seeds infected with Fungi *F. solani*-CS from 9.23±0.20 cm (in control) to 11.66±0.57 (with LAB- IDLAB7 cells treatment) as described in (Table 34). and The results indicates that soil treatment with LAB cells could enhanced the growth of the seedlings as observed by increased in seedling heights even though the seeds were infected with the fungi after two week incubation at room temperature at 28°C.

5.5.4.2 Seedling shoot

Generally, there was no significant difference (P>0.05) in the shoot size of germinated chili seeds infected with fungi *F. solani*-CS and *F. proliferatum* -LR sown in LAB treated soils added in above (Table 34). However, the shoot length of germinated seeds infected with fungi *F. oxysporum* f. sp. *lycopersici*- CL increased significantly (P<0.05) from 6.43±1.88 cm (control) to 9.16±1.04 cm sowed in soil with LAB-MSS5 cells. Similarly, the shoot length of seeds infected with fungi *F. acuminatum*-FC also increased significantly (P<0.05) when grown in soil treated with LAB-MSS1 from 6.00±1.00 (control) to 9.40±0.52 cm with LAB-MSS1 cells.

5.5.4.3 Seedling root

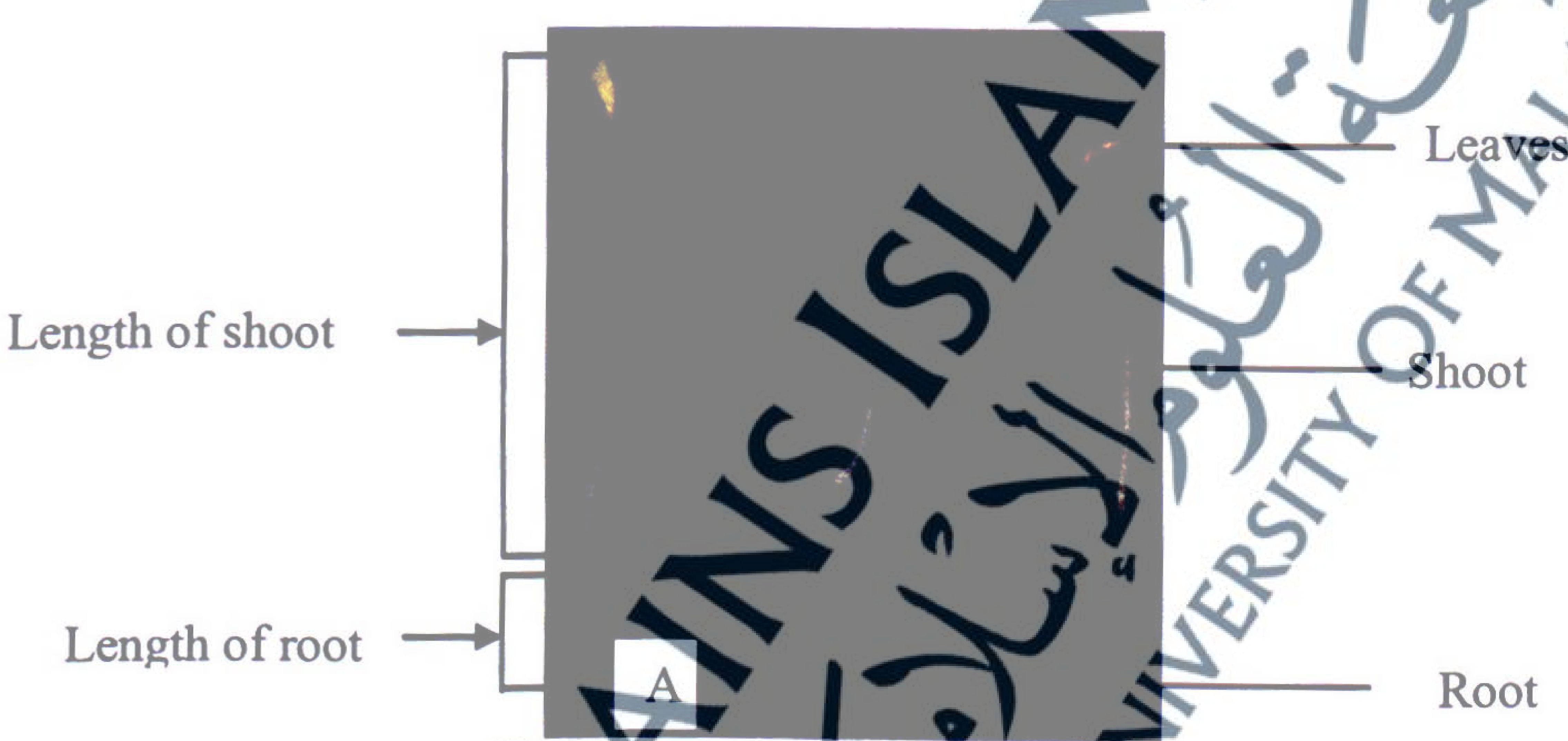
It was observed that sowing *Fusarium* infected seed in LAB treated soil increased the root length but was not significantly different (P>0.05) from control. However, it was interesting to note that root length of seeds infected with fungi *F. proliferatum* -LR was significantly (P<0.05) lowest with 0.60±0.36 cm compared to other treatments described in above (Table 34). In contrast, the root of seedling from fungi *F. solani*-CS infected seeds sowed in soil inoculated with LAB-IDLAB7 cells increased seedling roots of fungi-CS infected seeds from 1.43±0.05 to 3.60±0.94 cm even though it was of no significant different (P>0.05) after two week incubation.

