

## Effect of Soil Amendment with Urea and Calcium Oxide on Survival of *Ralstonia solanacearum*, the Causal Agent of Bacterial Wilt or Rhizome Rot of Ginger

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### ABSTRACT

The effect of soil amendment with urea and calcium oxide on survival of *Ralstonia solanacearum*, the causal agent of bacterial wilt or rhizome rot of ginger, was studied by mixing urea and calcium oxide at the rate of 80 : 800 kg/rai in the artificial infested soil with  $2.83 \times 10^7$  cfu/ml of bacteria. The treated soil was left one week before transplanting the two months old gingers. The soil was sampled during week 0-4 to evaluate the population of *R. solanacearum* and to compare with the control by serial dilution method and spread plate on SM-1 medium. The experiment showed that the population of *R. solanacearum* in the soil amendment with urea and calcium oxide decreased from  $0.88 \times 10^7$  cfu/ml to  $0.15 \times 10^5$  cfu/ml in week one,  $0.1 \times 10^4$  cfu/ml in week two and 0 cfu/ml in week three. The control treatment still contained high population level of  $0.26 \times 10^7$  cfu/ml in week one,  $0.13 \times 10^6$  cfu/ml in week two and three and  $0.11 \times 10^6$  cfu/ml in week four. This population level could cause typical wilt of the tested gingers. The tested gingers transplanted in the treated soil showed no symptom. The decrease of bacteria in the treated soil was due to the toxicity of ammonium, ammonia and nitrate degraded from urea in high pH soil condition (average 7.0-7.2). Therefore the soil amendment with urea and calcium oxide at the rate of 80 : 800 kg/rai is recommended to decrease population of *R. solanacearum* in the infested fields for bacterial wilt control. The treated soil should be left at least 3 weeks before planting a new crop to avoid toxicating to ginger seedlings.

**Key words:** ginger, bacterial wilt, rhizome rot, *Ralstonia solanacearum*, *Pseudomonas solanacearum*, soil amendment, urea and calcium oxide

### INTRODUCTION

Bacterial wilt or rhizome rot of ginger caused by the soilborne bacteria *Ralstonia (Pseudomonas) solanacearum* is the serious problem in ginger growing area in both tropical and sub tropical regions. This disease usually occurs in late rainy season. The diseased plant showed inward curling, yellowing and browning of the entire shoot and almost dead. The basal portion of the yellow stem

(shoot) is water-soaked and easily broken off from the underground rhizome and there is milky bacterial ooze exuding from cut stem or rhizome. According to the examination of the causal agent founded in Thailand by Koch's postulate and identification indicated that the causal agent was *Pseudomonas solanacearum*, ginger strain, belonging to biotype 3 and 4. It caused severe disease to tomato, potato, egg plant, phuttaraksa, edible ginger, creater-galangal, kachai, phai, and tumeric. All inoculated

plants died 100 % in two weeks. Ginger strain could not infect sesame, peanut, bird chille, cowpea, yard long bean, triploid banana and bird of paradise. The relationship between bacterial causal agent and other organisms such as *Fusarium oxysporum*, *Sclerotium rolfsii*, *Pythium sp.*, *Bacillus megatherium*, *Bacillus subtilis* etc are as follow : the symptom and diseased development are not different when inoculated with *P. solanacearum* alone and *P. solanacearum* mixed with the other organism. There were no other organisms in the experiment that could inhibit the growth of *P. solanacearum* or retard wilt symptom. On testing of 9 chemicals by petridish zonal inhibition and tube dilution technique, it was found that aureomycin at 50 ppm, streptomycin at 100 ppm and captan at 500 ppm could inhibit the growth of this organism. But in dipping ginger-seed into these chemicals before planting at concentration 2000 ppm for aureomycin and streptomycin, and 5000 ppm for captan, the chemicals could not prevent ginger-seed from disease (Chantaraotan, 1982). The infectivity tritration of *P. solanacearum* indicated that the concentration of inoculum at  $10^5$  -  $10^8$  cfu/ml could cause bacterial wilt symptom (Vudhivanich, 1997). Yabuuchi *et al.* (1992) reported some physical and biochemical characteristics and nucleotide sequences of *P. solanacearum* are different from *P. aeruginosa* which was the type strain of bacteria in genus *Pseudomonas*. Therefore, it was transfer to new genus, *Burholderia solanacearum*, and finally changed to *Ralstonia solanacearum* in 1995. The disease can widespread by water, rain and by seed (rhizome) transmission (Chantaraotan *et al.*, 1986). A wide host range have been described for this pathogen (Atabug and San Juan, 1981 ; Vudhivanich and Soontarasing, 1994). It could survive in debris in soil for many years (Smith, 1944 ; Kelman, 1953). Chemical control of the disease has been attemptd with little success, beacause the pathogen resisted to many chemicals and antibiotics (Garner *et al.*, 1917 ; Well and Roldan, 1922 ; Miller and

Harvey, 1932 ; Chantaraotan, 1982). This pathogen has high genetic variation, new race or strain often occurred (AVRDC, 1974).

