ISSN No:-2456-2165

Pepper Cili Kulai Seed Germination in Soil using Cells and Supernatants of Beneficial Microorganisms as Energy Sources and Inhibitory Activity against *Fusarium* Species

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Abstract:-Most of the microorganisms are known as beneficial to human and plants; one of them beneficial microorganism lactic acid bacteria (LAB) isolate cells and their cells free supernatants (CFS) have ability to inhibit the growth of pathogenic fungi and cells and their cells free supernatants (CFS) were observed that protect to the pepper Cili Kulai seeds germination which are used worldwide by consumers. In this study treatment of small volumes cells and CFS of LAB isolates; Lb. plantarum1-MSS, P. pentosaceus1-MSS, Lb. acidophilus ATCC314, Lb. plantarum ATCC8014 and Lb. plantarum1 were showed to improve the Cili Kulai seeds percentage germination; either seeds were infected artificially with Fusarium species; Fusarium sp. fus 124-FC, Fusarium oxysporum KAML01-CL, Fusarium sp. CID124-CS and F. Proliferatum-LR isolates. In vitro assay with cells and CFS of LAB were assayed on pepper Cili Kulai seeds germination in 160 eyes plastic tray. The highest percentage germination of seeds were noticed more than 97.00% when the soil was treated with Lb. plantarum1-FCF cells, even the Cili seeds were infected with pathogenic fungi Fusarium sp. CID124-CS and F. proliferatum -LR. The pathogenic Fusarium species infected with soil significantly (P≤0.05) improved percentage germination of Cili Kulai seeds from 80.0 to 97.0% predominantly for seeds treated with CFS of Lb. plantarum1-FCF (98.0%). The percentage germination of seed was infected with Fusarium species were reduced the germination of seeds between 56.00% and 66.00% compare to control (96.00%), when grown in soil seeds infected with cells of pathogenic fungi Fusarium sp. fus 124-FC and Fusarium sp. CID124-CS grown in soil without LAB cells. Effect of F. Oxysporum KAML01-CL was noticed that highly virulent to suppress the seeds germination. However, all LAB cells and CFS were observed that to enhance the percentage germination.

Keywords:- Cells and supernatant of *Lactic Acid Bacteria; Fusarium Species; Inhibitory Activity; Cili Kulai germination.*

I. INTRODUCTION

Most of the world food crops as well as pepper chillies are propagated by seeds and chilli plants are also highly susceptible to fungal and viral pathogens (1). Many seeds borne diseases caused by microorganisms such as pathogenic fungi and bacteria that can be adhesive with surface on seed or in seed and damaged germination rate of seeds caused infection with microorganisms would naturally result in significant final yield losses (2). Pathogenic Fungi *Fusarium*, *Aspergillus* and *Penicillium* are commonly known to produce mycotoxins that are harmful substances for humans, animals and plants (3). Genus *Fusarium* members are produced a variety of chemically different types of phyto-toxic compounds especially, fumonisins , fusaric acid (FA), beauvericin (BEA), (fumonisin B1, FB1), enniatin (ENN), moniliformin (MON) and trichothecenes ,these phyto-toxic compounds were reported that a variety of biological activities and cause morphological, physiological and metabolic effects as well as chlorosis, necrosis, wilting, growth inhibition, inhibition of seed germination rates and effects on calli (4). *Fusarium* infectious diseases have been seen in Malaysia where different vegetable crops rotting tissues including family: Solanaceae; red chilli (*Capsicum annuum*), Malvaceae; okra (*Hibiscus esculentus)*, Moringaceae; moringa *(Moringa olifel)*, Fabaceae; long bean (*Vigna sesquipedalis*) infection caused by *Fusarium species* (5). Fungicidal compounds can be produced by many beneficial microorganisms namely, strains of *Enterobacter*, *Pseudomonas Bacillus*, *Streptomyces* (6); (7); (8). Beneficial microorganisms such as Lactic Acid Bacteria an alternative plant diseases control method and their metabolites has been gained too much importance to control Phyto-pathogenic fungi (9). Species *Lb. paracasei* subsp. *tolerans* (LAB I) and *Lb. paracasei* sub sp. *paracasei* (LAB II) have been showed an enhancement in tomato seed germination to indicate plant growth promoting bacteria (PGPB) and good energy sources for seeds germination and protection without using of synthetic compounds which commercially available for use of in agricultures purposes (10). Interestingly, it has been encouraging that microorganisms have plenty of micronutrients specific characteristic which can acceptable in agricultural field in future. The LAB isolates could be used as bio-fertilizers and bio-regulators. The present study was conducted on chilli paper seed namely, Cili Kulai seed germination has been increased with treatments of cells and cells free supernatants (CFS) of LAB strains either Cili Kulai seeds were infected with *Fusarium* species. However, Cili Kulai seeds infected with pathogenic fungi *Fusarium* species survived in soil