5.5.5 Seedling heights of seeds soaked in CFS of LAB and sowed in soil infected with *Fusarium* species

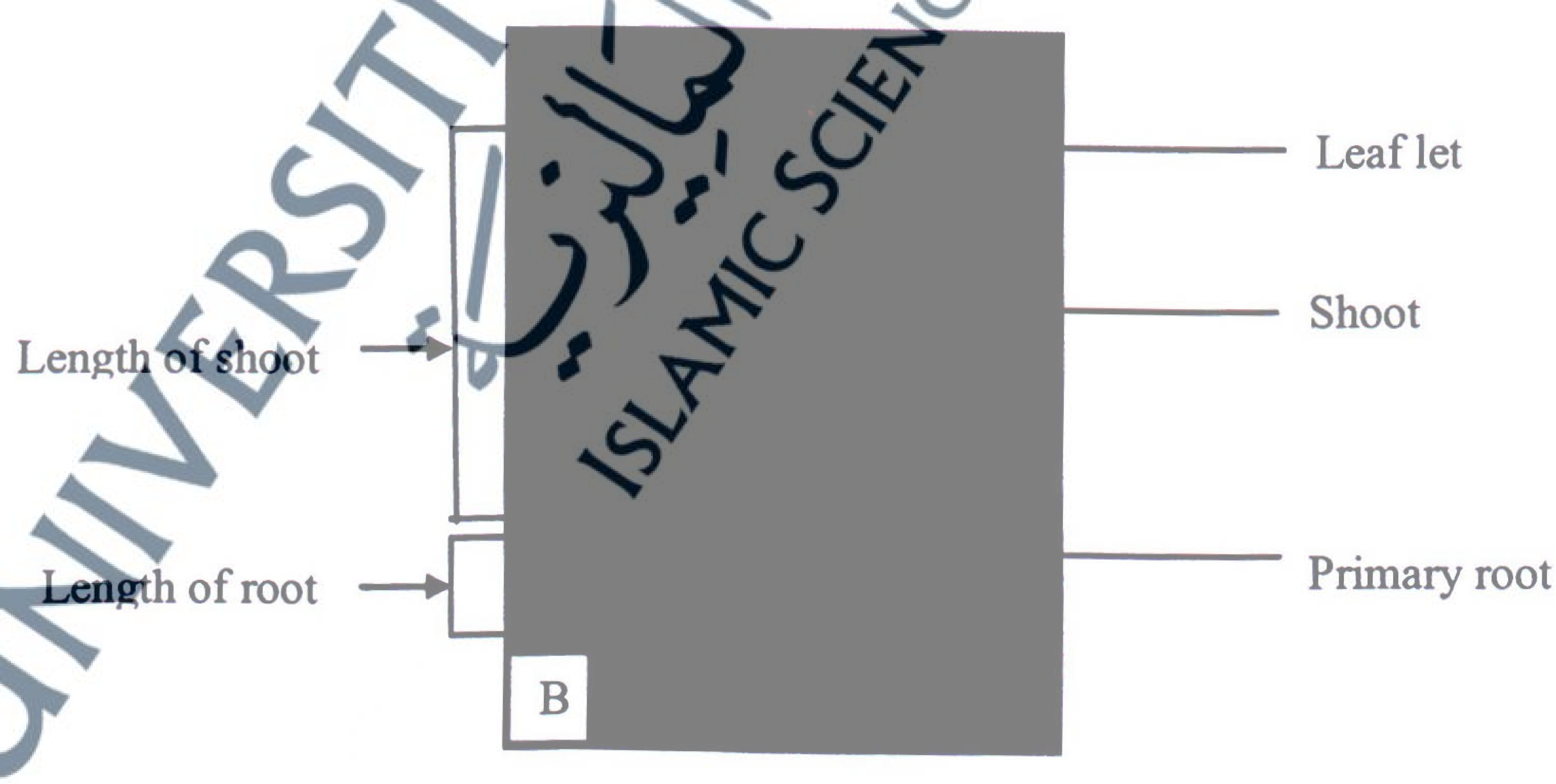
5.5.5.1 Seedling height

It was observed that the sowing seeds soaked in LAB-CFS in soils infected with fungi significantly (P<0.05) increased the seedling height. The increase in seedling height was variable depending on the CFS-LAB used to treat the soil and the fungi inoculated to the seeds. The seedling height of seeds infected with fungi *F. proliferatum* -LR increased from 7.56 cm (soil without LAB-CFS of LAB-MSS1) to 11.40 cm (soil with LAB-CFS of LAB-MSS1) as mentioned (Figure 22A & B); other LAB cells and *Fusarium* spp. on length of chilli seedlings systems of seeds as showed in Table 35 after two week incubation in soil. Likewise, seeds infected with fungi *F. oxysporum* f. sp. *Lycopersici*-CL treated with LAB-CFS -MSS1 also showed increase in seedling height when sowed in soil treated with LAB-CFS-MSS1 from 7.30±1.65 cm (without LAB-CFS-MSS1) treatment) to 10.56±1.50 cm with LAB-CFS-MSS1 treatment. Growth of seedling from seeds infected with fungi *F. proliferatum* -LR also increased form 8.90±0.79 (without LAB-CFS-FF1 treatment) to 9.50±0.86 cm (with LAB-CFS-FF11treatment). LAB-CFS-FF11 also improved slightly the seedling height of seeds infected with Fungi *F. solani*-CS from 9.23±0.20 cm (without LAB-CFS of LAB-FF11 treatment) to 10.83±0.76 cm (with LAB-FF11 treatment) as shown in same above (Table 35) and. Other fungi *F. acuminatum*-FC on Length of chilli seedlings systems of seeds also was effected after two week incubation in soil height of seedling value of 7.56 cm that less improvement compared to LAB treated seedling systems. Based on the results indicates that chilli seeds treatment with LAB-CFS could enhanced the growth of the seedlings as observed by increased in seedling heights even though the seeds were grown in soil infected with fungi.

FIGURE 22: Length of chilli seedlings inoculated with LAB-CFS after two weeks incubation



Notes:- (A) germinated seeds inoculated with CFS of LAB-MSS1 showing good appearance of leaves and root elongation



Notes:- (B) germinated seeds infected with *F. proliferatum*-LR showing not good areal shoot and root elongation growth and no leaf appeared

TABLE 35: Seedling heights of seeds soaked in LAB-CFS seeds and sowed in soils infected with *Fusarium* species