For many last years, the farmers have avoided the disease by shifting the ginger growing area to new location. It caused the increase of deforestation and widespread of the disease to the new cultivated area. One approach to help the farmer growing ginger in their own infested fields is soil amendment with some materials to decrease the population of the soilborne pathogen. There are many reports of soil amenment attempting to control the disease. Elphinstone and Aley (1993) reported the integrated control for bacterial wilt of potato race 1 biovar1 by crop rotation with maize and using 0.5 kg/ha metriburin, soil amendment with 5 ton/ha calcium oxide and 200 kg/ha urea could reduce the population of *R. solanacearum* and nematode in soil. It was also found that the population of *Pseudomonas cepacia*, the antagonistic bacteria increased. Michel (1997) reported that intercropping tomato with cowpea and the application 200 : 5000 kg/ha of urea and calcium oxide in soil amendment could reduce the population of *P. solanacearum* and bacterial wilt of tomato in Taiwan. However the experiment result of each location was not consistent. Patcharin (1997) reported soil amendment with urea and calcium oxide at the rate of 68.5 : 800 kg/rai could reduce the bacterial wilt of tomato by 60 % at greenhouse condition and 81 % at field condition. There were no significant differences among the types of calcium oxide such as calcium hydroxide, marl or dolomite. Furthermore the increase of calcium oxide have greater effect on the disease control than the increase of urea.

The objective of this experiment was to study the effect of soil amendment with urea and calcium oxide on survival of *R. solanacearum*, the causal agent of bacterial wilt or rhizome rot of ginger. The relationship between the population level and the bacterial wilt of ginger was also studied.

## MATERIALS AND METHODS

### Inoculum preparation

*Ralstonia solanacearum* was isolated from diseased ginger by cross streak on Tetrazolium medium (TZC) [Kelman, 1954] for 48 hour at room temperature (30-32°C). Virulent colony of small irregularly round, fluidal, white with pink in center were picked up and maintained in sterile distilled water and kept in a cooler at 13°C. The inoculum was prepared from virulent colony and increased on TTC medium for 30 hours and then suspended in sterile distilled water. The optical density (O.D.) was measured by spectrophotometer to reach 0.5 at wavelength 590 nanometer. Some of the inoculum was inoculated into disease -free ginger shoot by scalpel leaf clip method for pathogenicity test.

### Artificially infested soil preparation

Artificially infested soil was prepared by mixed inoculum 1 liter per 16.2 kilograms of sterilized soil. A half of infested soil was filled into 12 clay pots as the control and the rest used as the treatment for soil amendment with urea and calcium oxide.

### Survival of *R. solanacearum* in infested soil (control)

Two months old disease - free gingers were transplanted in artificial infested soil in 12 clay pots. Each clay pots had 4-5 shoots. Wilt symptom was checked everyday. The infested soil was sampled every week from week 0 (initial state) to week 4. The evaluation of population of *R. solanacearum* in infested soil was made by serial dilution method and spread plate on SM-1 medium (Granada *et al.*, 1983).

### Survival of *R. solanacearum* in infested soil amending with urea and calcium oxide

The infested soil was amended with urea and calcium oxide at the rate of 80 : 800 kg/rai (50 g urea mixing with 500 g calcium oxide per 16.2 kg of infested soil). The soil was watered and

wrapped with plastic sheet and left for 1 week. Afterward, the treated soil was divided into 12 clay pots. Two months old disease - free gingers were transplanted into those 12 clay pots. Wilt symptom was checked everyday. The treated soil was sampled every week from week 1 to week 4. The evaluation of population of the pathogen in treated soil was made by the same method as in the control. Soil pH was also recorded.

## RESULTS AND DISCUSSION

The disease-free gingers which inoculated with bacterial suspension at O.D. of 0.5 by Scalpel leaf clip method for pathogenicity test showed typical wilt in 3 days. The tested gingers transplanted in the artificial infested soil (control pots) showed wilt symptom in 4 days and died within one week. It confirmed that the inoculum used in this experiment was virulent and high pathogenicity. The diseased plants showed inward curling, yellow and wilt of the lower leaves, followed by complete yellowing and browning of the entire shoot. The rhizome of the infected gingers showing water soak area in the succulent part and having milky bacterial ooze exuding from the cut rhizome. The virulent colony which was small irregularly round, fluidal, white with pink in center could isolate from the inoculated plant (Figure 1). The bacterial population in the control pots slowly decreased from  $0.88 \times 10^7$  cfu/ml in the initial stage to  $0.26 \times 10^7$  cfu/ml in week one,  $0.13 \times 10^6$  cfu/ml in week two and three and  $0.11 \times 10^6$  cfu/ml in week four. All of the tested gingers showed typical wilt symptom in 4-5 days after transplanted into the control pots and died in a few days. The bacterial population in the infested soil amending with urea and calcium oxide at the rate of 80 : 800 kg/rai decreased from  $0.88 \times 10^7$  cfu/ml to  $0.15 \times 10^5$  cfu/ml in week one,  $0.1 \times 10^4$  cfu/ml in week two and was not founded in week three. The tested gingers transplanted into treated pots showed no symptom. The population dynamic of the pathogen during week 0 - 5 showed in Table



