

Lactic Acid Bacteria as Energy Sources and Inhibit to Fungi *Fusarium* Species on Seedling Systems in Soil Medium

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Abstract:- Pepper Cili Kulai of the variety chilli pepper belonging to family Solanaceae is an important vegetable and spice crop grown in Malaysia and produced and consumed as fresh or processed in worldwide. The production is chilli yield products constrained by various plant pathogens causing diseases on chilli seeds, seedling systems and plants including pepper Cili Kulai variety. The main diseases that lead to wilting in chilli are *Fusarium* wilt and caused huge losses in final products and damage to seedling systems, plant systems in agriculture. In this study, cells and cells free supernatants of the five strains of lactic acid bacterial were used as bio-control agent and energy sources to enhance the seedling systems of Cili Kulai seed. *In vitro* assay of beneficial microbial supernatants and cells of lactic acid bacteria strains were showed inhibitory activity against fungi *Fusarium* species and better improvements were noticed that on seedling systems of Cili Kulai seeds in 160 eyes plastic tray filled with soil medium. After two week incubation seeds of Cili Kulai with cells and cells free supernatants of Lactic acid bacteria especially, *P. pentosaceus*1-MSS, *Lb. plantarum*1-FCF, *Lb. acidophilus* ATCC314, *Lb. plantarum* ATCC8014 and *Lb. plantarum*1-MSS; significantly, were observed that to suppress *Fusarium* species patho-genicity. The positive impacts were noticed that on seedling systems of Cili Kulai seeds enhancements on seedling systems such as height, shoots and roots length. The negative effect were noticed that on height, shoots and roots elongation when Cili Kulai seeds were survived artificially infected with *Fusarium* species namely, *Fusarium proliferatum*-LR, *Fusarium sp. fus* 124-FC, *Fusarium sp.* CID124-CS and *Fusarium oxysporum* KAML01-CL. The better elongation and development on seedling systems were noticed that which Pepper Cili Kulai seeds were survived in presence of cells or cells free supernatants of Lactic acid bacteria either Pepper Cili Kulai seeds and soil were infected with pathogenic *Fusarium* species. The cells and cells free supernatants of all LAB strains were showed as good energy sources on seedling systems of Cili Kulai and to protect from pathogenic fungi *Fusarium* species in soil medium.

Keywords:- Lactic Acid Bacteria; Cells and supernatants; *Fusarium* Species; Pepper Cili Kulai; Seedling Systems and Inhibitory Activity.

I. INTRODUCTION

In agriculture most of the farmers and plants growers are rely on the synthetic fertilisers, synthetic plant hormones as energy sources to enhance, promote seedling systems, plants systems in agriculture or greenhouse to increase productivity as well as synthetic chemical fungicides toward protect from pathogenic organisms in worldwide (1). The National Institute of Environmental Health Sciences (NIEHS) was reported that excess use of synthetic fertiliser and chemical fungicides have both negative and positive effects for better yield products and consumers health (2). Avoiding to negative effect and consumers demands to search for alternative solutions for agriculture as well as for Phyto-pathogens have prompted researchers to take a next selective choice of beneficial microorganisms long known to afford advantages to agricultural production and motivating quick growth in markets for bio-pesticides and bio-fertilizers (3) also crop growth promoting microorganisms (4). Many plants growth improvement using a number of plants allied in soil microorganisms are associated with capability to work as “bio-fertilizer” and “bio-regulators” by enhancing the accessibility of nutrients materials into the Rhizosphere areas of plants (5). The good characteristics of beneficial bacteria's have to enhance synthesis of Phyto-hormones especially; auxins, gibberellins, and ethylene which useful enzymes that encourage earlier germination, especially, α amylase, which enhance starch assimilation (6); (7). Lactic acid bacteria (LAB) strains are selective microorganisms have been classified as food grade beneficial microorganisms and it is significant to stress that an broad work has been completed in developing diverse tools for the recombinant protein creation using micro bacterial LAB strains as cells factories (8). The LAB cells and CFS are consisted metabolites molecules which are acted as bio-control agent and bio-fertilizers in agriculture (9). Application of LAB isolates have been used on different plants however, the limit study was conducted on Solanaceous

plants especially, chilli plant is belonging to family Solanaceae and most important in human food in worldwide (10); (11). The wilt plant diseases have been well recognized universally in chilli plants because it caused serious economic loss due to the pathogenic fungi *Fusarium* species causative agent of wilt diseases in many plants (12); (13); (14). This patho-genicity in soil and on plants has been needed to remove by using beneficial microbial bio-control agents; LAB strains have been increased to plant development and final products simultaneously. Treatments of LAB cells and CFS in soil and soaked with seeds were indicated as bio-control agents and bio-fertilizers activity to improve seedling systems of pepper Cili Kulai seeds in this study.

II. MATERIALS AND METHODS

A. Preparation of Cells of Lactic Acid Bacteria

Cells of beneficial microorganism Lactic Acid Bacteria (LAB) species namely, *Lb. plantarum* ATCC8014, *Lb. acidophilus* ATCC314, *P. pentosaceus*1-MSS, *Lb. plantarum*1-MSS and *Lb. plantarum*1-FCF were survived in specific medium the (MRSB Oxoid,) using the technique was described by (15) with small modification and incubated in an incubator shaker for overnight at 37°C and cells concentration of each LAB isolates were observed with help of Nanophotometer- atOD₆₀₀^o before each treatments.

B. Cells Free Supernatants Preparation

For preparation of cells free supernatants(CFS) of *Lb. plantarum* ATCC8014, *Lb. acidophilus* ATCC314, *P. pentosaceus*1-MSS, *Lb. plantarum*1-FCF and *Lb. plantarum*1-MSS strains were inoculated into specific medium MRSB and incubated about 24h maintained at 37°C in aerobic shaker incubator using the method described by (15). Then cells cultures broth of all LAB strains were centrifuged at 11500×g rpm for 10 min at 4°C. The CFS of each LAB strains were filtrated with help of sterile filtered paper 0.45µm pore size Milli pore filter then used for further treatments with soil and pepper Cili Kulai seeds.

