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ORIGINAL ARTICLE

Compatibility studies on different endophytes of *Citrus limon* antagonistic to bacterial with plant pathogen

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ABSTRACT

A mutual compatibility study was undertaken to develop the most effective endophytic bioconsortium for the management of insect from Citrus limon. The promising endophytes selected from in planta experiment were tested for their mutual compatibility to develop the microbial consortia. The interactions were studied among and between the three Bacillus spp. of bacteria. The three bacterial isolated from steam, leaf and seed of Citrus limon, selected from in planta experiment were subjected to mutual compatibility test by cross streak method, all three isolates MSZLd, MSZLe and MSZPse were found compatible. Based on sporulation and growth of the organisms and further confirmation by plating on their respective media, the three isolates compatible to each other and therefore, all these isolates were selected for the development of different endophytic consortia.

KEY WORDS: *Citrus limon, Bacillus spp., Endophytes, Mutual compatibility, Pesticide resistance.*

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INTRODUCTION

Maggot and some insect cause damage the plant and fruits of *Citrus limon*. Bacterial wilt disease incited by *Ralstonia solanacearum* (Smith) Yabuuchi *et al.* is one of the most destructive diseases of solanaceous crops in tropics, subtropics, and warm temperate regions of the world[14]. In Kerala, the yield loss due to the bacterial wilt incidence ranged from 20 to cent per cent depends upon the varieties [12]. Biocontrol of plant pathogen is becoming an important component of integrated disease management. In view of the hazardous impact of pesticides and other agrochemicals on the ecosystem, biocontrol of plant diseases as an alternate strategy has received increasing attention in recent years.[14]Therefore, the focus on the management of plant diseases has been shifted from chemical pesticides to more ecofriendly biopesticides to reduce environmental hazards and minimize the risk of development of pesticide resistant strains of plant pathogens. [6] A novel method of biological control using endophytes has entered the arena of disease management with attempts to make the plant, defend itself from the pathogens. The beneficial effects that the endophytes can confer on plants have made their role highly significant in biological control of diseases in various crops[1] [5]. Recently, a greater thrust is given for the development of biological consortium since it consists of microbes with different biochemical and physiological capabilities, which permits interaction among themselves and will lead to the establishment of a stable and effective microbial community. It will further provide better management of diseases by way of synergistic effect and multiple mode of action. Therefore, a study was carried out on the compatibility of endophytes isolated from *Citrus limon* antagonistic for insect and pest control.

MATERIAL AND METHODS

The endophytes isolated from *Citrus limon* were subjected to both *in vitro* and *in vivo* experiment and the promising bacterial isolates selected were tested for their mutual compatibility *in vitro* using Nutrient Agar media. The interaction between the Bacillus spp. as endophytes was studied. Two different bacterial isolates were streaked vertically and horizontally on Nutrient Agar mediated plates by cross streak method. [9]Plates were kept in triplicates for each combination and incubated at ±37°C for 24-48 h to observe the compatibility reaction for lysis at the juncture of the streaks. The microbial population in each plate was recorded and compared with that of individual cultures. [9] Pesticide resistance test with these three isolates also performed by well diffusion method using 100ppb contains mixture of 13 different pesticides on Nutrient agar plate and incubated at ±37°C for 24-48 h to observe the zone of resistance. ANOVA software as statistical tool used for the average of error and slandered deviation.

RESULTS

The promising endophytes selected from *in plant* experiment were tested for their mutual compatibility todevelop the microbial consortia. The interactions were studied among and between the three Bacillus spp. of endophytes of *Citrus limon*.

Compatibility between bacterial antagonists

The three bacterial isolates selected from *in plant* experiment were subjected to mutual compatibility test by cross streak method (Figure 1)(Table 1). No lysis was observed at the juncture of MSZLd x MSZLe, MSZLd x MSZPse and MSZLe x MSZPse combinations, which indicated the compatibility among the isolates and these three bacterial isolates MSZLe, MSZLe and MSZPse were selected. The isolates were compatible to each other and therefore, all these bacterial endophytes were selected for the development of consortia. These three isolates show resistance with 14 different pesticides up to 100ppb (Figure 2) (Table 2).

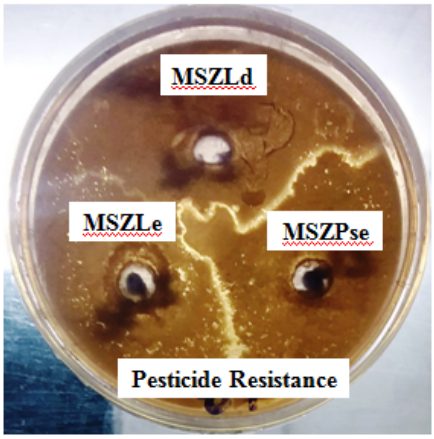

Figure 1: Pesticide resistance test

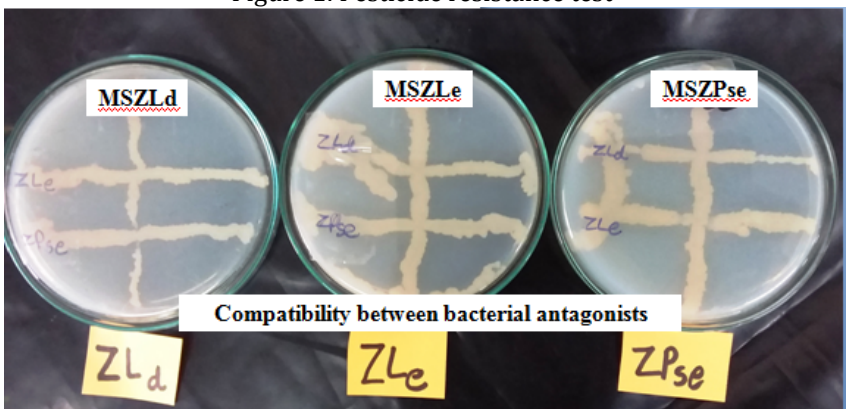

Figure 2: Compatibility between bacterial antagonists

Table 1: Mutual compatibility between endophytic bacterial isolates

Sr No.	Bacterial endophytes	Observations recorded	Interpretation
1	MSZLd x MSZLe	No change growth present	Compatible
2	MSZLd x MSZPse	No change growth present	Compatible
3	MSZLe x MSZPse	No change growth present	Compatible