ISSN No:-2456-2165

were noticed that to inhibit the germination rate and clear effects of patho-genicity was observed after two week incubation. These findings of encourage that to use cells and cells free supernatants of lactic acid bacteria in field experiment for future purposes.

II. MATERIALS AND METHODS

A. Preparation of Lactic Acid Bacteria for soil treatments

Lactic Acid Bacteria (LAB) strains; *Lb. plantarum* ATCC8014, *Lb. acidophilus* ATCC314, *P. pentosaceus*1-MSS, *Lb. plantarum*1-MSS and *Lb. plantarum*1-FCF were grown in de Man Rogosa and Sharpe Broth (MRSB) medium using the method was described by (11) with modification. Culture isolates were incubated in an incubator shaker for overnight at 37^{o}C. The cells concentration of each LAB isolates were determined before to use for soil treatments**.**

B. Preparation of Lactic Acid Bacteria cells free supernatants for chilli seeds treatment

Beneficial microorganisms namely, *Lb. plantarum* ATCC8014, *Lb. acidophilus* ATCC314, *P. pentosaceus*1-MSS, *Lb. plantarum*1-MSS and *Lb. plantarum*1-FCF strains were inoculated into MRSB medium using the method described by (11) and incubated for 24 h at 37°C in aerobic shaker incubator. The lactic acid bacteria cells free supernatants (LAB-CFS) were prepared by centrifuging the broth 11500 × g rpm for 10 min at 4^{o}C (Centrifuge Combi-514R, Korea). The supernatants of each LAB isolates were filtrated using sterile filtered 0.45µm-pore-size Millipore filter and ready to use for chilli seeds treatments**.**

C. Preparation of Fusarium Culture for inoculation with Cili Kulai seeds and soil

Pathogenic fungi *Fusarium* species especially, *Fusarium* sp. *fus* 124-FC, *Fusarium oxysporum* KAML01-CL, *Fusarium* sp. CID124-CS and *Fusarium proliferatum*-LR isolates were grown on potato dextrose agar (PDA) using the method described by (12) with modification and incubated at room temperature at 28^{o}C for 5 days. Sterilized distilled water (10 to 20 ml) was poured onto the plates. After that the fungal surfaces were gently scraped to loosen the spores and the spore suspensions were collected. The spore suspensions at concentration of 1×10^{5} spores/ml were obtained using serial dilution and then fungal spores were homogenized in a sterilized blender for few minutes and artificial infected on Cili Kulai seeds as well as in soil.

D. Preparation of Cili Kulai seeds

Pepper Cili Kulai seeds namely, Cili Kulai seeds were purchased from Market. The Cili Kulai seeds were prepared following the method described by (12) with modification. The seeds were washed with running tap water after that seeds surface sterilized with 1% sodium hypochlorite solution for 1-2 min then, washed with sterile distilled water 2-3 times. The seeds were air-dried in laminar flow cabinet to remove the moisture these surface sterilized Cili Kulai seeds were used for further treatments.