C. Surface Sterilization of Pepper Cili Kulai Seeds

The chilli seeds namely, Pepper Cili Kulai seed was purchased and surface sterilization was done using the method was mentioned by (16) with modification. The pepper Cili Kulai seeds were cleaned with running water and exterior sterilized with help of 1% sodium hypochlorite solution for 1-2 min. After sterilization of seeds were washed with pure sterile distilled water more than three times. The sterilized pepper Cili Kulai seeds were dried in air with help of laminar flow to remove the moisture from seeds after that treatments were done for seedling purposes in soil medium.

D. Pathogenic Fungi *Fusarium* Species Cultures Preparation

Fungi *Fusarium* species were prepared using highly virulent pathogenic fungi *Fusarium* species were selected namely, *Fusarium* sp. *fus* 124-FC, *Fusarium oxysporum* KAML01-CL, *Fusarium* sp. CID124-CS and *Fusarium*

proliferatum-LR were developed on specific PDA medium and incubation was done at room temperature at 28°C for 5 days adopting the processor described by (17) with slight modification. After that the sterilized distilled water 10 to 20ml were poured onto the PDA plate media. Then fungal surfaces were smoothly scraped to release the spores suspensions were collected in sterile bottle separately. The spores suspension were concentrated at 1×10⁵ spores/ml was maintained by serial dilution process. Afterward fungal spores of fungi species were homogenized mixing with help of sterilized blender machine for 1-2 min then the artificially infected on pepper Cili Kulai seeds and soil medium for patho-genicity tests.

E. Assay on Seedling Systems of Pepper Cili Kulai Seeds in 160 Eyes Plastic Tray

Effect of LAB cells and supernatants and patho-genicity effect fungi *Fusarium* species on seedling systems of Cili Kulai seeds were assayed *In vitro* as follows; surface sterilized pepper Cili Kulai seeds were treated adopting the described by (17) with modification. Pepper Cili Kulai seeds were separated into the six groups. The first group of chilli seed was survived in soil treated with 5ml of overnight bacterial cultures cells poured in the centre of the 160eye of plastic tray were filled with 25g potting soil without using any synthetic fertilizers at one seed per tray eye. The second group of Pepper Cili Kulai seeds infected with fungi *Fusarium* species and seeds were sown in soil treated with 5ml of LAB culture cells. The third group of the Pepper Cili Kulai seeds were treated with LAB-CFS for 1h and it was dried in air laminar flow. Then the seeds were sowed in soil without synthetic fertilizer. The fourth group of Pepper Cili Kulai seeds were treated with LAB-CFS about 60 min. Then seeds were dried in air and sowed in plastic tray eye filled with 25mg potting soil medium infected with fungi *Fusarium* species. The fifth group of Pepper Cili Kulai seeds were grown in soil infected with 5ml of the five days old fungal spore suspension and each tray eye was inoculated in the centre used as negative control. Then group six Pepper Cili Kulai seeds were soaked with water did not treated with any supplements used as positive control to comparison. After treatments all the seeded trays were in dark chamber at 30°C and sprayed with tab water, covered with plastic after that incubated for two week to allow seedling systems. The length of seedling systems such as height, shoot and root were measured using general scale and the treatments were done in triplicate.

F. Data Analysis

All data were analysed with mean ± standard deviation was achieved from each analyses were analyzed with help of one way analysis of variance (ANOVA) and the mean significant was calculated using the Tukey test at (P≤0.05). The statistical analysis was calculated by running the Minitab 16 software.

III. RESULTS

Improvement on seedling systems such as heights shoots and roots of pepper Cili Kulai seeds artificially were infected *Fusarium* species grown in soil treated with LAB Cells. On the other hands, pepper Cili Kulai seeds were soaked in cells free supernatants of LAB grown in soil artificially were infected *Fusarium* species and seedling systems such as heights shoots and roots were mentioned clearly as following;

A. Improvement on Seedling Systems by Cells of Lactic Acid Bacteria

Improvement on seedling systems of seeds pepper Cili Kulai was infected with spores of pathogenic fungi *Fusarium* species and sowed in soil medium treated with LAB-cells were mentioned as following;

➤ Seedling Heights

The improvements were recorded that the fungi *Fusarium* species infected with pepper Cili Kulai seeds sowed in soil medium treated with strains LAB-cells significantly ($P \leq 0.05$) improved the seedling height in soil medium. The improvement in seedling height was variable depending on the fungi *Fusarium* species infecting the Cili Kulai seeds and the beneficial LAB-cells inoculated to the soil medium. The seedling height of fungi *Fusarium* sp. *fus* 124-FC infected pepper Cili Kulai seeds were improved from 7.56 ± 1.36 cm in soil medium without *Lb. plantarum*1-MSS1 cells to 12.33 ± 2.84 cm in soil medium with *Lb. plantarum*1-MSS cells Figure 1 (A, B & C). Other LAB-cells and fungi *Fusarium* species on length of pepper Cili Kulai seedling systems of seeds were described in Table 1 after 14 days incubation in soil medium. In the same way, seedling height infected with fungi *F. oxysporum*KAML01-CL was sowed in soil medium mixed with *Lb. plantarum*1-MSS increased from 7.30 ± 1.65 cm (in control) to 10.30 ± 1.57 cm (with cells of *Lb. plantarum*1-MSS treatment). The seedling height of fungi *F. proliferatum*-LR infected with pepper Cili Kulai seeds increased from 9.00 ± 0.79 cm (in control) to 10.16 ± 0.76 cm with cells of *Lb. plantarum*1-MSS treatment. The bacterial Cells of *Lb. plantarum* ATCC8014 increased the seedling height of pepper Cili Kulai seeds infected with high virulent Phyto-pathogenic fungi *F. solani*-CS from 9.23 ± 0.20 cm (in control) to 12.00 ± 0.57 (with cells of *Lb. plantarum* ATCC8014 treatment) was described in Table 1 clearly. The results were indicated that soil medium treatment with LAB-cells could promoted the growth of the seedlings systems as recorded by enhancement in seedling heights while the pepper Cili Kulai seeds were artificially infected with the fungi *Fusarium* species before applying in soil and after 14days incubation at 28°C in soil medium at room temperature *In Vitro* method.