Treatments	Seedling Height (cm)	Seedling Shoot (cm)	Seedling Root (cm)
CONTROL	11.66±0.76^{a}	8.83±0.76^{a}	2.83±0.28^{a}
CFS of LAB Treatments			
LAB-MSS1	11.40±2.70^{a}	8.90±1.27^{a}	2.50±2.10^{a}
LAB-MSS5	8.46±0.45^{abc}	6.66±1.44^{ab}	1.80±1.05^{a}
IDLAB6	9.50±1.80^{abc}	7.66±1.79^{ab}	1.83±0.05^{a}
IDLAB7	7.03±2.91^{b}	8.50±1.00^{ab}	2.53±2.48^{a}
LAB-FF11	9.16±0.28^{abc}	7.00±0.95^{ab}	2.16±0.76^{a}
Seeds treated CFS of LAB and sowed in soil infected with Fungi-CL			
LAB-MSS1+CL	10.56±1.50^{abc}	9.30±1.37^{a}	1.26±0.20^{a}
LAB-MSS5+CL	9.50±1.32^{abc}	8.30±1.5^{ab}	1.20±0.26^{a}
IDLAB6+CL	9.60±0.40^{abc}	8.10±0.10^{ab}	1.50±0.36^{a}
IDLAB7+CL	8.73±0.64^{abc}	7.73±0.64^{ab}	1.00±0.20^{a}
LAB-FF11+CL	10.03±0.47^{abc}	8.93±0.25^{a}	1.10±0.26^{a}
Fungi-CL	7.30±1.65^{c}	6.43±1.88^{ab}	0.86±0.23^{b}
Seeds treated CFS of LAB and sowed in soil infected with Fungi-CS			
LAB-MSS1+CS	10.16±1.04^{abc}	8.33±0.76^{ab}	1.83±0.28^{a}
LAB-MSS5+CS	9.00±1.80^{abc}	8.00±1.77^{ab}	1.00±0.10^{a}
IDLAB6+CS	9.66±0.28^{abc}	8.46±0.49^{ab}	1.20±0.45^{a}
IDLAB7+CS	10.50±1.50^{abc}	8.00±0.50^{ab}	2.50±1.00^{a}
LAB-FF11+CS	10.83±0.76^{abc}	9.33±0.76^{a}	1.50±0.00^{a}
Fungi-CS	9.23±0.20^{abc}	7.80±0.20^{ab}	1.43±0.57^{a}
Seeds treated CFS of LAB and sowed in soil infected with Fungi-FC			
LAB-MSS1+FC	10.06±1.10^{abc}	8.53±1.70^{ab}	1.53±0.46^{a}
LAB-MSS5+FC	8.63±1.51^{abc}	7.16±1.04^{ab}	1.46±0.50^{a}
IDLAB6+FC	8.63±0.63^{abc}	7.33±0.76^{ab}	1.30±0.26^{a}
IDLAB7+FC	9.46±0.55^{abc}	8.00±0.50^{ab}	1.46±0.89^{a}
LAB-FF11+FC	11.16±1.15^{a}	9.13±1.26^{a}	1.70±0.26^{a}
Fungi-FC	7.56±1.36^{b}	6.00±1.00^{b}	1.56±0.60^{a}

Continued			
Seeds treated CFS of LAB and sowed in soil infected with Fungi-LR			
LAB-MSS1+LR	9.50±0.86[abc]	8.23±0.75[ab]	1.26±0.40[a]
LAB-MSS5+LR	9.50±0.50[abc]	8.20±0.40[ab]	1.30±0.45[a]
IDLAB6+LR	8.23±0.25[abc]	7.16±0.28[ab]	1.06±0.11[a]
IDLAB7+LR	7.66±1.44[b]	6.16±0.76[ab]	1.50±1.00[a]
LAB-FF11+LR	9.50±0.86[abc]	7.33±1.15[ab]	2.16±0.28[a]
Fungi-LR	8.90±0.79[abc]	8.30±1.12[ab]	0.60±0.36[b]

Notes:- Means with the same letter in the same column are not significant different (P>0.05) and Means with the different letter in the same column are significantly different (P<0.05). LAB-MSS1 = *Lb. plantarum*1, LAB-MSS5 = *P. pentosaceus*1, IDLAB6 = *Lb. acidophilus* ATCC314, IDLAB7 = *Lb. plantarum* ATCC8014, LAB-FF11 = *Lb. Plantarum*1; Fungi *Fusarium* species; CL = *F. oxysporum* f. sp. *lycopersici*, CS = *F. solani*, FC = *F. acuminatum* and LR = *F. proliferatum*

