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# Effect of Chemical and Soil Amendment for the Control of Bacterial Wilt of Potato in Nepal Caused by *Ralstonia solanacearum*

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## ABSTRACT

Management to control bacterial wilt of potato caused by *Ralstonia solanacearum* (RS) (Syn. *Pseudomonas solanacearum)* race 3, biovar II was carried out by amending infested soils with stable bleaching powder (SBP) and a mixture of urea and lime (urea-lime) at different concentrations and combinations under glasshouse and field conditions of Nepal. Soils infested with RS were treated with either SBP or urea-lime mix two weeks before planting with the highly susceptible and healthy potato seeds cultivar Kufri Jyoti. The SBP treatment at 25 kg/ha provided an effective disease control for both glasshouse and field conditions in which disease suppressions in glasshouse and field experiment were 66.96% and 71.87% for plant infection and 76.94% and 88.89% for tuber infection as compared to nontreated control, respectively. Tuber yields of the SBP treatment at 25 kg/ha were121.5 g/plant in the glasshouse and 13.54 kg/plot in the field which were not significantly different from the treatment of SBP at 12 kg/ha + urea-lime mix at 428 kg/ha urea and 5 ton/ha lime. The RS population was reduced in all treatments except in nontreated control in which the treatment SBP at 25 kg/ha demonstrated the lowest RS population of 3.01 and 2.06 log cfu/g dry soil at 120 days after amendment in glasshouse and field experiments, respectively. The results indicated that the use of SBP at the rate of 25 kg/ha was effective and suitable than the other treatments for the control of bacterial wilt in infested soils under glasshouse and field conditions. Alternatively,soil amendment with 428 kg/ha urea and 5 ton/ha lime can be used to effectively control the bacterial wilt disease where SBP is not available.

**Key words** : potato, bacterial wilt, *Ralstonia solanacearum*, *Pseudomonas solanacearum*, bleaching powder, soil amendment, urea, lime.

## INTRODUCTION

Nepal is a small country having a diverse climate ranging from very hot sub-tropics in the plain to very cold temperature in the mountain. The Nepalese economy depends largely on agriculture and related industries. Rice, maize, wheat, millet, and potato are the major economic crops. Potato is one of the important staple food crops grown in mid to high hill areas. Comparison of potato yield with other developed countries shows a big gap between potential yield and actual yield of Nepalese farmers. This low productivity of potato is associated with a number of reasons, among them, diseases

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are the most important factors.

Among seventeen important potato diseases, bacterial wilt caused by *Ralstonia solanacearum* (Syn. *Pseudomonas solanacearum)* is the second most important disease of potato which was first reported by Pushkar Nath in 1960 (Shrestha, 1977). In 1977, Shrestha also confirmed *R. solanacearum* as the causal organism of wilted potato plants by performing physical, biological and host range tests. The bacterium associated with potato in mid hill to high hill regions is race 3, biovar II or known as potato strain (Shrestha, 1977). However, race 1, biovar III is also found associated with the wilting of tomato, eggplant, pepper and tobacco from the mid hill areas of the country (Adhikari *et al.*, 1993).

Bacterial wilt of potato has presently become a more serious problem in major potato growing areas of the mid to high hills ranging from 900 - 2,500 m above sea level (asl). Yield losses have been reported as high as 75% depending upon management practices of potato growers (Dhital, 1995). The disease has been spreading in all potato growing areas due to the lack of proper management practices for the disease control. Farmers in irrigated areas of the mid hill and plain were advised to use a crop rotation with paddy rice for at least two seasons (Hoger and Shrestha, 1982). These recommended practices are not feasible for the high hill and upland areas of the mid hill because of cold climate and no irrigation facility. In addition, no potato lines/varieties are found resistant to the disease. Bacterial wilt is considered to be the most difficult to control due to several factors such as the bacterium has a wide host range, a long survival in the soil, less effective chemical control and a variable biological characters (Persley *et al.*, 1986). Thus, it is essential to acquire more knowledge for the effective control of this disease. The present investigation was designed to determine the appropriate integrated control management of bacterial wilt by using different soil amendment with urea and lime and chemical soil treatment of stable bleaching powder in the infested soil under glasshouse and field conditions of Nepal.

## MATERIALS AND METHODS

### Bacterial isolation and cultures

Infected potato stems or tubers (Figure 1 A and B) were cut into small pieces and placed in test tubes containing sterile distilled water for standard isolation of plant bacterial pathogen (Schaad, 1988). Bacteria were allowed to flow from the vascular bundles for 5-10 minutes (Figure 1 C). One loopful of bacterial suspension was streaked on tetrazolium chloride (TZC) agar medium (Kelman,1954) and plates were incubated for 48 h at 28°C for bacterial colony observation. Single colony of *R.solanacearum* (Figure 1 D) was selected and multiplied in TTC medium (without tetrazolium chloride) (Kelman, 1954). Isolated cultures were maintained as suspension in sterile distilled water in screw-cap tubes at room temperature (Kelman and Person,1961).