➤ Seedling Shoot

Normally, there was no different significantly ($P \geq 0.05$) in the shoot length of developed seeds of pepper Cili Kulai when infected with fungi *F. solani*-CS and *F. proliferatum* - LR were sown in LAB treated soil medium was mentioned in

Table 1. On the other hand, the seedling shoot sizes of germinated seeds infected with fungi *F. oxysporum*KAML01-CL was enhanced significantly ($P \leq 0.05$) in shoot length was measured from 6.43 ± 1.88 cm (control) to 9.16 ± 1.04 cm was sowed in soil medium with cells of *P. pentosaceus*1-MSS strain. Likewise, the length of shoot seeds were infected fungi specie *Fusarium* sp. *fus* 124-FC also promoted significantly ($P \leq 0.05$) when it was survived in soil medium treated with cells of *Lb. plantarum*1-MSS in range from 6.00 ± 1.00 (control) to 9.40 ± 0.52 cm with cells of LAB- *Lb. plantarum*1-MSS in soil medium.

➤ Seedling Roots

Root of seedling of Cili Kulai was measured that sowing fungi *Fusarium* species infected pepper Cili Kulai seeds in cells of LAB strains treated in soil medium enhanced the root length of Cili Kulai seed however, it was not significantly different ($P \geq 0.05$) compared from control. On the other hand, it was exciting to observe that root length seedling system of pepper Cili Kulai seeds were infected with spores of fungi *F. proliferatum*-LR was noticed that significantly ($P \leq 0.05$) smaller in range from 0.60 ± 0.36 cm compared to other treatments that has been mentioned in Table 1 evidently. whereas, the root of seedling system from fungi *Fusarium* sp. CID124-CS infected with pepper Cili Kulai seeds were grown in soil medium inoculated with cells of LAB-*Lb. plantarum* ATCC8014 was promoted seedling roots system of fungi-*Fusarium* sp. CID124-CS infected with pepper Cili Kulai seeds in range from 1.43 ± 0.05 to 4.00 ± 0.94 cm even though it was of no significantly differences in length ($P \geq 0.05$) after 14days incubation in soil medium at room temperature.

Notes:-In Figure 1 was displayed that(A) germinated Cili Kulai seeds inoculated with cells of *Lb. plantarum*1-MSS was showed good shoot, root and leaflet opened clearly of seedling system (B) germinated Cili Kulai seeds did not inoculated with cells of LAB or fungi (control) showing normal shoot and root enhancements and leaflet opened visibly (C) germinated seeds infected with *Fusarium* sp. *fus* 124-FC showing weak seedling systems shoot and root elongation, leaflet did not opened clearly and surface of root showing many nodes were known as *Fusarium* species diseases symptoms.

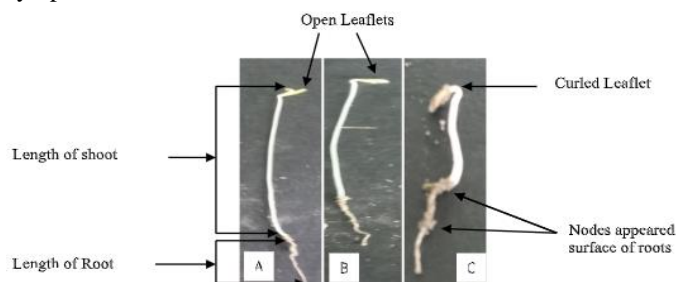


Fig 1:- Length of pepper Cili Kulai seeds inoculated with LAB-cells and seedling systems were observed after two weeks incubation

