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In Vitro Antagonistic Activity of Soil Bacteria Against *Fusarium* in Tomato

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Abstract

The study was carried out **the antagonistic activity** of soil bacteria isolated from the soil of peanut fields against *Fusarium* from the infected leaves of tomato *in vitro*. The infected leaves of tomato were collected from Shan Ka Lay Kyun village, Amarapura Township, Mandalay Region during August 2016. The fungal *Fasarium* were isolated by using the Potato Dextrose Agar (PDA) medium and identified by their morphology, colony colour and their spores. The soil bacteria were isolated on King's B medium and ATCC 552 medium. A total of 19 strains were obtained and identified as *Bacillus* and *Pseudomonas* by colony color, shape, margin, cell morphology and biochemical tests. Nineteen soil bacteria are MZ 1, MZ 2, MZ 3, MZ 4, MZ 5 MZ 6, MZ 7, MZ 8, MZ 9, MZ 10, MZ 11, MZ 12, MZ 13, MZ 14, MZ 15, MZ 16, MZ 17, MZ 18 and MZ 19. The bacterial isolates were screened for their antifungal activities. The assay for antagonism was performed on Potato Dextrose Agar (PDA) medium by Dual Culture Method. Among the isolated bacterial strains, *Pseudomonas* MZ 16 showed the most active antifungal activities against *Fusarium* fungi followed by MZ 17.

Keywords: Antagonistic activity, *Fusarium*, *in vitro*, *Pseudomonas*, Dual Culture Method.

Introduction

Fusarium wilt is caused by a fungus, *Fusarium oxysporum* f. sp. *lycopersici*, that enters the plant through the roots and grows up through the vascular tissue. The fungus destroys cells of the vascular tissue, causing starvation in nearby branches of the plant. Disease development is favored by warm temperatures, dry weather, acidic soil and root-knot nematodes. The Fusarium wilt fungus may be introduced to soils in several ways, such as through wind, water, wildlife or equipment. These fungi become established readily in most soils and can remain in the soil for years (Bost 2013).

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Fusarium oxysporum f. sp. *lycopersici* (FOL) is a highly destructive pathogen of both greenhouse and field grown tomatoes in warm vegetable production areas. The disease caused by this fungus is characterized by wilted plants, yellowed leaves and minimal or absent crop yield. There may be a 30 to 40% yield loss (Kirankumar *et al*. 2008 as cited in Asha *et al*. 2011). Management of seed-borne and soil-borne diseases such as wilt caused by *Fusarium* species has always been problematic (Rai *et al*. 1992 as cited in Asha *et al*. 2011).

Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that actively colonise roots and stimulate plant growth either directly or indirectly. Direct stimulation of plant growth takes place by providing phytohormones (Mordukhova *et al*, 1991 as cited in Saravanan *et al.* 2013) or by solubilisation of mineral phosphate and other nutrients (Glick 1995 as cited in Saravanan *et al.* 2013), while indirect stimulation takes place through suppression of phytopathogens by the production of siderophores (Scher & Baker 1982 as cited in Saravanan *et al*. 2013) or by producing antibiotics (Thomashow & Weller 1996 as cited in Saravanan *et al.* 2013). The rhizobacteria that control soil-borne pathogens are called biocontrol rhizobacteria (Saravanan *et al*. 2013).

Soilborne diseases have been controlled more recently by means of certain beneficial bacteria that are indigenous to the rhizosphere of plants (Thomshaw 1996 as cited in Elmahdi *et al.* 2015). The rhizosphere, representing the thin layer of soil surrounding plant roots and the soil occupied by the roots, supports large and metabolically active groups of bacteria (Villacieros *et al*. 2003 as cited in Elmahdi *et al.* 2015) known as plant growth promoting rhizobacteria (PGPR) (Kloepper *et al*. 1980 as cited in Elmahdi *et al.* 2015). PGPR are known to rapidly colonize the rhizosphere and suppress deleterious microorganisms as well as soilborne pathogens at the root surface (Rangajaran *et al*. 2003 as cited in Elmahdi *et al*. 2015). These organisms can also be beneficial to the plant by stimulating growth (Bloemberg and Lugtenberg 2001 as cited in Elmahdi *et al*. 2015).

Materials and Methods

Collection of Soil Samples

The soil samples and infected leaves of tomato were collected from Shan kalay kyun village, Amarapura Township, Mandalay Region from August to December, 2016.

Isolation of Fungi from Infected Leaves

Isolation of infected pathogenic fungi in tomato leaves was studied by using of Potato Dextrose Agar (PDA) medium. These fungus were identified by the morphological characters ,colony colour and their spores (Barnett 1955).