Based on pathogenicity and biochemical characters as described by He *et al.* (1983) and Hayward (1964), all strains were classified as race 3, biovar II. The strain "NSPC 3" of *R. solanacearum* obtained from Nigale (2500 m asl.) was used for soil infestation in glasshouse and field experiments.

### Planting materials and experimental design

The experiment on bacterial wilt management of potato by chemical and soil amendment was conducted both under glasshouse and field conditions. Highly susceptible of recommended potato cultivar "Kufri Jyoti" was used in all experiments as a planting material. Potato tuber seeds used in the experiment were second generation from disease-free, pre-basic seed produced from *in vitro* tissue culture scheme. The tuber seeds were

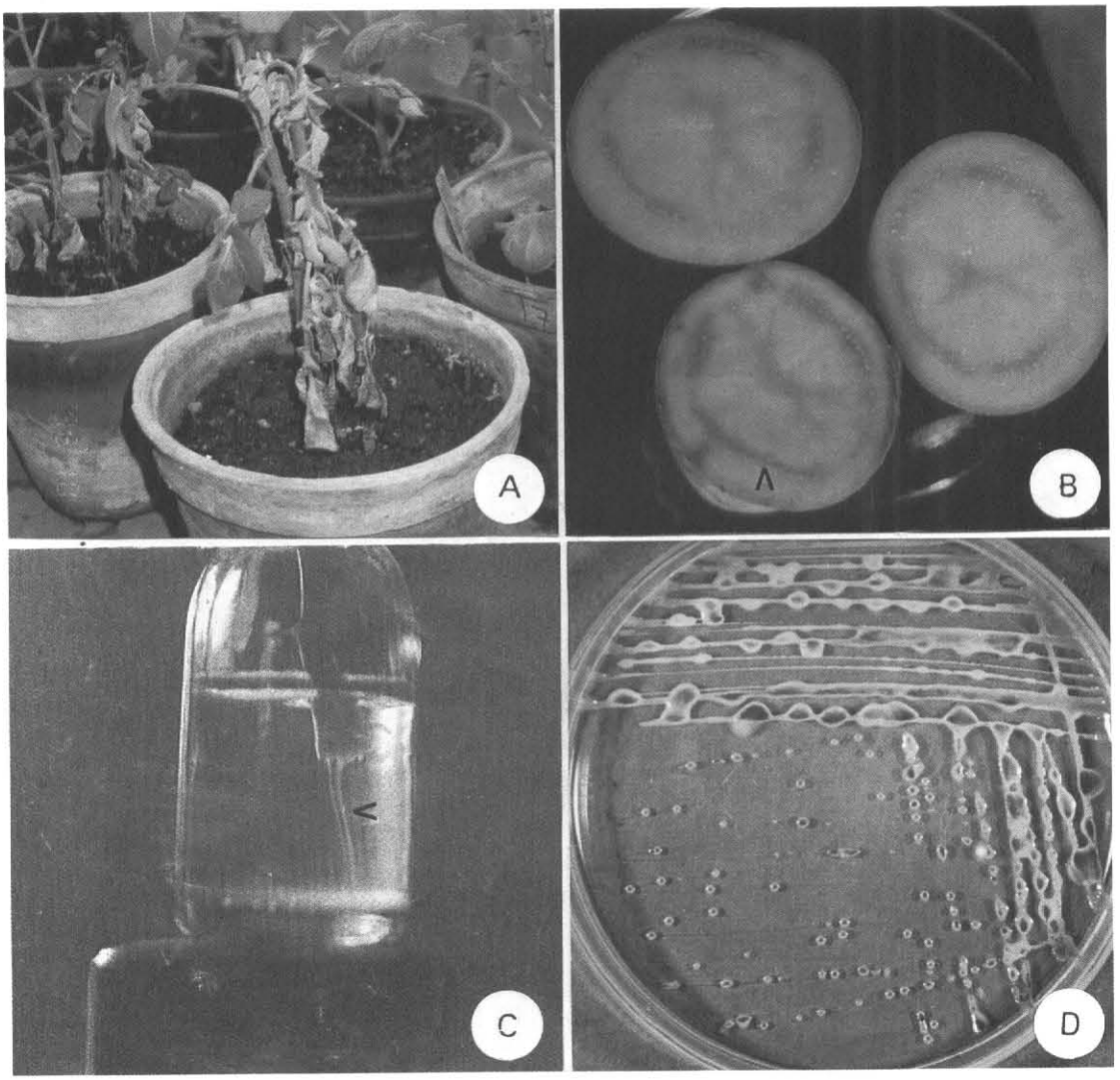


**Figure 1** Bacterial wilt of potato and colony of *Ralstonia solanacearum.*

(A) Symptoms of infected potato plant showing the drooping wilted leaves

(B) Naturally infected potato tuber showing brown discoloration of vascular bundles and bacterial exudate (arrow)