E. In vitro assay of Cili Kulai seeds germination in 160 eyes plastic tray

Assay of LAB cells and cells free supernatants on Cili Kulai seeds germination and fungi *Fusarium* species infected Cili Kulai seeds and in soil as follows; surface sterilized of Cili Kulai seeds were treated using method described (12) with modification and treatments of Cili Kulai seeds were separated into the six groups. The first group of Cili Kulai seeds were grown in soil treated with 5ml of overnight LAB culture cells poured in the centre of the eyes of plastic tray was filled with 25g potting soil with no fertilizer at one seed per tray eye. The second group of Cili Kulai seeds infected with fungi and seeds were grown in soil treated with 5ml of overnight LAB culture cells as energy sources. The third group of the Cili Kulai seeds were soaked with LAB-CFS for 1 h, air-dried in laminar air flow cabinet then sowed in soil with no fertilizer. The fourth group of Cili Kulai seeds were soaked in CFS of LAB for 1 h air dried in laminar cabinet and survived in tray eye was filled with 25g potting soil infected with fungi. The fifth group of Cili Kulai seeds were survived 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. Finally, the group six Cili Kulai seeds were soaked with water did not treated with any supplements used as positive control. All the seeded trays were sprayed with water, covered with dark colour plastic bags and placed in dark cabinet at room temperature at 30^{o}C for two weeks to allow seed germination. The Cili Kulai seeds germination was counted and the percentage germination was calculated using the equation; [GS (%)] = [TNGS ÷ TNTS] × 100 where; GS (%) = Percentage germination of seeds, TNGS = Total number of germinated seeds and TNTS = Total number of treated seeds. The treatments were done in triplicates.

F. Data analysis

The data was analysed mean ± standard deviation gained from each analysis was analyzed using one-way analysis of variance (ANOVA) and the mean significant was done by the Tukey test at ($P\leq0.05$). The statistical analysis was performed using Minitab 16 software.

III. RESULTS

A.Percentage germination of Cili Kulai seed infected with Fusarium species survived in soil treated with LAB cells

The percentage germination of Cili Kulai seeds that were infected with highly pathogenic fungi *Fusarium* species were reduced seeds germination between range 56% and 66.00% compare to control (96.00%). When Cili Kulai seeds were grown in soil seeds infected with fungi *Fusarium* sp. *fus* 124-FC and fungi *Fusarium* sp. CID124-CS artificially infected Cili Kulai seeds sowed in soil without treated LAB cells inoculation was showed less than 63.00% germination of Cili Kulai seeds. Whereas, germination of Cili Kulai seeds infected with fungi *F. oxysporum* KAML01-CL and fungi *F. proliferatum*-LR were noticed that between 56.00% and 66.00%, respectively compare to control as shown in (Figure 1). Before sowing the Cili Kulai seeds significantly ($P\leq0.05$) improved that the percentage germination of all the fungi infected seeds by 95.00% to 96.00% when Cili Kulai seeds were treated with cells of LAB strains and survived in soil medium. The inhibitory activity of LAB cells seems to be related to the LAB and the pathogenic fungi. The percentage germination of *F.*

ISSN No:-2456-2165

oxysporum KAML01-CL infected Cili Kulai seeds were increased to more than 96.00% when the soil was treated with strain *Lb. plantarum*1-FCF cells. Similarly, treatment of soil with *Lb. plantarum*1-FCF increased percentage germination of fungi *F. proliferatum*-LR and fungi *Fusarium* sp. CID124-CS infected Cili Kulai seeds by 97.00% and 96.00%, respectively. *Lb. acidophilus* ATCC314 cells treatments to potting in soil of also improved the percentage germination of fungi *F. proliferatum*-LR infected Cili Kulai seeds by 96.60%. A slight reduction in percentage germination (95.00%) was observed for Cili Kulai seeds infected with Fungi *Fusarium* sp. CID124-CS when the soil was treated with cells *Lb. acidophilus* ATCC314, *P. pentosaceus*1-MSS, and *Lb. plantarum* ATCC8014. The *Fusarium* infected soil significantly ($P \leq 0.05$) improved percentage germination from 80.00% to 97.00% particularly, for Cili Kulai seeds treated with LAB-cells - *Lb. plantarum*1-FCF (98.00%) and this LAB-CFS was indicated better engergy sources for improvmet of Cili Kulai seeds. In contrast, treating the potting soil with cells of *Lb. acidophilus* ATCC314, and *Lb. plantarum* ATCC8014 allowed lower percent germination (80 to 83%) of Cili Kulai seeds infected with pathogenic fungi *F. oxysporum* KAML01-CL and fungi *Fusarium* sp. CID124-CS and fungi *Fusarium* sp. *fus* 124-FC. Similarly, cells of *Lb. plantarum*1-MSS reduced the percentage germination Cili Kulai seeds (83%) of fungi *F. proliferatum* -LR infected chilli seeds compare to control after two week incubation. Finally, Cili Kulai seeds percentage germinations were noted that to enhance when survived in presence of all LAB cells.