Table 2: Pesticide present in 100ppb mixture

Sr. No.	Name of Pesticide	Concentration present in mixture µg/ml
1	α BHC	100±0.04
2	γ BHC	100±0.15
3	βBHCAldrin	100±0.24
4	Dieldrin	50±0.12
5	4 4DDT	80±0.22
6	α Endosulphon	50±0.04
7	β γ chlordane	80±0.16
8	α chlordane	40±0.10
9	4 4 DDE	80±0.26
10	Endrin	100±0.18
11	4 4 DDD	80±0.06
12	Endrinaldehyde	100±0.15
13	Endosulfun sulphate	50±0.22
14	Endrin ketone	40±0.09

Each value is the average of three analyses ± standard deviation

DISCUSSION

It is likely that, most cases of naturally occurring biological control results from mixtures of antagonists, rather than from high population of a single antagonist. Mixtures of antagonists are considered to account for protection in disease suppressive soils. Combinations of biocontrol agents for plant diseases include mixtures of bacteria. Most of the reports on bioconsortia showed that, combinations of antagonists resulted in improved biocontrol. However, there are also reports of combinations of bioagents that do not result in improved suppression of disease compared with the individual antagonist. Incompatibility of the coinoculants can arise because biocontrol agents may also inhibit each other as well as the target pathogen. Thus an important prerequisite for successful development of microbial consortia appears to be the compatibility of the coinoculated microorganisms.

Therefore, the endophytes selected from *in planta* experiment were subjected to mutual compatibility test for the development of efficient microbial consortia. Total twenty seven bacterial endophytes isolated from steam, leaf and seed respectively from *Citrus limon* out of them three potent isolates were tested *in vitro* for their compatibility. The interactions among the endophytes showed observations like heavy sporulation at the meeting point, diffusion of metabolite. Intermingling of hyphae, yellow pigmented band at the interaction point, clear demarcation at the meeting point, and dark green spores turned olive green by James in 2017 [3].Similar interactions were observed between endophytic *Trichoderma* isolates of black pepper by Mathew (2007).The fungi were noncompatible of which one showed suppression of growth and two exhibited overgrowth mechanism reported by James in 2017 [3]. The noncompatibility among the fungal isolates was also noticed by Mohammed in 2011 [7]in a composting process of oil palm waste where they observed the combinations of *T. viride* and *Penicillium* sp., *T. viride* and Basidiomycete M, *T. reesei* and *P. tigrinus* may interact as compatible, while *A. niger and T. viride*, *A. niger*and *T. reesei*, *T. viride* and *T. reesei* and *Penicillium* sp. and *P. tigrinus* were partially compatible and the other combinations were incompatible or inhibited by each other. Likewise, the three bacterial isolates were found compatible witheach other. Similar observations have been reported previously also. For example, *T. viride*/*T. harzianum* and *P. fluorescens* were reported to be compatible and improved plant growth, as well as suppressed seedling disease of chilli and tomato significantly when these were applied together Rini and Sulochana in 2007; Chaube and Sharma, in 2002 [10] [2].Later Rini and Sulochana in 2008 [11][6] also reported compatibility between *Trichoderma* spp. and fluorescent pseudomonads in dual cultures testing their efficacy against *Rhizoctonia solani* and *Fusarium oxysporum* infecting tomato by James in 2017 [3]. All three Bacillus spp. Bacillus subtilis (MSZLd), Bacillus vallismortis (MSZLe) and Bacillus licheniformis (MSZPse) show highly compatible to each other and also show tolerance with chemical pesticides up to 100ppb. Kale in 1989 [4] reported that the growth of A. chroococcum in nitrogen-containing culture medium was not affected by carbofuran at 0.5 and 5 ppm concentrations. Nawab in 2003 [8] also tested the growth of the Pseudomonas isolates in the presence of cHCH (c-hexachlorocyclohexane) at concentrations ranged from 15 to 120 µg/ml and also found that growth declined at higher concentrations. Azotobacter strains were able to tolerate the concentration of 1600 µg/ml for all the three pesticides Carbofuran, Endosulfan and Malathion by Shafianiin [13].

CONCLUSION

In view of these studies, the endophytes isolated from *Citrus limon* were subjected to both *in vitro* and *in vivo* experiment and the promising bacterial isolates selected were tested for their mutual compatibility *in vitro.* It is evident that the bacterial strains isolated from agricultural soil were able to grow in the presence of pesticides. High concentrations of the pesticides in soils have already been reported by Nawab in 2003 [8]. This property of pesticide tolerance in these bacteria may be important in the decontamination of agricultural soil polluted by the pesticides. This Bacillus spp. consider as compatibility to develop good consortium for antagonistic to bacterial with pathogen. It may work as biopesticide.

CONFLICT OF INTEREST

The authors have no conflict of interest in preparing of this article.

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