(C) Milky white steaming of bacterial mass (arrow) from vascular tissue of infected potato stem in clear water

(D) Typical virulent colonies of *R. solanacearum* on TZC medium after 24 h incubation showing irregular, fluidal, creamy white with pink at center

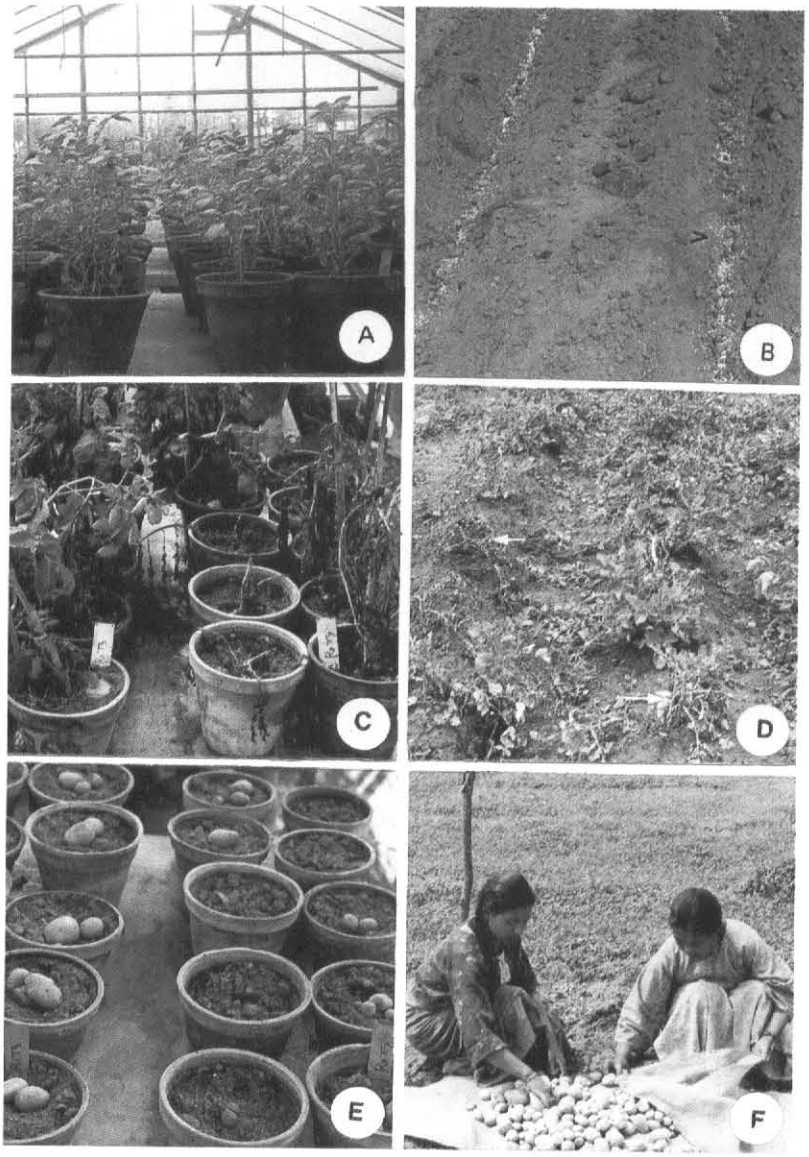


**Figure 2** Glasshouse (A,C,E) and field (B,D,F) experiments on the effect of stable bleaching powder (SBP) and soil amendment (urea + lime) for the control of bacterial wilt of potato caused by *Ralstonia solanacearum.*

(A) Potato plants growing in glasshouse for observation of wilting incidence in each treatment
(B) Furrow application of SBP, urea and lime (arrow) in the field experiment
(C) Potato plants showing wilting symptom and died (right) and normal (left)
(D) Potato plants showing wilting symptom (arrow) in the field experiment
(E) Harvested potato tubers of SBP (25 kg/ha) treatment (left) and nontreated control (right)
(F) Harvesting of potato tubers and disease inspection from field experiment

well sprouted and uniformed in size with the weight of 30-40 g each. Complete fertilizer N,P,K was applied at a rate of 100,80,60, kg per ha as a basal dose in the form of urea (46% N), diammonium phosphate (18, 48% N and $P_2O_5$) and murate of potash (60% $K_2O$) together with 20 ton/ha decomposed farm yard manure. Fertilizer and manure were seperately applied at the time of soil treatment.The experiment was carried out in randomized complete block design (RCBD) with three replications. The soil amendment used in the experiment was a mixture of urea ($NH_2CONH_2$) and lime (CaO) as reported by Elphinstone and Aley (1993). Similarly, chemical used in soil treatment was stable bleaching powder ($Ca(OCl)_2$ ) as mentioned by Verma and Shekhawat (1991), Shekhawat *et al.* (1988) and Ali (1995). Seven treatments of different combinations of soil amendment and chemical soil treatment used in both experiments were as followed:

1. Mixture of urea at the rate of 428 kg/ha and lime at the rate of 5 ton/ha (full rate of urea and lime),
2. Mixture of urea at the rate of 214 kg/ha and lime at the rate of 2.5 ton/ha (half rate of urea and lime),
3. Stable bleaching powder (SBP) at the rate of 25 kg/ha (full rate of SBP),
4. Stable bleaching powder at the rate of 12 kg/ha (half rate of SBP),
5. Mixture of urea at the rate of 428 kg/ha and lime at the rate of 5 ton/ha and supplemented with SBP at the rate of 12 kg/ha (full rate of urea and lime and half rate of SBP),
6. Mixture of urea at the rate of 214 kg/ha and lime at the rate of 2.5 ton/ha and supplemented with SBP at the rate of 12 kg/ha (half rate of urea and lime and half rate of SBP) and
7. Nonchemical treatment as a control.

**Glasshouse experiment**

The glasshouse experiment was conducted at Potato Research Program (PRP), Khumaltar, Lalitpur (1350 m asl.). Naturally infected potato tubers and soil were collected from potato field from the high hill Nigale at the end of potato growing season (July, 1996) for preparing the infested soil. In order to get uniform pathogen population in the infested soil, artificial inoculation was done as described by Shekhawat *et al.*(1993). Freshly prepared bacterial suspension (~$10^8$ cfu/ml) of *Ralstonia solanacearum* race 3, biovar II was used for soil drenching. The total volume 678 kg air-dry soil was throughly mixed with 20 liter of bacterial suspension. After mixing, the soil was divided into 7 equal parts (96.9 kg) for the seven treatments. Excepting the nontreated served as control, six treatments of SBP and urea - lime amendment were incorporated to the soil at the rate as required in each treatment then filled in clay pots of 20 cm diameter and kept in glasshouse for two weeks. Fertilizer and manure were applied at the same time when amended the soil. Ten pots were carried out for each treatment with 3 replications.

A 500 g of soil from each treatment was taken for pH measurement (20 g), determination of *R. solanacearum* population (10 g) and the remaining soil for NPK and organic matter analysis at the Soil Science Division, Khumaltar, Lalitpur.

In order to detect the population of *R. solanacearum* from soil, a 10 g sub-sample was suspended in 90 ml of sterile distilled water in 250 ml flask as mentioned by Klement *et al.* (1990). The soil suspension was vigorously shaked for 20-30 minutes and allowed to stand for 10-20 minutes to settle. A ten fold serial dilution was done by mixing 0.5 ml of soil suspension with 4.5 ml of water. A 100 microliter suspension from each dilution was spread with a flamed glass spreader on the modified SM-1 (mSM-1) selective medium (Granada and Sequeira, 1983) as described by

Leksomboon (1994) which was composed of 5 ppm crystal violet, 50 ppm polymyxin B sulphate and 50 ppm cycloheximide in TZC medium as a selective ingredient. The plates were incubated at 28°C for 2-4 days and the bacterial population as colony forming unit (cfu) per gram of dry soil was determined. The colony morphology was similar to colony found in TZC medium. For determination of soil pH and population of *R. solanacearum* (cfu/g dry soil) in the soil, composite samples containing 100 g of soil from each treatment (10 pots) were taken from a depth of 2-10 cm before planting and one month interval until the end of experiment at 120 days after amendment.

Potato was planted on September 2, 1996 two weeks after application of chemical and soil amendment and harvested on December 20, 1996. Plants were watered in alternate day until 15 days before harvesting. Disease incidence was recorded at weekly interval. In addition, soil and air temperatures were recorded daily. After harvestation, infected and noninfected tuber numbers and weight per plant were recorded and statistically analyzed.

**Field experiment**

The field experiment was conducted at Nucleus Seed Potato Centre (NSPC), Nigale, Sindhupalchok (2500 m asl). The selected field was naturally infested with *R. solanacearum* for more than 15 years. About 15-20 % of infected plants were observed during potato growing season in 1996.