Isolation of Bacteria from Soil Samples

The bacteria were isolated from rhizosphere soil samples and cultured on ATCC 552 and King's B medium. Among them, 11 strains were taken from ATCC 552 medium and 8 strains were isolated from King's B medium.

These strains were identified by the morphological characters and some biochemical tests (Atlas 1993 and Buchanan 1974).

Preparation of Inoculums

Pure cultures of *Bacillus sp*. and *Pseudomonas sp.* isolated from rhizospheric soil of peanut field, the experiments were carried out at the microbiology laboratory of Botany Department, University of Mandalay. All the test strains were maintained on ATCC Medium 552 and King's B Medium and sub-cultured once in every two-week. These bacteria served as test pathogens for antibacterial activity assay.

Preparation of Assay Medium

Pure culture of *Fusarium* sp isolated from the infected leaves of tomato plants the experiments were carried out at the Microbiology Laboratory of Botany Department, University of Mandalay. Isolated pathogenic fungi were maintained on Potato Dextorse Agar (PDA) Medium and sub-cultured once in every two-week. The Potato Dextorse Agar (PDA) Medium was sterilized at 121ºC for 15 minutes. . After cooling to about 65ºC, 20 ml of the medium was poured into 90 mm petridish and was allowed to solidify by Dual Culture Method on Potato Dextorse Agar (PDA) Medium (Figure 1).

Dual Culture Method

The assay for antagonism was performed on potato dextrose agar (PDA) by dual culture method. Isolate of *Fusarium* sp. was inoculated on PDA plates. After incubation for seven days, the mycelia and the agar were cut into small pieces using a cork borer with diameter 5 mm. Each piece of mycelia was placed on PDA plate, about 3 cm from the edge. On the opposite of the fungi, about 3 cm from the fungi, a loop full of one isolate of rhizobacteria was streaked to make a line about 2 cm long. The isolate of rhizobacteria was replaced with sterilized distilled water as control treatment. Inoculated plates were incubated in room temperature for 5-7 days. The diameter colony (DC) of the fungi was measured every day from 3 days after inoculation. The diameter of inhibition zones were calculated by measuring the clear zone between the bacterial colony and the mycelia of fungi when the growth of mycelia toward the edge of petri dish were maximum 3 cm (Figure 1). All treatments were replicated three times (Nawangshi *et al*. 2013).

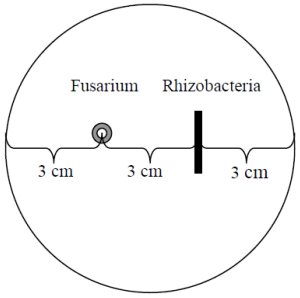

Figure 1. Composition of pathogen and rhizobacteria on PDA plate in antibiosis activity test (Nawangshi *et al*. 2013)

Growth Inhibition Assay by Dual Culture Method

Interaction between antagonistic efficacy of bacteria and pathogenic fungi by the method of Nawangshi *et al*. (2013) as the following:

$$\text{Percentage Suppression (\%)} = \frac{\text{DC control - DC treatment}}{\text{DC control}} \times 100$$

DC = dual culture

Measurement of Inhibition Zone

Inhibition zones were measured by the modifined Rukhasana Rating Scale (2011) (Table 1).

Table 1. Modified Rukhsana Rating Scale (2011)

Diameter of inhibition zone (mm)	Effectiveness
0-5 mm	Non effective
5.1-14 mm	Less effective
14.1-22 mm	Intermediately effective
>22 mm	Highly effective

Results

Total of nineteen bacterial strains were isolated from peanut fields. Among the nineteen isolated bacterial strains, MZ 1 to MZ 11 were identified as *Bacillus* and eight of them MZ 12 to MZ 19 were characterized as *Pseudomonas*. These bacterial strains were tested by Dual Culture Method against *Fusarium* fungi isolated from tomato plants for antifungal activity.

After 6 days, the Dual Culture Methods showed that the isolated bacterial strains MZ 1, MZ3, MZ 4, MZ 5, MZ 6, MZ 7, MZ 9, MZ 10, MZ 11, MZ 13, MZ 14, MZ 15, MZ 16, MZ 17 and MZ 18 had the antifungal activity. The MZ 16 and MZ 17 strain showed the strong antifungal activity followed by MZ 13 and MZ 14.

The results have showed that the isolated bacterial strains MZ 17 possess the effect on pathogenic fungi of leaf disease in tomato, the most antagonistic inhibition occurred on MZ 16 (Table 2-3 and Figure 2-7).