B. Percentage germination of Cili Kulai seed treated in LAB-CFS and grown in soil artificially infected with pathogenic fungi Fusarium species

Pepper Cili Kulai Seeds were grown in soil infected with *Fusarium* species significantly ($P \leq 0.05$) reduced the percentage germination of Cili Kulai seeds range between 56.00 to 66.60% compared with the control which was showed around 97% germination Cili Kulai Seed as mentioned in (Figure 2) clearly. However, treatments of Cili Kulai Seeds were observed with LAB-CFS before sowing in soil medium. The *Fusarium* infected with soil significantly ($P \leq 0.05$) improved percentage germination from 80.00% to 97.00% particularly for Cili Kulai Seeds treated with CFS of *Lb. plantarum*1-FCF (98.00%). The inhibitory effect of LAB-CFS depends on the species of LAB and fungi similar to that observed when applying LAB-CFS in soil medium. It was observed that Cili Kulai Seeds treated with LAB-CFS of *Lb. acidophilus* ATCC314, *Lb. plantarum*1-MSS, and *P. pentosaceus*1-MSS enhanced seeds germination by ranges about 83.00%, 90.00%, and 96.00%, respectively in soil infected with fungi *Fusarium* sp. CID124-CS. Similarly, It was observed that CFS of LAB-CFS *P. pentosaceus*1-MSS was affective against fungi *Fusarium* sp. *fus* 124-FC, LAB of CFS- *Lb. acidophilus* ATCC314 was affective against fungi *F. proliferatum* –LR. While, the LAB-CFS of *Lb. plantarum* ATCC8014 and *Lb. plantarum*1-FCF were affected against fungi *F. oxysporum* KAML01-CL and allowed to better Cili Kulai seeds percentage germination in *Fusarium* infected in soil medium. Overall results cells and CFS of LAB strains were noticed that to enhance the percentage germination Cili Kulai Seeds after two incubations. Either the Cili Kulai Seeds were survived in soil treated with cells of LAB or CFS of LAB strains as energy sources for better germination of pepper Cili Kulai Seeds.