To ensure uniformity of inoculum distribution in the soil, disease infested plot was developed by growing most susceptible potato plant (var. Kufri Jyoti) from August to November, 1996 in the experimental plot. After 45 days of planting, potato plants were inoculated with *R. solanacearum* by stem inoculation method as described by Valdez (1986). After more than 80 % plants showed wilting symptom, all potato plants were incorporated into the soil as described by McCarter (1973). Following incorporation the infested field was divided in to 21 plots (3 × 3.25 m), each plot was surrounded by a drainage ditch in a randomized block design with 3 replications. The field was left fallow for two months in winter (December, 1996 to January, 1997). At the time of land preparation, the addition of bacterial suspension prepared from artificial inoculation of susceptible tomato plant (var. Pusha Ruby) in the Khumaltar glasshouse was drenched uniformly into the experimental plots.

Before planting and after harvesting, composite samples containing 1 kg of soil from each of five sites per plot were removed from a depth of 5-20 cm (Elphinstone and Aley, 1993) for determination of pH, NPK, organic matter and population of *R. solanacearum* (cfu/g dry soil). Similarly, after planting a composite sample containing 1 kg soil was removed at one month interval for pH and *R. solanacearum* population determination as described earlier. Each treatment was individually incorporated with SBP or urea + lime and combinations into a depth of 30 cm (Figure 2 D) as described by Elphinstone and Aley (1993) and Verma and Shekhawat (1991).

Two weeks after treatment, potato tubers were planted on the ridge with a spacing of 25 cm between plants and 60 cm in a row. The planting and harvesting dates were on March 10 and on July 30, 1997, respectively. Other agronomical practices, including weeding and hilling were done according to cultural requirements. Disease incidence was recorded at 15 days interval after planting whereas soil temperature, air temperature, humidity and rainfall were recorded daily. After harvesting, infected and noninfected tuber numbers and tuber yield per plot were recorded and statistically analyzed.

## RESULTS AND DISCUSSION

### Glasshouse experiment

The application of SBP, urea and lime at various combinations in artifical infested soils showed a remarkable reduction of bacterial wilt incidence, tuber infection and pathogen population and an increase of tuber yield when compared with nontreated control (Table 1). The wilt symptom was firstly observed in nontreated control followed by less effective treatment while the more effective treatment occurred lastly. Treatment of full rate of SBP (25 kg/ha) showed lowest disease incidence at 24.80% and tuber infection at 7.17% which were

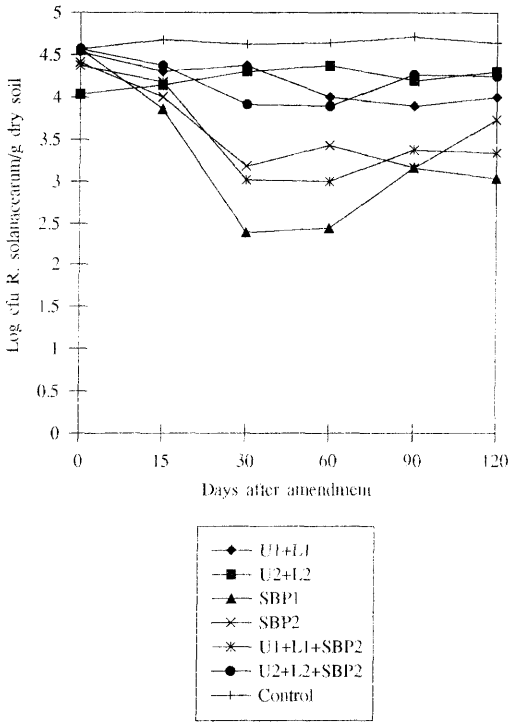


**Figure 3** Effect of chemical and soil amendment on population of *Ralstonia solanacearum* (log cfu/g dry soil) under glasshouse at Khumaltar, Nepal for 120 days after amendment.

U1+L1 = Urea 428 kg/ha+lime 5t/ha, U2+L2 = Urea 214 kg/ha+lime 2.5t/ha, SBP1 = SBP 25 kg/ha, SBP2 = SBP 12 kg/ha, U1+L1+SBP2 = Urea 428 kg/ha+lime 5t/ha + SBP 12 kg/ha, U2+L2+SBP2 = Urea 214 kg/ha+lime 2.5t/ha + SBP 12 g/ha, Control = Nontreated

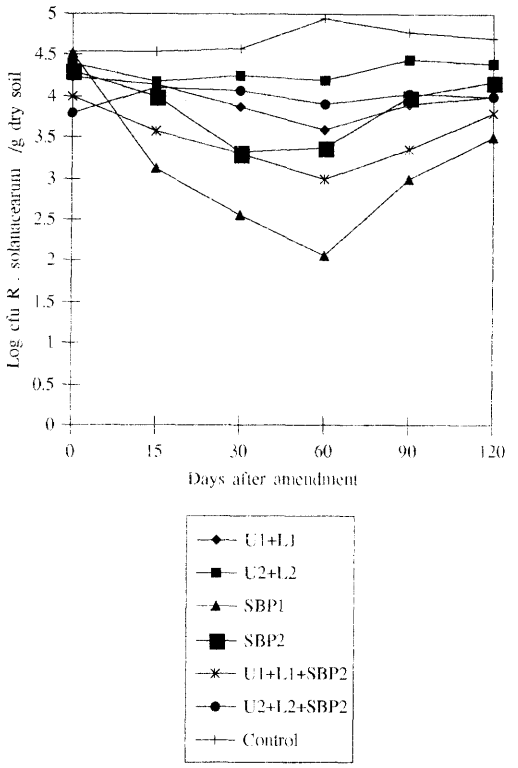


**Figure 4** Effect of chemical and soil amendment on population of *Ralstonia solanacearum* (log cfu/g dry soil) under field condition at Nigale, Nepal for 120 days after amendment.