Table 2. The antagonistic activity of isolated *Bacillus* sp. strains from peanut fields

Method / Strains	MZ 1	MZ 2	MZ 3	MZ 4	MZ 5	MZ 6	MZ 7	MZ 8	MZ 9	MZ 10	MZ 11
Dual culture	+	–	+	+	+	+	+	–	+	+	+

Table 3. The antagonistic activity of isolated *Pseudomonas* strains from peanut fields

Method / Strains	MZ 12	MZ 13	MZ 14	MZ 15	MZ 16	MZ 17	MZ 18	MZ 19
Dual culture	–	++	++	+	+++	+++	+	–

+++ = Highly effective ++ = Intermediate effective

\+ = Less effective – = Non effective

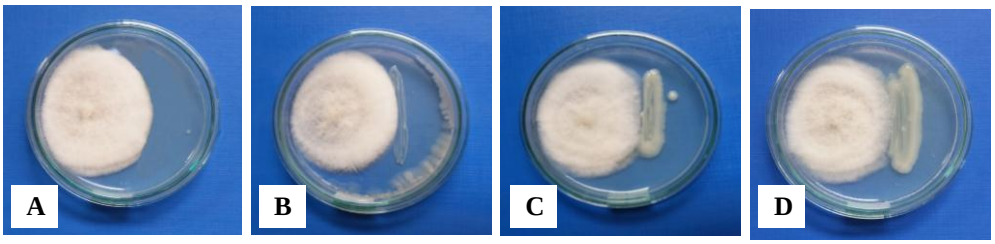

Figure 2. Dual Culture Method

A. Control

B. Fungus mycelium in test inoculated MZ 1

C. Fungus mycelium in test inoculated MZ 2

D. Fungus mycelium in test inoculated MZ 3

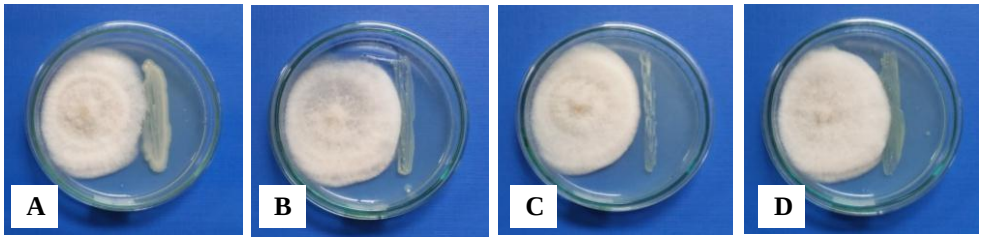

Figure 3. Dual Culture Method

A. Fungus mycelium in test inoculated MZ 4

B. Fungus mycelium in test inoculated MZ 5

C. Fungus mycelium in test inoculated MZ 6

D. Fungus mycelium in test inoculated MZ 7

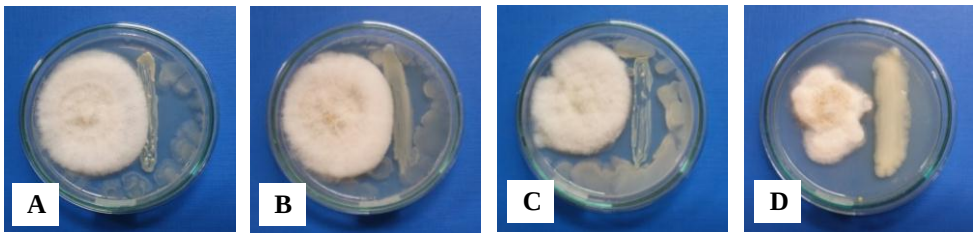

Figure 4. Dual Culture Method

A. Fungus mycelium in test inoculated MZ 8

B. Fungus mycelium in test inoculated MZ 9

C. Fungus mycelium in test inoculated MZ 10

D. Fungus mycelium in test inoculated MZ 11

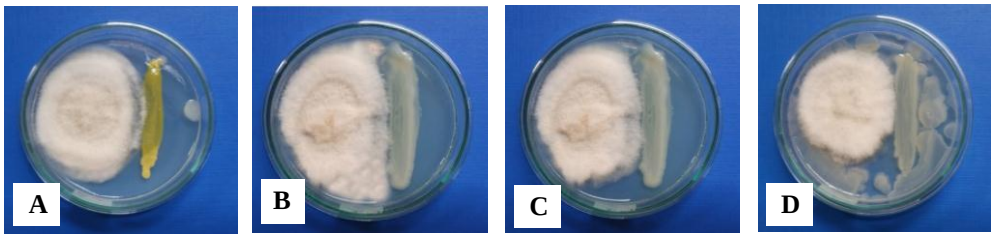

Figure 5. Dual Culture Method

A. Fungus mycelium in test inoculated MZ 12
B. Fungus mycelium in test inoculated MZ 13
C. Fungus mycelium in test inoculated MZ 14
D. Fungus mycelium in test inoculated MZ 15