Treatments	Measurement of seedling systems		
	Seedling height (cm)	Seedling shoot (cm)	Seedling root (cm)
Control	12.00±0.76 ^a	9.00±0.76 ^{ab}	3.00±0.28 ^{ab}
Cells of LAB treated with seeds			
<i>Lb. plantarum</i> 1MSS	9.03±0.50 ^{ab}	7.33 ±0.76 ^{ab}	2.00±0.26 ^{ab}
<i>P. pentosaceus</i> 1MSS	9.23±1.16 ^{ab}	8.00±1.40 ^{ab}	2.00±0.01 ^{ab}
<i>Lb. acidophilus</i> ATCC314	10.00±0.68 ^{ab}	8.00±1.20 ^{ab}	2.10±0.55 ^{ab}
<i>Lb. plantarum</i> ATCC8014	10.00±1.68 ^{ab}	8.83±1.41 ^{ab}	2.06±0.95 ^{ab}
<i>Lb. Plantarum</i> 1FF	10.36±1.89 ^{ab}	7.50±0.81 ^{ab}	3.00±1.10 ^{ab}
Seed infected with fungi-CL and grown in soil medium treated with strains of LAB-cells			
<i>Lb. plantarum</i> 1MSS + Fungi-CL	10.30±1.57 ^{ab}	9.73±1.41 ^{ab}	2.00±0.51 ^{ab}
<i>P. pentosaceus</i> 1MSS + Fungi-CL	10.23±1.06 ^{ab}	9.16±1.04 ^a	1.06±0.20 ^{ab}
<i>Lb. acidophilus</i> ATCC314 + Fungi-CL	10.00±0.65 ^{ab}	8.16±0.35 ^{ab}	2.00±0.64 ^{ab}
<i>Lb. plantarum</i> ATCC8014 + Fungi-CL	9.13±1.00 ^{ab}	9.00±0.64 ^{ab}	1.06±0.40 ^{ab}
<i>Lb. Plantarum</i> 1FF + Fungi-CL	10.16±0.57 ^{ab}	9.00±0.11 ^{ab}	1.16±0.69 ^{ab}
<i>Fungi-CL</i>	7.30±1.65 ^b	6.43±1.88 ^{ab}	0.86±0.23 ^{ab}
Seed infected with fungi-CS and grown in soil medium treated with LAB strains cells			
<i>Lb. plantarum</i> 1MSS + Fungi-CS	9.33±0.28 ^{ab}	8.30±0.34 ^{ab}	1.03±0.05 ^{ab}
<i>P. pentosaceus</i> 1MSS + Fungi-CS	9.43 ±0.20 ^{ab}	8.03±0.41 ^{ab}	1.23±0.11 ^{ab}
<i>Lb. acidophilus</i> ATCC314 + Fungi-CS	9.06±0.92 ^{ab}	8.23±0.46 ^{ab}	1.16±0.76 ^{ab}
<i>Lb. plantarum</i> ATCC8014 + Fungi-CS	12.00±0.57 ^a	8.06±0.40 ^{ab}	4.00±0.94 ^a
<i>Lb. Plantarum</i> 1FF + Fungi-CS	11.00±0.25 ^{ab}	9.00±0.76 ^{ab}	2.10±1.01 ^{ab}
<i>Fungi-CS</i>	9.23±0.20 ^{ab}	8.00±0.76 ^{ab}	1.43±0.05 ^{ab}
Seed infected with fungi-FC and grown in soil medium treated with LAB strains cells			
<i>Lb. plantarum</i> 1MSS + Fungi-FC	12.33±2.84 ^a	9.40±0.52 ^a	3.00±2.92 ^{ab}
<i>P. pentosaceus</i> 1MSS + Fungi-FC	10.36±0.41 ^{ab}	9.00±0.17 ^{ab}	1.46±0.25 ^{ab}
<i>Lb. acidophilus</i> ATCC314 + Fungi-FC	11.00±0.63 ^{ab}	9.00±0.17 ^{ab}	2.00±0.73 ^{ab}
<i>Lb. plantarum</i> ATCC8014 + Fungi-FC	9.46±0.55 ^{ab}	8.00±0.50 ^{ab}	2.00±0.55 ^{ab}
<i>Lb. Plantarum</i> 1FF + Fungi-FC	11.16±1.15 ^a	9.30±1.53 ^a	1.86±0.55 ^{ab}
<i>Fungi-FC</i>	7.56±1.36 ^b	6.00±1.00 ^b	1.56±0.60 ^{ab}
Seed infected with fungi-LR and grown in soil medium treated with LAB strains cells			
<i>Lb. plantarum</i> 1MSS +Fungi-LR	10.16±0.76 ^{ab}	9.00±0.86 ^{ab}	1.16±0.28 ^{ab}
<i>P. pentosaceus</i> 1MSS + Fungi-LR	9.33±1.15 ^{ab}	8.00±1.00 ^{ab}	2.00±0.50 ^{ab}
<i>Lb. acidophilus</i> ATCC314 + Fungi-LR	9.16±0.76 ^{ab}	8.16±1.25 ^{ab}	1.00±1.86 ^b
<i>Lb. plantarum</i> ATCC8014 + Fungi-LR	9.16±0.28 ^{ab}	8.00±0.57 ^{ab}	2.00±0.50 ^{ab}
<i>Lb. Plantarum</i> 1FF + Fungi-LR	10.16±1.25 ^{ab}	9.40±1.51 ^a	0.76±0.25 ^{ab}
<i>Fungi-LR</i>	9.00±0.79 ^{ab}	8.30±1.12 ^{ab}	0.60±0.36 ^b

Table 1:- Seedling systems of pepper Cili Kulai seed infected with fungi *Fusarium* species and grown in soil treated with cells of LAB strains

Notes:-In Table 1 showed that means with the same letters are did not significantly differences ($P \geq 0.05$) and means with the different letters are showed significantly differences ($P \leq 0.05$); Fungi *Fusarium* species: CS = *Fusarium* sp. CID124, CL = *F. oxysporum* KAML01, FC = *Fusarium* sp. *fus* 124 and LR = *F. proliferatum*.

B. Improvement on Seedling Systems by Cells Free Supernatants of Lactic Acid Bacteria

On the other hand, improvement on seedling height of pepper Cili Kulai seeds soaked in LAB- CFS and it was grown in soil medium infected with fungi *Fusarium* species were mentioned as following;

➤ Seedling height

It has been observed that the sowing pepper Cili Kulai seeds soaked in LAB-CFS in soils infected artificially with fungi

Fusarium species significantly ($P \leq 0.05$) increased the seedling height. The improvement in seedling height was variable depending on the LAB- CFS used to treat in soil and the fungi inoculated to pepper Cili Kulai seed. The height of seedling systems of seed infected with fungi *F. proliferatum*-LR improved from 8.00cm (in soil medium without LAB-CFS of *Lb. plantarum*1-MSS) to 11.40cm (in soil medium with LAB-CFS of *Lb. plantarum*1-MSS) as mentioned in Figure 2 (A, B & C) clearly.