5.5.5.2 Seedling shoot

There was no significant difference (P>0.05) in the shoot size of seeds with and soaking prior to seed sowing the in fungi *F. solani*-CS and *F. proliferatum* -LR infected soil as listed above (Table 35). However, the shoot length of seeds soaked in LAB-CFS-MSS1 when sown in soil infected fungi *F. oxysporum* f. sp. *lycopersici*-CL increased significantly (P<0.05) from 6.43±1.88 cm (without soaking in LAB-CFS-MSS1) to 9.30±1.37 cm (with soaking in CFS-LAB-MSS1). Similarly, the shoot length of seeds soaked in LAB-CFS-FF11 increased significantly (P<0.05) from 6.00±1.00 cm (without soaking in LAB-CFS-FF11) to 9.13±1.26 cm (with soaking in LAB-CFS-FF11) when sowed in soil infected with *F. acuminatum*-FC after two week incubation at room temperature 28°C.

5.5.5.3 Seedling root

It was observed that sowing seeds treated with LAB-CFS increased the seedling root length even though the seeds were sowed in soil infected with fungi however; there was no significantly difference ($P>0.05$) from control. It was interesting to note that seedling root length of seeds sowed in soil infected with *F. proliferatum* -LR and *F. oxysporum* f. sp. *lycopersici*- CL were significantly ($P<0.05$) lowest 0.60±0.36 cm and 0.86±0.23 cm, respectively, compared to other treatments as mentioned in above (Table 35). In contrast seeds soaked in LAB-CFS-IDLAB7 that were sowed in soil infected with either *F. solani*-CS or *F. acuminatum*-FC increased seedling roots to 2.50±1.00 cm and 1.56±0.60 cm, respectively, compared to 1.43±0.57 cm for control. For seeds sown in soil were infected with Fungi *F. solani*-CS seedling roots increased to 1.43±0.57 cm. Similarly, the seedling roots of seeds grown in soil infected fungi *F. acuminatum*-FC 1.56±0.60 cm (soil without LAB-CFS) was noted more pathogenic on seedling roots.

5.6 DISCUSSION

This study indicates that LAB isolates showed ability to prevent fungal pathogenicity and promote germination of chilli seeds. Soaking the seeds with LAB cells or supernatant enhanced the germination of chilli seeds whether the seeds are infected with the *Fusarium* or sowed in *Fusarium*-infected soil. Even though seeds of chilli Bangi were treated and grown in presence fungi *Colletrotrichum capsici* LAB C5 isolated from durian used as fungal control and seed promoter was showed good germination (El-Mabrouk et al., 2012). Similarly, in this study the cells free supernatants LAB-CFS improved the seeds germination growth compared with chilli seeds infected with *Fusarium* species. The percentage of seed germination was very good in variety chilli seeds hot chilli pepper (Cili Kulai) (93.3%) with LAB-CFS-FF11 which was isolated from fermented food. Other LAB-CFS were noticed that to improve germination percentage but less than LAB-CFS-FF11.

In addition, other varieties of chilli seeds were observed also similar germination with LAB-CFS-MSS1 and LAB-CFS-FF11was showed good percentage germination details description added in (Table 29). LAB-CFS-MSS1 and LAB-CFS-FF11 was noticed that more suitable to improve the percentage germination of chilli seeds of all three verities compare to *Fusarium* species infected seeds. Thus, percentage germination of all chilli seeds were noted to inhibit the percentage germination when chilli seeds were artificially infected with plant pathogenic *Fusarium* species; those were isolated from different plants parts; when the pathogenicity effect of *F. solani*-CS was observed more pathogenicity compared to other *Fusarium* species. Similar results were also detected by Latiffah et al. (2013); Arun & Mathew, 1991) suggested that seeds germination of pigeon pea and gram pea varieties. In addition, the fungi *Fusarium* species did not only suppressed the germination of chilli seeds it was also noticed that *Fusarium* species effected to early germination because germination of chilli seeds appeared late compare to grown chilli seeds in presence of LAB-CFS isolates in Figure 17 (A, B & C). Similarly, the seed samples of pigeon pea were treated with culture filtrates of *F.*

oxysporum udum and their effect on percentage of seed germination and seedling growth has been observed clearly during seeds germination (Jalander & Gachonde (2011).