ISSN No:-2456-2165

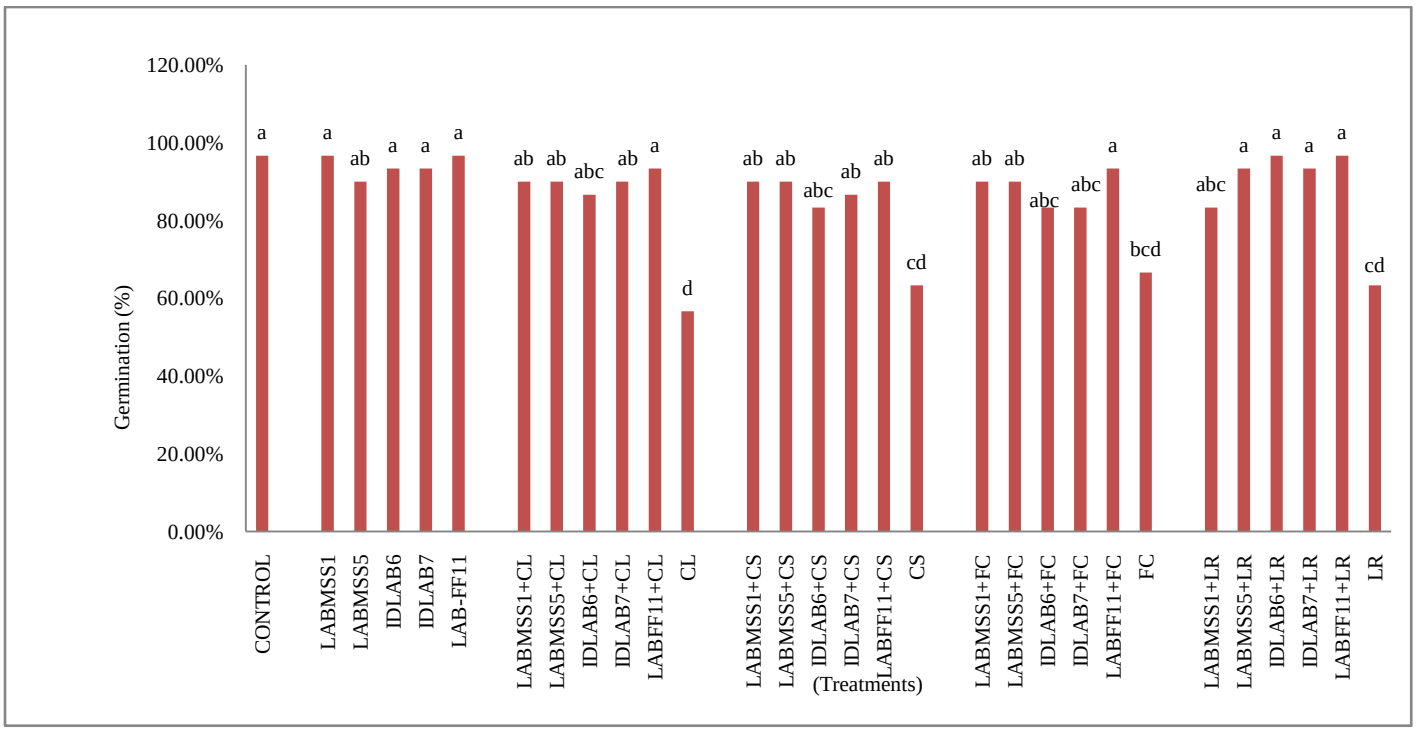

Figure 1:-Percentage germination of Cili Kulai seeds infected with pathogenic fungi *Fusarium* species survived in soil treated with LAB cells.

Notes:- Means with the same alphabetic letters are not significant different (P≥0.05) and Means with the different alphabetic letters are significantly different (P≤0.05); Cells of 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* KAML01-CL, CS = *Fusarium* sp. CID124-CS, FC = *Fusarium* sp. *fus* 124-FC and LR = *F. Proliferatum* specie.