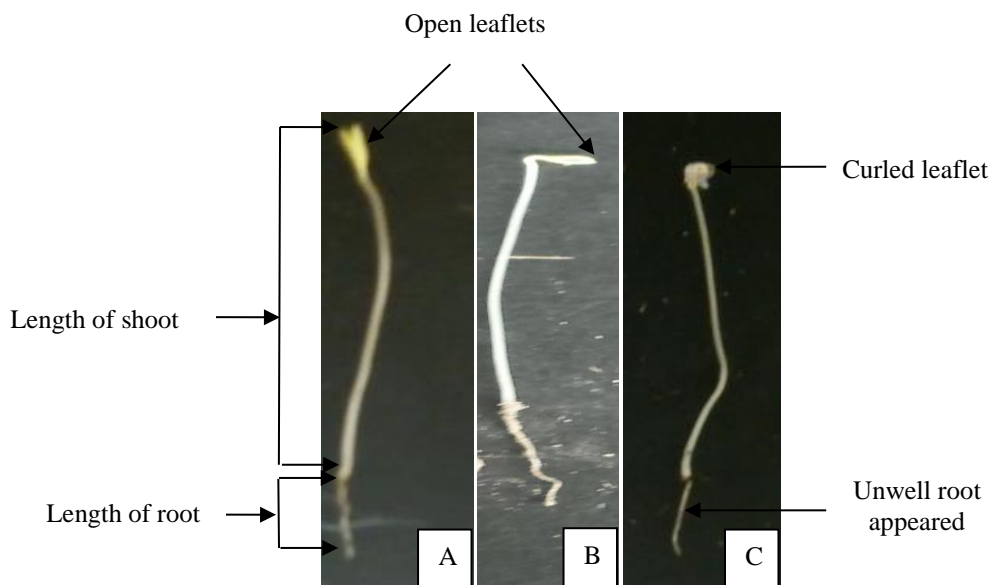


Fig 2:- Length of seedling systems pepper Cili Kulai seeds treated with LAB-CFS and it was sowed in soil observed after two weeks incubation

Notes:-In Figure 2 was displayed that (A) Germinated pepper Cili Kulai seeds were treated with CFS of *Lb. plantarum*1-MSS indicating good manifestation of leaves, root improvements and leaflet has been opened clearly, (B) Germinated pepper Cili Kulai seeds have not been inoculated with cells of LAB or fungi (control) indicating good shoots, root improvements and leaflet has been opened visibly (C) Germinated pepper Cili Kulai seeds infected with fungi *F. proliferatum*-LR did not showed good areal shoot and root improvements and appeared as curled leaflet did not opened preferably.

Likewise, pepper Cili Kulai seeds were infected with *Fusarium oxysporum* KAML01-CL treated with LAB-CFS - *Lb. plantarum*1-MSS also was showed to enhance the seedling height when grown in soil medium mixed with CFS of *Lb. plantarum*1-MSS strains was improved seedling hieght from 7.30 ± 1.65 cm (without LAB-CFS- *Lb. plantarum*1-MSS)

treatment) to 11.00 ± 1.50 cm with LAB-CFS of *Lb. plantarum*1-MSS strain treatments. Growth of seedling height of pepper Cili Kulai seed infected with fungi *F. proliferatum*-LR also has been improved form 9.00 ± 0.79 (with no LAB-CFS- *Lb. plantarum*1-FCF treatment) to 10.00 ± 0.86 cm (with LAB-CFS- *Lb. plantarum*1-FCF treatment). Isolate LAB-CFS- *Lb. plantarum*1-FCF also has been improved seedling height of pepper Cili Kulai seeds infected with *Fusarium* sp.CID124-CS from 9.23 ± 0.20 cm (with no LAB-CFS of *Lb. plantarum*1FCF treatment) to 11.00 ± 0.76 cm (with LAB- *Lb. plantarum*1-FCF inoculation) has been mentioned in Table 2 clearly.