Additionally infected chilli seeds with *Fusarium* spp. and CFS of LAB applied on different alteration of day showed improvement in chilli seeds germination but applied LAB-CFS at 3 d and 7 d on incubated chilli seeds infected with *Fusarium* species did showed the better germination. However, the alteration of day applications LAB-CFS-MSS1 on 0 d incubated chilli seeds infected with *F. oxysporum* f. sp. *lycopersici*-CL was showed better germination 98.0% (Table 30). In addition, applied LAB-CFS-MSS5 and LAB-FF11 after 3 d on incubated chilli seeds infected with *F. solani*-CS were showed good germination compare to other LAB-CFS. Therefore the application of LAB-CFS showed to be suitable to enhance the percentage of germination. Similarly, when LAB-CFS were applied 0 d on infected chilli seeds with *Fusarium* species. This finding of agreed with earlier report from (Laitila et al., 2002) showed that *Lb. plantarum* was showed effective in reducing the many plant pathogens of *Fusarium* species on the germination of cereals crops.

Furthermore, the germination percentage of selected hot chilli pepper (chilli kulai) seeds was evaluated in soil treated with cells and LAB-CFS. The germination percentage were recorded better either chilli seeds artificially infected with *Fusarium* species or uninfected with *Fusarium* species and grown in soil treated with LAB cells because, cells of LAB work in two action. Firstly, by reduce the pathogenicity of *Fusarium* species and secondly to promote seeds germination and seedling systems. For instance, chilli seeds were grown in soil treated with cells of both LAB-MSS1 and LAB-FF11 showed good germination more than 97.0% in (Figure 19). The other LAB cells were showed the ability to improve the germination percentage either chilli seeds infected or not infected with fungi *F.* oxysporum f. sp. *lycopersici*-CL, *F. solani*-CS, *F. acuminatum*-FC and *F. proliferatum*-LR in soil. Because, infected chilli seeds with *Fusarium* species or none infected with *Fusarium* species showed good germination in soil treated with cells of LAB isolated from different sources such soil sample, fermented chilli fruits and ATCC culture.

It was obviously clear that reduction of fungal infection pathogenicity and exotic seeds germination were come from inoculated cells of LAB due to, when infected chilli seeds with *Fusarium* species were grown in soil without treated cells of LAB showed poor germination percentage compare to control and cells of LAB treated seeds. In this case pathogenicity effect of all *Fusarium* species was noticed during germination time. In addition, Hamed et al. (2011) reported that isolated LAB1, LAB2, LAB3 LAB4 and LAB5 cells showed ability to increase another Solanaceae plant such as tomato plant seeds germination either tomato seeds were survived in presence of phytopathogenic fungi *Fusarium* species *F. oxysporum*-1 *R. solani*-1 and *F. oxysporum*-2 , *S. rolfsii*; *R. solani-2*; in soil treatments. Furthermore, report from Muhaildin et al. (2011) observed that the variability in cells mass reduction could be related to specificity of metabolites produced by LAB that may have caused failure of conidia germination and mycelium proliferation. For instance, cell mass of *A. oryzae,* a food spoilage fungi was inhibited by LAB-CFS of LAB Te010 and G004 in the liquid system. Additionally, Yousef and Lloyd

(2008) reported that *Lb. paracaseis* sp. *Tolerans* completely inhibited the growth of *F. proliferatum* M5689, M5991 and *F. graminearum* R 4053 compared to control. In addition, Phytopathogenic was Inhibited the seed germination of tomato of variety ICP-2376 by10% and the effects were seen on seedling systems; there was reduction in length of roots, shoots and seedling growth (Jalander & Gachonde, 2011). Similarly, Strains of lactic acid bacteria reported to have antifungal activity (El. Mubroke et. al., 2012). The LAB can be as biocontrol in chili seeds and plants as well as improve the plant growth and productivity (Anupama et al., 2014). Thus, LAB was noticed that to show ability of production fungicidal compound and plants improvement nature compound. Because report from Gould (1992; De Vuyst & Vandamme (1994); Holzapfel et al. (1995) bacteria produce chemicals that are anti-pathogenic (e.g. diacetyl, hydrogen peroxide, lactic acid, acetic acid, propionic acid, bacteriocins, carbon dioxide and chitinase enzyme). Similarly, phytohormones amiability includes production of plant growth regulators (like auxin, gibberellin, and ethylene), siderophores, HCN and antibiotics (Arshad et al., 1992). Bacteria synthesize auxins in order to perturb host physiological processes for their own benefit (Shih-Yung, 2010).