U1+L1 = Urea 428 kg/ha+lime 5t/ha, U2+L2 = Urea 214 kg/ha+lime 2.5t/ha, SBP1 = SBP 25 kg/ha, SBP2 = SBP 12 kg/ha, U1+L1+SBP2 = Urea 428 kg/ha+lime 5t/ha + SBP 12 kg/ha, U2+L2+SBP2 = Urea 214 kg/ha+lime 2.5t/ha + SBP 12 kg/ha, Control = Nontreated

no significant differences with full rate of urea (428 kg/ha) and lime (5 ton/ha) plus half rate of SBP (12 kg/ha) (Table 1). However, tuber yield of the full rate of SBP treatment was 121.50 g/plant which was slightly lower than the treatment of full rate of urea and lime plus half rate of SBP (139.17 g/ plant). The highest disease incidence was 75.07% in nontreated pots (Table 1) which was significantly different with all treatments (Figure 2 C).

On the basis of percent tuber infection, the lowest infected tubers was observed with an average of 7.17% on pots treated with full rate of SBP (Figure 2 E) and this was followed by 7.78% on pots amended with a full rate of urea and lime plus half rate of SBP whereas the highest infection was 31.10% in nontreated pots. The highest disease control on plant and tuber infections were 66.96% and 76.94% in pots treated with full rate of SBP, respectively. The least disease control was 25.40% for plant infection and 51.92% for tuber infection in the treatments of half rate of urea (214 kg/ha) and lime (2.5 ton/ha) and half rate of SBP (12 kg/ha), respectively.

The highest tuber yield (139.17 g/plant) was also observed in the pots treated with full rate of urea and lime supplemented with half rate of SBP and followed by the full rate of SBP treatment (121.50 g/plant). However, the two treatments were not significantly different from half rate of urea and lime supplemented with half rate of SPB (Table 1). Nontreated pot produced lowest yield of 72.67 g/ plant which was significantly different to all

**Table 1** Effect of soil amendment and chemical soil treatment for the control of bacterial wilt of potato caused by *Ralstonia solanacearum* under glasshouse condition at Khumaltar, Nepal.

| Treatments | Disease incidence (%) | | Disease control (%) | | Tuber yield/plant (g) |
|---|---|---|---|---|---|
| | Plant | Tuber | Plant | Tuber | |
| Urea (428 kg/ha)+lime (5 ton/ha) | 39.71 c[1] | 11.12 c | 47.10 | 64.24 | 115.33 b |
| Urea (214 kg/ha)+lime (2.5 ton/ha) | 56.00 b | 14.53 bc | 25.40 | 53.32 | 112.17 b |
| Stable bleaching powder (SBP) (25 kg/ha) | 24.80 d | 7.17 d | 66.96 | 76.94 | 121.50 ab |
| SBP (12 kg/ha) | 42.53 c | 14.95 b | 43.35 | 51.92 | 118.67 b |
| Urea (428 kg/ha)+lime (5 ton/ha + SBP (12 kg/ha) | 26.01 d | 7.78 d | 65.35 | 74.98 | 139.17 a |
| Urea (214 kg/ha)+lime (2.5 ton/ha) + SBP (12 kg/ha) | 33.30 cd | 12.19 bc | 55.64 | 60.80 | 120.17ab |
| Nontreated control | 75.07 a | 31.10 a | 0.00 | 0.00 | 72.67 c |
| CV% | 9.86 | 19.99 | | | 14.14 |
| F-Test[2] | ** | ** | | | ** |

[1] Mean values in the same column followed by a common letter were not significantly different at the $P < 0.05$ level by Duncan's new multiple range test

[2] ** = Significant at $P < 0.01$

treatments.