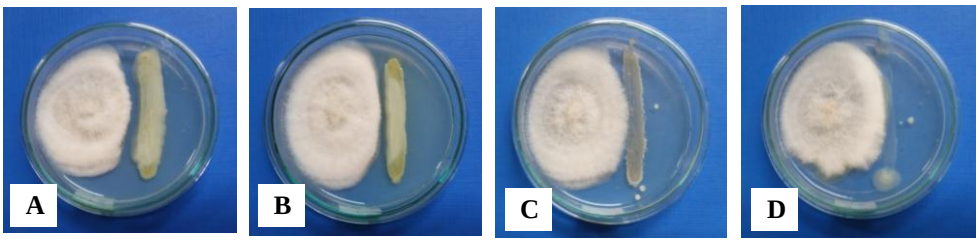

Figure 6. Dual Culture Method

A. Fungus mycelium in test inoculated MZ 16
B. Fungus mycelium in test inoculated MZ 17
C. Fungus mycelium in test inoculated MZ 18
D. Fungus mycelium in test inoculated MZ 19

Figure 7. Cell Morphology of *Fusarium* sp. for 6 days on PDA medium (MZ 16)

A. Microscopic structure of conidia in control
B. Microscopic structure of conidia in test inoculated
C. Microscopic structure of hyphae in control
D. Microscopic structure of hyphae in test inoculated

Discussion and Conclusion

The study was carried out the antagonistic activity of soil bacteria isolated from the soil of peanut fields against *Fusarium* from the infected leaves of tomato *in vitro* during August 2016. According to Lugtenberg & Kamailova (2009), to provide an environmentally friendly *Fusarium* disease control system, the use of antagonistic microorganisms represents an alternative disease management strategy. The present results showed that total of 19 bacterial strains were isolated from the rhizospheric soil of peanut fields.

The isolated bacterial strains were Nineteenth soil bacteria are MZ 1, MZ 2, MZ 3, MZ 4, MZ 5 MZ 6, MZ 7, MZ 8, MZ 9, MZ 10, MZ 11, MZ 12, MZ 13, MZ 14, MZ 15, MZ 16, MZ 17, MZ 18 and MZ 19. Among them, MZ 1 to MZ 11 were identified as *Bacillus* species and MZ 12 to MZ 19 were *Pseudomonas* species. Waites *et al*. (2008) Stated that *Bacillus* species are of considerable importance because they produce a number of antibiotics like chloramphenical, erythromycin, neomycin, nystatin. In the present study, the antifungal activity of isolated bacterial strains was performed by Dual Culture Method.

In the Dual Culture, fifteen isolates showed the antagonistic activity and MZ 13, MZ 14, MZ 16 and MZ 17 were more effective against fungal *Fusarium*. Antagonisms might be due to the production of biologically active compounds in media (Castilo *et al*. 2002). In this research, both *Bacillus* species MZ 1 to MZ 11 and *Pseudomonas* MZ 12 to MZ 19 showed the antifungal activities. Monda (2002) reported that the bacterial biocontrol agents with promising biocontrol activities against *Fusarium oxysporum* f. sp. *Lycopersici* include *Pseudomonas fluorescence*, *P. putide*, *P. chlororophis*, *Bacillus subtilis*, *Sterptomyces pulcher*, *S. corhorusii* and *S. mutabilis*.

In the present work, the isolated soil bacteria could be promising source of antifungal activity on *Fuasrium* wilt in tomato. It would be considered that comparison of all isolates, *Pseudomonas* strain MZ 16 had more active antifungal activity against *Fusarium* fungi. The use of bioagents was reported quite effective to control *Fusarium* wilt disease on tomato (Freeman *et al*. 2002). Pal & Gardener (2006) also reported that the mechanism adopted by biological control agents could be direct, indirect or mixed.

In conclusion, the isolated MZ 16, *Pseudomonas* bacterial strain obviously showed the antagonistic activity against Fusariam fungus which occur in tomato. Therefore, the bacterial strains isolated from the rhizospheric soil of peanut fields would be considered as the biocontrol agents for the infected plants and agricultural crops.

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