Similarly, treated chilli seeds with LAB-CFS isolated from different sources were noticed that to enhance the germination percentage of chilli seeds even though it was grown in soil infected with fungi *Fusarium* species or not infected with fungi *Fusarium* species (Figure 21). Chilli seeds treated with LAB-CFS-FF11 was germination nearly 96.0% when it was survived in soil infected with *F. oxysporum* f. sp. *lycopersici*-CL. It was observed that LAB-CFS other LAB also showed the ability to improve the seeds germination but the germination percentage were noticed that vary depend on sources of isolates. However, the percentage germination of selected Chilli Kulai seeds was observed better in presence of cells of LAB compare to LAB-CFS. Similar report showed by El-Mobrok el al. (2012) was reported that the *Lb. plantarum* C5 cell and their supernatants was showed ability to prevent fungal pathogenicity and promote the seeds germination of chilli seeds even though chilli seeds were grown in presence fungi *Colletotrichum capsici* and LAB-CFS of LAB C5 isolated from durian was showed good germination of chilli seeds. Similarly, Anasol et al. (1981) reported that toxic metabolites of Drechslera rostrota and *Fusarium* equiseti retarded root growth of wheat. Reduction in percentage seed germination of soybean seeds was observed in seeds soaked in filtrates of Phomopsis phaseoli (Hilty & Lee, 1988). Because, fungi of the genera *Fusarium*, *Aspergillus*, *Penicillium* and *Rhizoctonia* are commonly known to produce toxic substances (Singh et al., 1991).

Additionally, in the present study also was isolated four phytopathogenic fungi *Fusarium* species were tested for pathogenic effect on germination of selected hot chilli pepper (Cili Kulai) seeds in soil. The *F.* oxysporum f. sp. *lycopersici*-CL, *F. acuminatum*-FC, *F. solani*-CS and *F. proliferatum-LR* homogenized culture cells were artificially infected with chilli seeds or infected in soil thus, both cases seeds germination were observed to suppress the percentage of seed germination and seedling systems because of, the soil and seeds did not treat with LAB-CFS and cells of LAB strains. The poorest germination of chilli seeds was noted 63.0% when it was grown without treated with LAB-CFS in soil infected with fungi *F. acuminatum*-FC and *F. solani*-CS. However, the soil infected with *F.* oxysporum f. sp. *lycopersici*-CL and *F. proliferatum-LR,* and chilli seeds were non treated LAB-CFS and cells of LAB germination were suppressed moderately. So the fungi *F.* oxysporum f. sp. l*ycopersici*-CL and *F. proliferatum-LR* shown to be less effective on chilli seeds germination compared to *F. acuminatum*-FC and *F. solani*-CS. The above results conformed the findings (Gopinath & Shetty, 1988) fungi is known to degrade seed germination and effect the physiology. The similar results were also observed by (Arun & Mathew, 1991; Gachande and Jadhav 2010). In addition the LAB isolates were treated as against four fungi *Fusarium* spp. on hot chilli pepper seeds (Chilli Kulai) germination to enhance the seedling plant system (seedling plant height, length of shoot and roots) as described in Table 25. For example, Narasimha et al., 2012 reported that the potential activities of two Lactic acid Bacteria such as *Lb. paracasei* subsp. *tolerans*, *Lb. paracasei* sub sp. *paracasei,* have abilities to suppress the wilt pathogen and these LAB play important role as plant growth promoting bacteria (PGPB) in tomato seeds against *R. solanacerum* that is caused bacterial wilt and deemed to be one of the most important plant diseases in tropical agriculture. (Saranraj et al., 2013) detected that other bacteria *Pseudomonas* species are important plant growth promoting rhizobacteria (PGPR) have ability to enhance crop by direct and indirect mechanisms and this bacteria isolated from rhizosphere of paddy grown was produced phyto-hormones substances that are indole acetic acid (IAA) and siderophores.