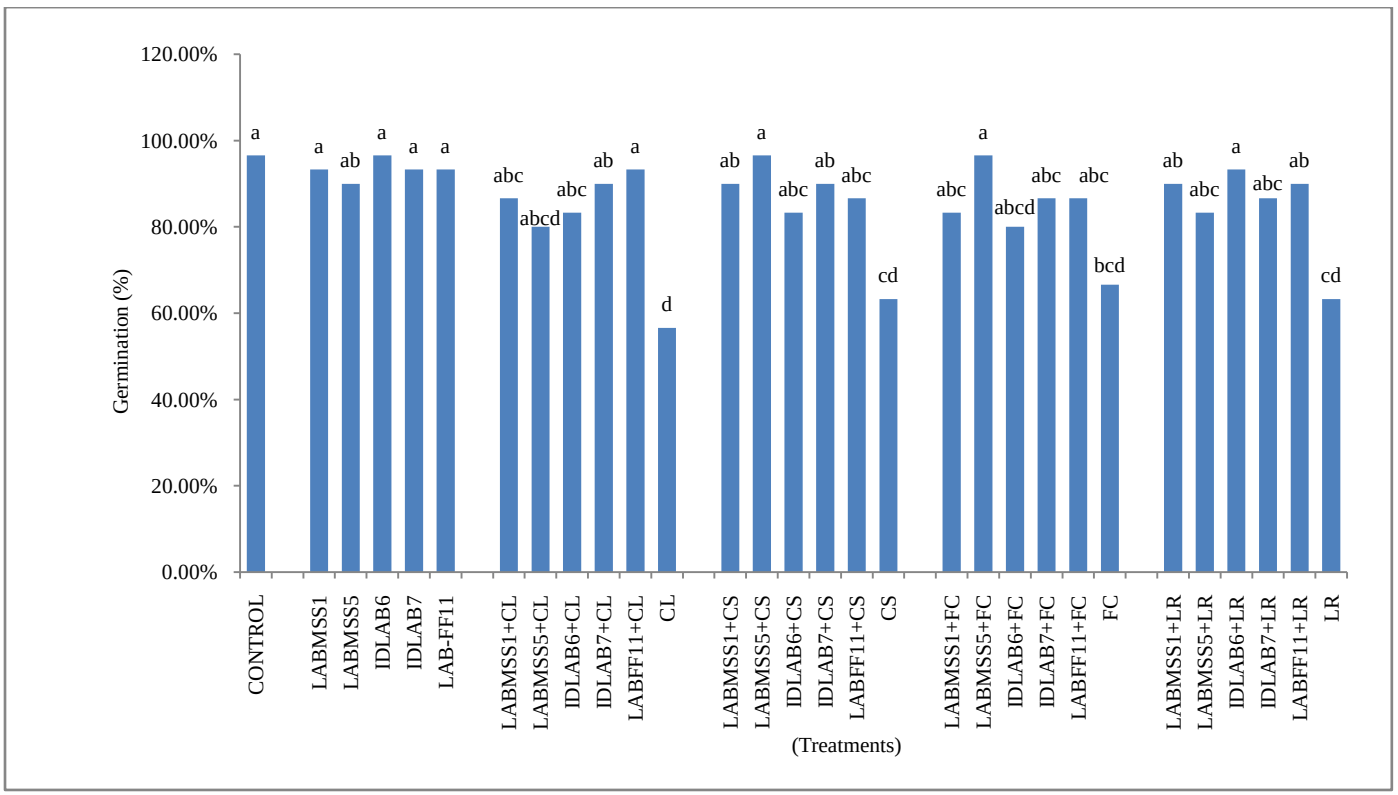

Figure 2:-Percentage germination of Cili Kulai seeds treated with LAB-CFS and sowed in soil artificially infected with pathogenic fungi *Fusarium* species.

ISSN No:-2456-2165

Notes:-There are means with the same alphabetic letters are not significant different (P≥0.05) and Means with the alphabetic different letters are significantly different (P≤0.05); cells free supernatants of 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* species; CL = *F. oxysporum* KAML01-CL, CS = *Fusarium* sp. CID124-CS, FC = *Fusarium* sp. *fus* 124-FC and LR = *F. Proliferatum* specie.