Treatments	Measurement of Seedling Systems		
	Seedling height (cm)	Seedling shoot (cm)	Seedling root (cm)
Control	12.00±0.76 ^a	9.00±0.76 ^a	3.00±0.28 ^a
CFS of LAB strains treated with seeds			
<i>Lb. plantarum</i> 1MSS	11.40±2.70 ^a	9.00±1.27 ^a	3.00±2.10 ^a
<i>P. pentosaceus</i> 1MSS	8.46±0.45 ^{abc}	7.00±1.44 ^{ab}	2.00±1.05 ^a
<i>Lb. acidophilus</i> ATCC314	10.00±1.80 ^{abc}	8.00±1.79 ^{ab}	2.00±0.05 ^a
<i>Lb. plantarum</i> ATCC8014	7.03±2.91 ^b	9.00±1.00 ^{ab}	3.53±2.48 ^a
<i>Lb. Plantarum</i> 1FF	9.16±0.28 ^{abc}	7.00±0.95 ^{ab}	2.16±0.76 ^a
CFS of LAB strains treated seeds and grown in soil medium infected with Fungi-CL			
<i>Lb. plantarum</i> 1MSS + Fungi-CL	11.56±1.50 ^{abc}	9.30±1.37 ^a	1.26±0.20 ^a
<i>P. pentosaceus</i> 1MSS + Fungi-CL	10.00±1.32 ^{abc}	8.30±1.50 ^{ab}	1.20±0.26 ^a
<i>Lb. acidophilus</i> ATCC314 + Fungi-CL	10.00±0.40 ^{abc}	8.10±0.10 ^{ab}	2.00±0.36 ^a
<i>Lb. plantarum</i> ATCC8014 + Fungi-CL	9.00±0.64 ^{abc}	8.73±0.64 ^{ab}	1.00±0.20 ^a
<i>Lb. plantarum</i> 1FF + Fungi-CL	10.03±0.47 ^{abc}	9.00±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
CFS of LAB strains treated seeds and grown in soil medium infected with Fungi-CS			
<i>Lb. plantarum</i> 1MSS + Fungi-CS	10.16±1.04 ^{abc}	8.33±0.76 ^{ab}	2.00±0.28 ^a
<i>P. pentosaceus</i> 1MSS + Fungi-CS	9.00±1.80 ^{abc}	8.00±1.77 ^{ab}	1.00±0.10 ^a
<i>Lb. acidophilus</i> ATCC314 + Fungi-CS	10.00±0.28 ^{abc}	8.46±0.49 ^{ab}	1.20±0.45 ^a
<i>Lb. plantarum</i> ATCC8014 + Fungi-CS	11.00±1.50 ^{abc}	8.00±0.50 ^{ab}	3.00±1.00 ^a
<i>Lb. plantarum</i> 1FF + Fungi-CS	11.00±0.76 ^{abc}	9.33±0.76 ^a	2.00±0.00 ^a
Fungi-CS	9.23±0.20 ^{abc}	7.80±0.20 ^{ab}	1.43±0.57 ^a
CFS of LAB strains treated seeds and grown in soil medium infected with Fungi-FC			
<i>Lb. plantarum</i> 1MSS + Fungi-FC	10.06±1.10 ^{abc}	9.00±1.70 ^{ab}	2.00±0.46 ^a
<i>P. pentosaceus</i> 1MSS + Fungi-FC	9.00±1.51 ^{abc}	7.16±1.04 ^{ab}	1.46±0.50 ^a
<i>Lb. acidophilus</i> ATCC314 + Fungi-FC	9.00±0.63 ^{abc}	7.33±0.76 ^{ab}	1.30±0.26 ^a
<i>Lb. plantarum</i> ATCC8014 + Fungi-FC	9.46±0.55 ^{abc}	8.00±0.50 ^{ab}	1.46±0.89 ^a
<i>Lb. plantarum</i> 1FF + Fungi-FC	11.16±1.15 ^a	9.13±1.26 ^a	2.00±0.26 ^a
Fungi-FC	7.56±1.36 ^b	6.00±1.00 ^b	2.00±0.60 ^a
CFS of LAB strains treated seeds and grown in soil medium infected with Fungi-LR			
<i>Lb. plantarum</i> 1MSS + Fungi-LR	10.00±0.86 ^{abc}	8.23±0.75 ^{ab}	1.26±0.40 ^a
<i>P. pentosaceus</i> 1MSS + Fungi-LR	10.00±0.50 ^{abc}	8.20±0.40 ^{ab}	1.30±0.45 ^a
<i>Lb. acidophilus</i> ATCC314 + Fungi-LR	8.23±0.25 ^{abc}	7.16±0.28 ^{ab}	1.06±0.11 ^a
<i>Lb. plantarum</i> ATCC8014 + Fungi-LR	8.00±1.44 ^b	6.16±0.76 ^{ab}	1.50±1.00 ^a
<i>Lb. plantarum</i> 1FF + Fungi-LR	10.00±0.86 ^{abc}	7.33±1.15 ^{ab}	2.16±0.28 ^a
Fungi-LR	9.00±0.79 ^{abc}	8.30±1.12 ^{ab}	0.60±0.36 ^b

Table 2:- Seedling heights of pepper Cili Kulai seeds treated in CFS of LAB strains and grown in soil medium infected with fungi *Fusarium* species

Notes:-In Table 2 have been mentioned clearly means with same letter in same column are did not significantly differences ($P \geq 0.05$) however, means with different letter in same column are showed significantly differences ($P \leq 0.05$); Fungi *Fusarium* species; CS = *Fusarium* sp. CID124, CL = *F. oxysporum* KAML01, FC = *Fusarium* sp. *fus* 124 and LR = *F. proliferatum*

However, other fungi *Fusarium* sp. *fus* 124-FC on length of pepper Cili Kulai seedlings systems of seeds also was effected after two week incubation in soil height of seedling value of 8.00cm that less improvement compared to LAB treated seedling systems. Resulting that the pepper Cili Kulai seeds inoculated with CFS of LAB could improve the growth of seedling systems have been noticed that improved in seedling height although the seeds of pepper Cili Kulai were survived in soil medium infected with pathogenic fungi *Fusarium* species.

➤ Seedling shoot

It was observed that shoot of seedling systems did not showed significantly differences ($P \geq 0.05$) in the shoot length of pepper Cili Kulai seeds with and soaking prior to seed sowing in fungi *Fusarium* sp. CID124-CS and *F. proliferatum*-LR infected soil medium has been mentioned in Table 2. However, the length of shoot pepper Cili Kulai seeds treated in CFS- *Lb. plantarum*1-MSS while sown in soil medium infected *F. oxysporum*KAML01-CL improved significantly ($P \leq 0.05$) in length size from 6.43 ± 1.88 cm (without soaking in CFS- *Lb. plantarum*1-MSS) to 9.30 ± 1.37 cm (with soaking in CFS of *Lb. plantarum*1-MSS strain). In the same way, the shoot length of pepper Cili Kulai seeds soaked in CFS- *Lb. plantarum*1-FCF enhanced significantly ($P \leq 0.05$) from 6.00 ± 1.00 cm (without soaking in LAB-CFS- *Lb. plantarum*1-FCF) to 9.13 ± 1.26 cm (with soaking in LAB-CFS- *Lb. plantarum*1-FCF) when survived in soil medium infected with *Fusarium* sp. *fus* 124-FC after 14days incubation at 28°C in soil medium.

➤ Seedling Root

Obviously, in seedling systems were recorded that the sowing pepper Cili Kulai seeds inoculated with CFS of LAB strains have been improved the seedling root although pepper Cili Kulai seeds were grown in soil medium infected with pathogenic fungi on the other hand, there has not been significantly differences ($P \geq 0.05$) from control seedling systems. It was recorded that length of seedling root of pepper Cili Kulai seeds were grown in soil medium infected with fungi *F. proliferatum*-LR and *F. oxysporum* KAML01-CL were significantly ($P \leq 0.05$) smallest in length size 0.60 ± 0.36 cm and 0.86 ± 0.23 cm correspondingly, compared to other treatments have been mentioned in Table 2 noticeably. On the contrary pepper Cili Kulai seeds were soaked in CFS- *Lb. plantarum* ATCC8014 strain was grown in soil medium artificially infected with either *Fusarium* sp. CID124-CS or *Fusarium* sp. *fus* 124-FC improved the seedling roots length to

3.00 ± 1.00 cm and 2.00 ± 0.60 cm, correspondingly, compared to 1.43 ± 0.57 cm for control seedling systems. For pepper Cili Kulai seeds grown in soil medium were infected with *Fusarium* sp. CID124-CS seedling roots also enhanced to 1.43 ± 0.57 cm. the seedling roots of pepper Cili Kulai seeds were grown in soil medium infected fungi *Fusarium* sp. *fus* 124-FC 2.00 ± 0.60 cm (soil medium without LAB-CFS) was noted more pathogenic on seedling roots improvement after 14days incubation in soil medium.