The major enhancements were measured when the infected chilli seeds with fungi *F. solani*-CS and grown in soil treated with cells of LAB-MSS1 on seedling plant height of 12.33 cm. Then the LAB-MSS1 which was isolated from soil more beneficial to promote seedling growth compare to other LAB isolates. Similarly, treatment of soil with LAB may trigger systematic acquired resistance (SAR) which develops when plants successfully activate their defense mechanism, in presence of a pathogen infection, resulting in an enhanced synthesis of plant defense chemicals which support plant growth and make stronger plant cell wall strength (Stephane et al., 2005). Likewise, chilli seeds were neither infected with *Fusarium* species nor inoculated with LAB cells used (as control) showed enhancement on seedling plants height about 11.66 cm that results was same as LAB cells and LAB-CFS effect on enhancement on chilli seedling plant systems were noticed after two weeks of incubation (Figure 28). Similarly, good improve was noticed on length of seedling shoot which chilli seeds were infected with *F. proliferatum-LR* and grown in soil treated with cells of LAB-FF11. The treated chilli seeds with LAB-CFS-MSS1 and grown in soil without infected *F. acuminatum-FC* was showed better improvements on seedling plant height, length of shoot and roots even the seeds were grown in soil infected with *Fusarium* species details were given in Figure 20 (A, B & C). Finally, the chilli seeds when were grown in presence of cells and LAB-CFS to improve the seedling systems whether the seeds were infected with/without *Fusarium* species (Table 35). Many researchers were proved that chilli is susceptible to several diseases by *Fusarium* species including wilt, root rot and powdery mildew caused by the fungus *Leveillula taurica* (Mushtaq & Hashmi, 1997). Similarly, root and collar rot produces by *Phytophthora capsici* (Saleem et al., 1998; Than et al. 2008). Similarly, Asalmol et al. (2001) suggested that seed borne fungi in the chilli seeds such as *Fusarium moniliformae, A. flavus, Rhizopus stolonifer, C. capsici* and *A. niger* was more pathogenicity effect on chilli seeds and inhibited the germination percentage.

Likewise, chilli seeds were infected with *F. acuminatum*-FC and survived in soil uninoculated with cells of LAB showed poor development on length of seedling shoot and length of root when sowed in soil treated with LAB-MSS1 cells (Table 34). The less development on length of root of infected chilli seeds with *F. proliferatum-LR* and grown in soil untreated with cells of LAB strains. It was clear that *F. acuminatum*-FC and *F. proliferatum*-LR were showed sever reduction on length of seedling root but did not effected the seedling plants system when treated chilli seeds grown in presence of cells and LAB-CFS of LAB isolates. These results were agree with El. Mobrok el al. (2012) that reported the *Lb. plantarum* C5 cells and their supernatants showed ability to inhibit fungal pathogenicity and promote the germinated plants systems (seedling height, shoot and root) of chilli seeds were grown in presence of fungi *Colletrotrichum* sp. Finally, the seedling systems of Chilli Kulai seeds treated with cells and LAB-CFS of LAB either the chilli seeds were grown in presence of pathogenic *Fusarium* species were noticed that to improve the percentage germination and seedling systems. It was not only improved the percentage germination and seedling systems; it was observed that also the length of shoots and roots stronger and thickness than compare to infected chilli seeds were grown in absence of cells and LAB-CFS of LAB isolates.

5.7 CONCLUSSION

The germination of chilli seeds was enhanced when the seeds were treated with both the cells and LAB-CFS of the LAB isolates as observed by the good germination percentage and elongation of chilli seedling plant systems (seedling height, shoot length and root length) in conditions where seeds were infected with the fungi or seeds sowed in fungi infected soils. Thus this findings further support the LAB-MSS1, LAB-MSS5, IDLAB6, IDLAB7 and LAB-FF11 can be used as biocontrol agents against *Fusarium* species which were isolated from different plants systems such as *F. oxysporum* f. sp. *lycopersici*-CL, *F. solani*-CS, *F. acuminatum*-FC and *F. proliferatum*-LR and LAB can be used as a plant growth promoting bacteria (PGPB) mainly Solanacae plants and other plants. Furthermore, cells and LAB-CFS of LAB were showed improvement on seed germination and seedling systems. Then these LAB isolates can be used to enhance the plant growth and plant systems that is mentioned in further chapter.