**Figure 1** Bacterial wilt or rhizome rot of ginger, symptom and colony of *R. solanacearum*.

- Tested ginger transplanted in the infested soil showing inward curling and complete yellowing in 4 days.
- Typical wilt symptom in the ginger field occurred during rainy-season.
- Rhizome of the infected ginger showing water soak area in the succulent part.
- Milky bacterial ooze exuding from the cut rhizome.
- Virulent colony of *R. solanacearum* on TZC medium formed an irregularly round, fluidal, white with pink in center.
- Virulent colony of *R. solanacearum* on SM1 medium formed round, pulvinate, fluidal and tan in color.

1 and Figure 2.

Soil amending with urea and calcium oxide at the rate of 80 : 800 kg/rai could decrease the population of *R. solanacearum* in the infested soil due to the toxicity of ammonium, ammonia and nitrate degraded from urea in high pH soil condition (average 7.0 - 7.2). The experimental result was confirmed the conclusion of Elphinstone and Aley (1993) ; Michel (1997) and Patcharin (1997) that soil amendment with urea and calcium oxide could reduce the population of *R. solanacearum* and bacterial wilt of tomato . However the rate of urea and calcium oxide was different depending on soil

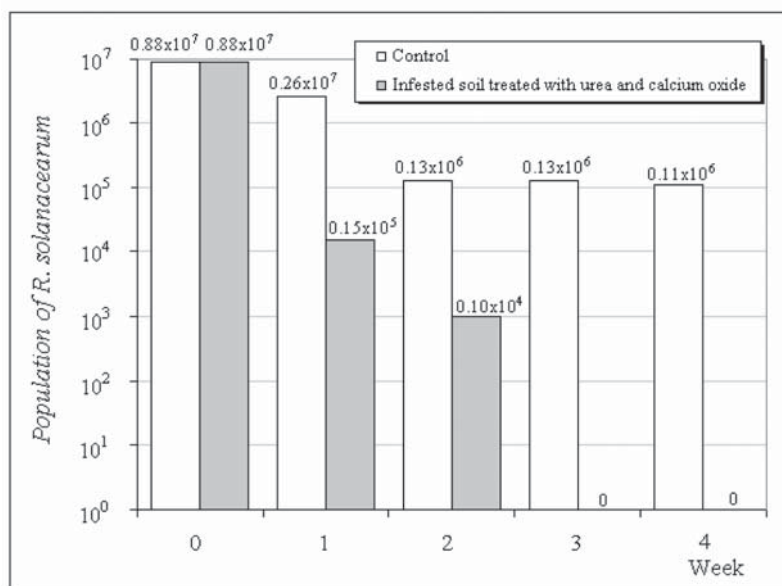
type, soil pH, soil moisture and soil microorganisms. Thus the soil amendment with urea and calcium oxide at the rate of 80 : 800 kg/rai is recommended to reduce the bacterial wilt of ginger in the severe infested area. The treated soil should be left 3 weeks before planting to avoid toxicating to ginger seedlings.

## CONCLUSION

The soil amendment with urea and calcium oxide at the rate of 80 : 800 kg/rai could decrease population of *R. solanacearum* in the infested soil

**Table 1** Population of *R. solanacearum* in the control and treated soil during week 0 – 4.

Week	Control (cfu/ml)	Treated soil (cfu/ml)
0	$0.88 \times 10^7$	-
1	$0.26 \times 10^7$	$0.15 \times 10^5$
2	$0.13 \times 10^6$	$0.1 \times 10^4$
3	$0.13 \times 10^6$	0
4	$0.11 \times 10^6$	0



**Figure 2** Population of *R. solanacearum* in the control and treated soil with urea and calcium oxide at the rate of 80:800 kg/rai.

in greenhouse condition. The decrease of bacteria was due to the toxicity of ammonium, ammonia and nitrate degraded from urea. This method can be recommended to decrease bacteria in the infested area for bacterial wilt control. The treated soil should be left at least 3 weeks before planting a new crop to avoid toxicating to plant.

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