Under glasshouse condition, *R. solanacearum* population was considerably high (more than 4.0 log cfu/g dry soil) in all treatments at initial stage and decreased sharply in all treatments until 30 days after amendment. Thereafter, population was gradually increased until harvesting. However, the lowest population was 2.38 log cfu/g dry soil in the full rate of SBP and followed by 3.01 log cfu/g dry soil in the full rate of urea and lime supplementing with half rate of SBP at 30 days after treatment (Figure 3). On the other hand, population of *R. solanacearum* was remained at the highest level throughout the growing period in nontreated plots. However, the last observation showed that the lowest *R. solanacearum* population was detected in the full rate of SBP and followed by the full rate of urea and lime amended with half rate of SBP. The highest number of *R. solanacearum* population was detected in treatments of half rate of urea and lime and nontreated pots.

**Field experiment:**

The lowest wilt incidence was 12.75% in the plot treated with full rate of SBP which was significantly different with all treatments. The highest disease incidence was 45.32% in nontreated plots ( Table 2, Figure 2 D).

The highest disease control on plant infec-

**Table 2** Effect of soil amendment and chemical soil treatment for control of bacterial wilt of potato caused by *Ralstonia solanacearum* under field condition at Nigale, Nepal.

| Treatments | Disease incidence (%) | | Disease control (%) | | Tuber yield/plant (g) |
|---|---|---|---|---|---|
| | Plant | Tuber | Plant | Tuber | |
| Urea (428 kg/ha)+lime (5 ton/ha) | 22.50 cd[1] | 2.02 c | 50.35 | 80.23 | 13.04 ab |
| Urea (214 kg/ha)+lime (2.5 ton/ha) | 30.05 b | 4.02 b | 33.69 | 60.85 | 11.98 bc |
| Stable bleaching powder (SBP) (25 kg/ha) | 12.75 e | 1.14 c | 71.87 | 88.89 | 13.54 a |
| SBP (12 kg/ha) | 27.46 bc | 4.94 b | 39.41 | 51.89 | 12.06 bc |
| Urea (428 kg/ha)+lime (5 ton/ha) + SBP (12 kg/ha) | 20.39 d | 1.38 c | 55.00 | 86.56 | 14.12 a |
| Urea (214 kg/ha)+lime (2.5 ton/ha) + SBP (12 kg/ha) | 24.68 bcd | 4.10 b | 45.54 | 60.07 | 11.65 c |
| Nontreated control | 45.32 a | 10.27 a | 0.00 | 0.00 | 11.02 c |
| CV% | 18.35 | 23.98 | | | 8.00 |
| F-Test [2] | ** | ** | | | * |

1 Mean values in the same column followed by a common letter were not significantly different at the $P<0.05$ level by Duncan's new multiple range test

2 ** = Significant at $P < 0.01$

* = Significant at $P<0.05$

tion was 71.87% in plots treated with full rate of SBP (25 kg/ha) and followed by 55.00% in plots amended with full rate of urea (428 kg/ha) and lime (5 ton/ha) supplemented with half rate of SBP (12 kg/ha).

Lowest tuber infection was 1.14% in plots treated with full rate of SBP whereas highest infection was 10.27% in nontreated control plots. Highest disease control on tuber infection was 88.89% in the full rate of SBP treatment. However, there were not significantly different in tuber infection among treatments of full rate of SBP, full rate of urea and lime supplemented or nonsupplemented with half rate of SBP.

The highest tuber yield was 14.12 kg/plot in plot amended with full rate of urea and lime supplemented with half rate of SBP. However, it was not significantly different from treatments of full rate of urea and lime and full rate of SBP (Figure 2 F). The lowest tuber yield was 11.02 kg/plot in nontreated plots which was not significantly different with the treatments of half rate of urea and lime, half rate of SBP and half rate of urea and lime plus half rate of SBP (Table 2).

Population of *R. solanacearum* in full rate of SBP treatment was drastically reduced until 60 days similarly with the result of glasshouse experiment except in nontreated soil (Figure 4). However, the lowest population was 2.06 log cfu/g dry soil in plot treated with full rate of SBP at 60 days after treatment. After 60 days of amendment the population of *R. solanacearum* was gradually incresed in all treatments at different levels until harvesting.

Regarding bacterial wilt management, research results of various workers indicated that the wilt disease can be managed in an effective way by treating the soil with SBP and soil amendment with urea and lime. In both glasshouse and field experiments, the lowest population of *R. solanacerum* was observed when percentage of disease control for plant and tuber was high in the treatment of full rate of SBP and followed by the treatment with a mixture of full rate of urea and lime supplemented with half rate of SBP (Figure 3 and 4). In this study the high rate of SBP at 25 kg/ha was more effective than the low rate of SBP at 12 kg/ha in both experiments. This may be due to the high concentration of chlorine generated by SBP into the soil. Our research results were similar to the findings of Ali (1995) from Bangladesh that in field experiment, plant infection was only 5.62% when treated with full rate of SBP whereas in nontreated plot was 26.04%. It was also agreed with the report from India (Verma and Shekhawat, 1991) that application of SBP in furrow soil can control the wilt disease by 68.4%.