IV. DISCUSSIONS

Soil medium is the good sources for germination of many seeds verities one of them pepper Cili Kulai seeds were observed with cells and cells free supernatants of lactic acid bacteria as energy sources without supplicating any synthetic energy sources. However, in previous study was observed that Cili Kulai seeds on Petri dishes showed that LAB-CFS of *P. pentosaceus*1-MSS, *Lb. plantarum* ATCC8014, *Lb. acidophilus* ATCC314, *Lb. plantarum*1-FCF and *Lb. plantarum*1-MSS isolates have ability to suppress biomass of fungal patho-genicity and promote the percentage germination of different varieties of chilli seeds; one of them Cili Kulai seeds on Petri Dishes and it has been encouraged that germination of Cili Kulai seeds were needed to survive in soil medium because, this medium is better sources for any seeds germination (11). Therefore, present study indicates that soaking the seeds with LAB cells or cells free supernatants both were promoted the percentage germination of Cili Kulai seeds whether, the Cili Kulai seeds are artificially infected with the pathogenic fungi *Fusarium* species or survived in *Fusarium* species infected in soil medium that is shown in Figure 1 and Figure 2. Even though, other chilli seeds were treated and grown in presence of fungi *Colletrotrichum capsici* and strain LABC5 used as fungal control and seeds promoter was showed good percentage germination (13). Likewise, lactic acid bacteria cells free supernatants (LAB-CFS) improved the seeds germination growth was compared with Cili Kulai seeds infected with *Fusarium* species. In this case, percentage germination of Cili Kulai seeds were noted to inhibit the percentage germination when Cili Kulai seeds were artificially infected with plants pathogenic *Fusarium* species; namely, these fungi species are *F. oxysporum* KAML01-CL, *F. proliferatum*-LR, *Fusarium* sp. *fus* 124-FC and *Fusarium* sp. CID124-CS. Whereas, the patho-genicity effect of *Fusarium* sp. CID124-CS was observed that highly pathogenic compared to other *Fusarium* species; *F. oxysporum* KAML01-CL, *F. proliferatum*-LR and *Fusarium* sp. *fus* 124-FC. Similarly, Akanmu et al. (14) also was detected on the millet varieties the *Fusarium* species have been demonstrated a different types of pathogenic effects that is namely, caused by *Fusarium subglutinans, F. dlamini, F. fujikuroi, F. beomiforme, F. verticillioides,F. anthophilum F. oxysporum, F. nygamai* and *F. scirpi* communicated a more pathogenic fungal effects and the most patho-genicity has been noticed on the millet varieties by *Fusarium subglutinans, F. verticillioides, F. anthophilum, F. scirpi* and *F. Oxysporum*. In addition, in this finding also were noticed that the fungi *Fusarium* species did not only suppressed the germination of Cili Kulai seeds it was also noticed that *Fusarium* species effected to early germination. Because, germination of Cili Kulai seeds were appeared late and very slower compare to grown Cili Kulai seeds in presence of LAB-CFS and LAB cells isolates treatments. This results are agreed with earlier report from Hamed et al. (12) reported that lactic acid bacteria specifically, LAB1, LAB2, LAB3 LAB4 and LAB5 cells have been noticed that to increase another Solanaceous plant seeds of percentage germination such as tomatoes plant seeds either tomato seeds were survived with artificially infected of pathogenic fungi *Fusarium* species *Fusarium oxysporum*-1 *R. solani*-1, *F. rolfsii*; *F. oxysporum*-2 and *R. solani-2*; in soil medium inoculations that findings are found strongly agreed with this study. Because, inhibitory activities and enhancement of seeds germination indicate that cells and supernatants of LAB was consisted chemical compounds which responsible to inhibit fungi and to promote seeds germinations were promoted of Cili Kulai seeds and control to the pathogenic fungi *Fusarium* species when were infected artificially on Cili Kulai seeds before sowing in soil medium. Additionally, Yousef and Lloyd (15) reported that lactic acid bacteria *Lb. paracasei*s sp. *Tolerans* has been inhibited completely the growth of *Fusarium graminearum* R 4053 *F. proliferatum* M5689 and M5991 compared to control. Therefore, the strains LAB showed plenty of chemical activity and can be used as bio-control on protection of chilli seeds, plants from pathogenic fungi and improve the plant growth and yield product finally (16). Thus, the LAB isolates were noticed that to indicate the ability of fungicidal compounds and plants improvement nature compounds, because, report from (17); (18); (19) bacteria produce chemicals that are anti-pathogenic (e.g. diacetyl, hydrogen peroxide, lactic acid, acetic acid, propionic acid, bacteriocins, carbon dioxide and another compounds which also were recognised to useful for plants heath and energy sources such as chitinase enzyme and Phyto-hormones (like indole acetic acid (IAA), auxins, gibberellins, and ethylene), HCN siderophores and antibiotics (20). Finally, LAB isolates were used in this study may have been consisted fungicidal compounds and bio-fertilizer as well as Phyto-hormones. Because, cells and CFS of lactic acid bacteria were used as energy sources to improve the percentage germination and avoiding synthetic fertilizers, plant regulators and synthetic fungicides to control *Fusarium* pathogenic effects during Cili Kulai seeds germination.

V. CONCLUSIONS

The germination of Cili Kulai seeds were noticed that to improve with treatments of cells and CFS of lactic acid bacteria. When the Cili Kulai seeds were treated with both the cells and LAB-CFS of the LAB has been showed good percentage germination. In conditions where Cili Kulai seeds were infected with the fungi or Cili Kulai seeds were sowed in fungi infected in soil medium to suppress the germination of seeds. Thus, this findings further support the *Lb. Plantarum*1FCF, *P. pentosaceus*1 MSS, *Lb. acidophilus* ATCC314 *Lb. plantarum*1MSS and *Lb. plantarum* ATCC8014 can be used as bio-control agents against

Fusarium species such as *Fusarium* sp. CID124-CS, *F. proliferatum* LR, *F. oxysporum* KAML01-CL, and *Fusarium* sp. *fus* 124-FC. In addition, 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. For further investigations are recommended to these LAB isolates can be used to enhance the seedling systems, plant growth and plant systems that would be mentioned in further study.

VI. ACKNOWLEDGEMENT

This work was financially supported by the Department of Science & Technology, University Sains Islam Malaysia and Ministry of Education Malaysia through Exploratory Research Grant Scheme (ERGS55087) is duly acknowledged.

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