IV. DISCUSSIONS

Microbial LAB-cells and LAB-CFS of microorganism are known as helpful to improve the seedling systems of Solanaceous plants in agriculture where plants have ability to interact directly with microorganisms when microbial inoculants were applied on seeds treatments deliver microorganisms straight to plants rhizo-sphere in soil medium which surroundings plant root areas (18). Because, microorganisms were produced nutritional supplements it was proved by Narasimha et al. (19) the potential activities of LAB strains namely *Lb. Paracasei* sub sp. *Paracasei*, *Lb. Paracasei* subsp. *tolerance*, have been recognised to inhibit bio-mass growth of various fungi which casing wilt diseases on plants and these LAB play an important actions such as microbial Phyto-hormones on Solanaceous plant seeds for instance tomato seeds against *R. solanacerum* that is caused plant wilting and considered one of the most significant crops ailments in agriculture. Similarly, in this study was found that cells and CFS of LAB strains were showed ability to inhibit fungal patho-genicity effects and enhance seedling systems of pepper Cili Kulai seeds soaking with cells or supernatant of LAB were enhanced the germination of pepper Cili Kulai seedling systems whether the pepper Cili Kulai seeds have been artificially were infected by species of *Fusarium* or grown in soil medium infected with fungi *Fusarium* were noticed that to improve the seedling height, length of shoot and roots have been mentioned in Table 1 & 2 apparently. Because, many micro-organism have been recognised and identified to produce plant hormones, bio-fertiliser and fungicidal compounds for examples, (20) detected that micro-organism *Pseudomonas* species were produced Phyto-hormones essences that are recognized as an hormone especially indole acetic acid and siderophores that are an important for crop development and enhancement of plants by direct and indirect mechanisms and necessary for agriculture uses. Similarly, treatments in soil medium by LAB strains could be triggered for systematic acquired resistance (SAR) that expands when plants successfully trigger with defence mechanism by self immunities, in presence of pathogenic contamination, resultant an enhancement synthesis of plant defence chemicals compounds that help to plant development and make stronger cell wall of plants (21). However, seedling systems were noticed to show poor growth which pepper Cili Kulai seeds were did not treated with cells and lactic acid bacterial cells free supernatants but infected with *Fusarium* species with showed poor seedling systems of pepper Cili

Kulai seeds. Because, it was proved that many number of *Fusarium* species causative agent of different diseases on plants as well as seedling systems and seeds germination for example, chilli is susceptible to several diseases that have been caused by plant pathogenic fungi is *Fusarium* species including root rot, wilting and mildew of powdery degasses have been caused with fungi namely *Leveillula taurica*). Same as the root rot and collar rot causes by *Phytophthora capsici* (22); (23); (24). Similarly, Asalmol et al. (25) were reported that seed borne fungi in the chilli seeds and seedling systems such as fungi *F. moniliformae*, *C. capsici*, *A. flavus*, *Rhizopus stolonifer* and *A. Niger* has been recognised that more pathogenicity effect on chilli seeds and suppress to percentages of germination in resulting showed poor seedling systems. However, in this study *Fusarium* species did not suppressed the seedling systems when pepper Cili Kulai seeds were survived in presence of LAB cells and cells free supernatants even the pepper Cili Kulai seeds artificially were infected *Fusarium* species but, patho-genicity effect on pepper Cili Kulai seedling systems were noticed when the Cili Kulai seeds were grown in absence of LAB cells and cells free supernatants after two week incubation in soil medium.

V. CONCLUSION

The germination of pepper Cili Kulai seeds were enhanced their seedling systems well when the seeds of pepper Cili Kulai were inoculated by LAB-cells and LAB-CFS have been showed better percentages germination and developments of pepper Cili Kulai seedling systems; height, shoot length and root length of Cili Kulai seeds. Because, Cili Kulai seeds were infected with the fungi or Cili Kulai seeds were grown in fungi infected with soil medium. Therefore, findings of this experiment support that lactic acid bacterial cells and CFS of *Lb. plantarum* ATCC8014, *P. pentosaceus*1-MSS, *Lb. acidophilus* ATCC314, *Lb. plantarum*1-MSS and *Lb. plantarum*1-FCF could be used as removal agents to suppress pathogenic effect of fungi *Fusarium* species for example; *F. acuminatum*-FC, *F. solani*-CS, and *F. oxysporum* f. sp. *lycopersici*-CL, *F. proliferatum*-LR. Simultaneously, LAB strains could be used as a plant hormone to better development of chilli plants seedling systems as well as other plants seedling systems. Furthermore, cells and CFS of LAB were showed improvement on seed germination and seedling systems. Then these LAB isolates could be used to enhance the plant and plant systems in agriculture for future uses.