Higher incidence of plant and tuber infections were observed earlier in the glasshouse than in the field may be due to the more favourable conditions for the pathogen growth and infection. However, disease control for plant and tuber infections was promisingly found in both conditions when treated with full rate of SBP. In the field experiment, plant infection was observed at 75 days after planting and tuber yield was not greatly different among the treatments even the plant infection in nontreated plots was high (45.32%). The most effective treatment was the full rate of SBP whereas the least effective treatment was the half rate of urea and lime. Similarly, Lalithakumari and Jehan (1987) reported that a significant decrease in the number of bacterial and actinomycetes colonies was observed when applied SBP at 3 and 6 inches depths which were 40 to 80 percent in case of bacterial colonies and 20 to 50 percent in case of actinomycetes. They also reported that SBP was affective against bacterial leaf blight of paddy rice and the use of SBP as a soil treatment appeared to safeguard the productivity of the soil. The SBP with 35 percent chlorine was also reported to be an effective chemical for the control of many bacterial

plant diseases. The results of this experiments showed that the bacterial population in the soil was low when treated with the high rate of SBP chemical may be due to the toxic effect of chlorine in the soil.

The results of this experiment were corresponded with other workers that the use of a mixture of urea and lime also help to control the bacterial wilt in potato. Elphinstone and Aley (1993) reported that soil amendement with 0.5% (w/w) CaO and/or 0.1% urea prevented the development of bacterial wilt in tomato. Urea was readily degraded by soil microorganisms into ammonia and carbon dioxide. Ammonia generated by hydrolysis of urea was toxic to various pathogens (Chun and Loockwood, 1985).This result also supported the finding of Chang and Hsu (1988) that control of bacterial wilt by soil amendments containing urea and CaO was associated with indirect effects on the activity of other soil-borne microorganisms which influence the survival of *R. solanacearum* in the soil. A number of factors were suggested to be involved in the control of several soil-borne disease in amended soil.

In an anaerobic condition, urea was hydrolyzed by certain soil bacteria (*Micrococcus urease*) and converted into ammonium ($NH_4$) and ammonia ($NH_3$) through ammonification. Again, ammonia gas was converted into nitrite ($NO_2$) by *Nitrosomonas sp.* This $NO_2$ was very toxic to fungi and bacteria when the soil pH was between 5.0 to 7.0. Likewise, when the soil pH was more than 9.0, $NH_3$ and/or $NH_4$ was highly toxic to fungal and bacterial growth in the soil ( Tsao and Oster, 1981; Sequeira,1963) In this study, the soil pH was between 5.9 to 7.4 before and after amendment indicating the soil pH was not greatly changed after amendment under both glasshouse and field conditions. Therefore, $NO_2$ may play a role on the reduction of pathogen population instead of $NH_4$ or $NH_3$ since only at high soil pH (about 9.0) $NH_4$ and $NH_3$ will be toxic to bacteria especially the *R. solanacearum* in the soil.

This results also supported the findings of Thaveechai *et al.* (1997) that soil amendment with a mixture of urea and CaO at 428 kg/ha and 5,000 kg/ha, respectively showed the promising control of *R. solanacearum* in tomato under glasshouse condition. Tomato survival in amended soil at 21 days after transplanting was 63% whereas in nonamended soil survival was only 6.7%. They also mentioned that the alkaline soil (pH 7.7) may have an indirect effect in the reduction of pathogen population in the experiment.

Efficacy of stable bleaching powder and a mixture of urea and lime remained upto 60 days after application because the population of *R. solanacearum* was reduced until 60 days after application. After that the bacterial population and wilt incidence were steadily increased until the end of experiments in both glasshouse and field.

Results of this experiment suggested that the use of SBP at 25 kg/ha was more effective than the other combination of treatments. However, a mixture of urea at 428 kg/ha and lime at 5 ton/ha could be used effectively as SBP at 25 kg/ha.

## CONCLUSION

The results of this study indicate that the use of full rate of SBP at 25 kg/ha in infested soil is highly effective for the control of bacterial wilt of potato caused by *R. solanacearum* race 3,biovar II in terms of reduction of disease incidence (%) and pathogen population (cfu/g soil). On the other hand, total tuber yield is higher when planted in soil amended with a mixture of full rate of urea (428 kg/ha) and lime (5 ton/ha) supplemented with half rate of SBP (12 kg/ha) which is not significantly different with the full rate of SBP treatment. Whereas, soil amended with a mixture of half rate of urea and lime or at half rate of SBP was the least effective for

the control of bacterial wilt in the soil. Therefore, the use of full rate of SBP is the most effective and suitable than the other treatments especially in remote and high hill areas of Nepal. However, if the SBP is not available the use of full rate of urea and lime as a soil amendment can be recommended to use in the farmers' field in Nepal or in other potato growing areas facing similar bacterial wilt problem.

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