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REFERENCES

- [1]. E.C. Oerke, "Crop losses to pests *J. Agric. Sci.* Vol. 144:p.31-43, 2006.
- [2]. T. Galare, J. Caradus, W. Gelernter, T. Jackson, N. Keyhani, J. Kohl, P. Marrone, L. Morin, A. Stewart, "Have bio-pesticides come of age? *Trends Bio-technol.* Vol. 30: p. 250–258, 2012.
- [3]. P. Lehr, "Bio-pesticides: The gold market. Report code CHM029B, *BCC Research, Wellesley, Massachusetts*, 2010.
- [4]. G. Berg, "Plant microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. *Appl. Microbiol. Biotechnol.* Vol. 84: p. 11-18, 2009.
- [5]. J.K. Vessey, "Plant growth promoting rhizo-bacteria as bio-fertilizers. *Plant. Soil.* Vol. 255: p. 571-586, 2003.
- [6]. M. Arshad & W.T. Jr. Frankenberger, "Microbial production of plant growth regulators" *In meeting: F.B. Jr. (eds). Soil Microbial Ecol. Marcel Dekker Inc. New York.* p.307-347, 1992.
- [7]. Y. Ma, M. Rajkumar, Y.M. Luo, H. Freitas, "Inoculation of endophytic bacteria on host and non host plants effect on plant growth and Ni uptake. *Journal of Hazardous Materials*, Vol. 195: p. 230- 237, 2011.
- [8]. E. Garcia Fruitos, "lactic acid bacteria: a promising alternative for recombinant protein production. *Microb. Cells Fact.* Vol. 11: p. 157, 2012.
- [9]. H. Shih-Yung, "IAA production by *Streptomyces* scabies and its role in plant microbe interaction. *Msc Thesis, Cornell University*, 2010.
- [10]. G.B. Dias, V.M. Gomes, T.M. Moraes, U.P. Zottich, G.R. Rabelo, A.O. Carvalho, M. Moulin, L.S. Goncalves, R. Rodrigues, & M. Da Cunha, "Characterization of *Capsicum* species using anatomical and molecular data", "Characterization of *Capsicum* species using anatomical and molecular data". *Genetic Molecular Research*, (Online first, 2013).
- [11]. Y. Wahyuni, A.R. Ballester, E. Sudarmonowati, R.J. Bino & A.G. Bovy, "Secondary metabolites of *Capsicum* species and their importance in the human diet". *Journal of Natural production.* DOI: 10.1021/. p. 300898z (online first), 2013.
- [12]. R.K. Horst, "Plant Diseases and Their Pathogens". *Westcott's Plant Disease Handbook. XIV, 90 illus., Hardcover, ISBN.* p.978-1-4020-4584-4.p.1318. <http://www.springer.com/978-1-4020-4584-4>, 2008.
- [13]. M. Joshi, R. Shrivastava, A.K. Sharma & A. Prakash, "Screening of resistant varieties and antagonistic *Fusariumoxysporum* in chilli for bio-control of

- Fusarium*wilt of chilli”. *Journal of plant pathology and Microbiology*. Vol. 3. p. 134, 2012. doi: 10.4172/2157-7471.1000134.
- [14]. F. Hussain, S.S. Shaukat, M. Abid, F. Usman & M. Akbar, “Control of Meloidgyne javanica and *Fusarium solani* in chilli (*Capsicum annuum* L.) with the application of chitin”. *Pakistan Journal of Namatology*. Vol. 31. p. 165-170, 2013.
- [15]. B.J.A. Muhialdin, Z. Hassan & S. Sadon Kh, “Antifungal activity of *Lactobacillus fermentum* Te007, *Pediococcus pentosaceus* Te010, *Lactobacillus pentosus* G004, and *L. paracasi* D5 on Selected Foods”, *Journal of Food Science*, Vol. 76, p. 493–499, 2011.
- [16]. M.N. Khare, “Methods to test seeds for associated fungi”. *Indian Phytopathology*. Vol. 49. p. 319-328, 1996.
- [17]. M.A. Hamed, “Inflorescence rot disease of date palm caused by *Fusarium proliferatum* in Southern Iraq”. *African Journal of Biotechnology*. Vol.11. p. 8616-8621, 2012.
- [18]. L. Philippot, J.M. Raaijmakers, P. Lemanceau, W.H. vander Putten, “Going back to the roots: the microbial ecology of the rhizosphere. *Nat Rev Microbiol*. 11:789–799, 2013.
- [19]. M.K. Narasimha, J. M. Malini, Savitha & C. Srinivas, “Lactic acid bacteria (LAB) as plant growth promoting bacteria (PGPB) or of wilt of tomato caused by *Ralstonia solanacearum*”. *Pest Management in Horticultural for the control*. Vol. 18. p. 60-65, 2012.
- [20]. P. Saranraj, P. Sivasakthivelan & S.S. Siva, “Prevalence and Production of Plant Growth Promoting Substance by *Pseudomonas fluorescens* Isolates from Paddy Rhizosphere Soil of Cuddalore District, Tamil Nadu”. *India African Journal of Basic & Applied Sciences*. Vol. 5. p. 95-101, 2013.
- [21]. C. Stephane, D. Brion, N. Jerzy, C. Christophe & A. Essaid, “Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanism of action, and future prospects”. *Applied Environmental Microbiology*. Vol. 71. p. 4951-4959, 2005.
- [22]. M. Mushtaq & M. H. Hashmi, “Fungi associated with disease of capsicum in Sindh, Pakistan”. *Pakistan Journal of Botany*. Vol. 29. p. 217-222, 1997.
- [23]. A. Saleem, M. Ansar, K. Hamid & F.F. Jamil, “Effect of Physical parameters on the incidence of root and collar rot disease in Chillies”. *Pakistan Journal of Botany*. Vol. 30. p. 39-43, 1998.
- [24]. Thane , P.H. Prihastuti, S. Phoulivong, W. Paul, J. Taylor & K.D. Hyde, “Chilli anthracnose disease caused by *Collectotrichum* species”. *J. Zhejiang. University of Scienc*. Vol. 9. p. 764-778, 2008.
- [25]. M.N. Asalmol, V.P. Kale & S.T. Ingle, “Seed borne fungi of chilli, incidence and effect on seed germination”. *Seed Resources*. Vol. 29. p. 76-79, 2001.