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Management of *Ralstonia* wilt of tomato through microbes, plant extract and combination of cake and chemicals

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ABSTRACT: The experiment was conducted on wilt of tomato (*Lycopersicon esculentum* L.), caused by *Ralstonia solanacearum* Yabuuchi, during post monsoon season for four consecutive years (1998-99 to 2002-03) to record the impact of three groups viz; (i) microbes (ii) plant extract (iii) cake and chemicals on the yield of tomato as well as on the viability of inocula. The pooled data revealed that there was significant effect in all the treatments in reducing the primary inocula. Microbes viz; *Glomus mosseae*, *Triochoderma viride* and *Azotobacter+Phosphobactrin* resulted in reduction of primary inocula (41.4, 29.0 & 37.3% at 90 days over initial population, respectively) of *Ralstonia solanacearum* in soil and increased the yield by 141.1, 142.1 & 89.9%, respectively. Similar trend was observed in plant extracts viz; Asafoetida +Turmeric, Onion and Garlic in which the yield increase was noted to be 71.2, 71.2 & 64.4%, respectively over control. Karanj cake along with bleaching powder was more effective in reducing the apparent bacterial growth rate at flowering stage and increased the yield by 123.4% followed by bleaching powder and lime 107.5% over control.

Key words: *Ralstonia* wilt, tomato, microbes, cake and chemicals, plant extracts

Soil is a heterogeneous habitat of beneficial, plant pathogenic and saprophytic microbes, which plays an important role in the spread of soil borne diseases. *Ralstonia solanacearum* Yabuuchi is the causal agent of wilt of solanaceous vegetables (Samaddar *et al.*, 1998). Among them, tomato (*Lycopersicon esculentum*) is one of the important vegetables, which suffers badly from this disease in summer, rainy and winter seasons. Infested soil and surface water, irrigation water, are the main sources of spread of inocula. The pathogen can infect undisturbed roots of susceptible hosts through microscopic wounds caused by the emergence of lateral roots. After infection, the bacterium colonizes the cortex and makes its way towards the xylem vessel; from there it rapidly spreads throughout the plant (Momol *et al.*, 2005). It has been reported that infected roots present in soil released vast number of *R. solanacearum* cells into the rhizoshere and invades subsequent crops to cause of spread the disease (Mc Carter, 1976). The race 1 of *R. solanacearum* can survive for more than 6 months on seed surface and in the soil (Samaddar *et al.*, 1998) and also reported to be spread through seed transmission (Singh *et al.*, 1995). The meager information on management by microbes (Kobayaashi, 1999), plant extracts (Bora *et al.*; 1997), cakes (Sharma and Kumar, 2000) and chemicals (Michel *et al.*, 1997; Mazumder, 1998) are available. Hence the study was undertaken on effect of microbes, plant extracts, cake and chemicals on primary inoculums and impact of yield reported here under.

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MATERIALS AND METHODS

The experiment was conducted in R.B.D. with 3 replications during post monsoon seasons of 1998-99 to 2002-03 on cv. Pusa Ruby with treatments; (i) microbes (a) mycorrhiza (*Glomus mosseae*) @500 g/m^2 of dry root mass multiplied in finger millet (ragi) *Penisetum typhoides* in pot (b) *Trichoderma viride* @5 kg/ha mixed in FYM (c) *Azotobacter chroococcum* + *Phosphobactrin* @2 kg each mixed in 50 kg FYM + 50 kg soil (ii) Plant extracts viz; (a) asafotida (*Ferula foetida)* @1 g +

turmeric (*Curcuma longa)* @5 g in 10 litres water (b) onion (*Allium cepa*) @5% extract (c) garlic (*Allium sativum)* @ 5% extract (iii) cake and chemicals viz; (a) karanj (*Deris indica*) cake @10 q/ha + bleaching powder ($CaOCl_2$) @30 kg/ha and (b) bleaching powder ($CaOCl_2$) @30 kg/ha + lime @2500 kg/ha (c) urea @200 kg/ha + lime ($CaCO_3$) @5000 kg/ha (v) control. The microbes as well as cake and chemicals (viz; bleaching powder and lime) were applied 15 days earlier to transplanting. The plant extracts were used for seedling dip for 30 minutes and sprayed three times at 10 days interval after one month of transplanting. Twenty-one days seedlings were transplanted and recommended doses of NPK were applied. The soil samples were collected at 0, 30, 60 & 90 days and the bacterial count was made on TTC media (Kelman, 1954) by dilution method and data were statistically analysed. The apparent bacterial growth rate (r) was calculated by following the formula given below after conversion of bacterial counts at different days in to LOGIT value described by Van der Plank (1963) and reported in here with:

$$r = 1/t_2\text{-}t_1 \; [\log_e (X_2 / X_1)]$$

Where, X_1 = initial count (cfu x 10^4/g soil)

X_2 = final count (cfu x 10^4/g soil)

The apparent infection rate was presented graphically. The yield recorded year wise were pooled, statistically analyzed and presented herewith.

RESULTS AND DISCUSSION

Effect of microbes

Mycorrhyza (*Glomus mosseae*): It is revealed from the mean of four years (1998-99 to 2002-03) data that there was a significant effect of treatments on reduction of *Ralstonia solanacearum* population in soil. Addition of VAM mycorrhyza (*Glomus mosseae*) results in maximum reduction (41.4%) of *Ralstonia solanacearum* population. The VAM was effective at 30 days in which the apparent bacterial growth rate (BGR) was (r = -0.032 unit/day), at flowering stage (r = -0. 011 unit/day) at 60 days interestingly later on it reduced at 90 days (r = -0.009 unit/day) (Fig.1). The trend resulted in a

Table 1. Effect of microbes, plant extracts and cake and chemicals on primary inoculuar of bacterial wilt of tomato cv. Pusa Ruby (1998- 99 to 2002-03)

Treatments		*Ralstonia solanacearum* (cfu x 10^4/g soil) at days					% decrease by 90 days	% decrease over control	Yield* (g/ha)	% Increase over control
		Initial	30	60	90	Av of 30,60 & 90 day				
Microbes	*Glumos mosseae*	84.4	64.4	56.4	49.5	56.8	41.4	39.41	76.18	141.1
	Trichoderma viride	83.3	61.0	70.2	59.2	63.5	29.0	27.52	76.50	142.1
	Azotobacter + *Phosphobactrin*	81.5	72.2	56.1	59.1	59.8	37.3	27.62	60.00	89.9
Plant extracts	Asafoetida + turmeric	75.3	69.0	53.0	45.7	55.9	39.3	44.01	54.10	71.2
	Onion extract	70.8	77.5	52.3	50.5	60.1	32.7	38.09	54.10	71.2
	Garlic extract	97.4	76.1	72.9	73.6	74.2	24.4	9.80	51.95	64.4
Cake & chemicals	Karanj cake + bleaching powder	78.8	67.0	56.3	53.2	58.8	32.5	34.87	70.60	123.4
	Bleaching powder + lime	80.1	61.1	64.8	51.1	59.0	36.2	37.37	65.58	107.5
	Urea +lime	91.2	63.8	65.6	63.1	63.5	30.8	22.72	48.23	52.6
Control		81.6	72.2	55.4	54.2	60.6	33.6	-	31.60	
CD(P 0.05)					6.1			260.17		
CV (%)								30.66		

*Mean of four years (1998-99 to 2001-02)

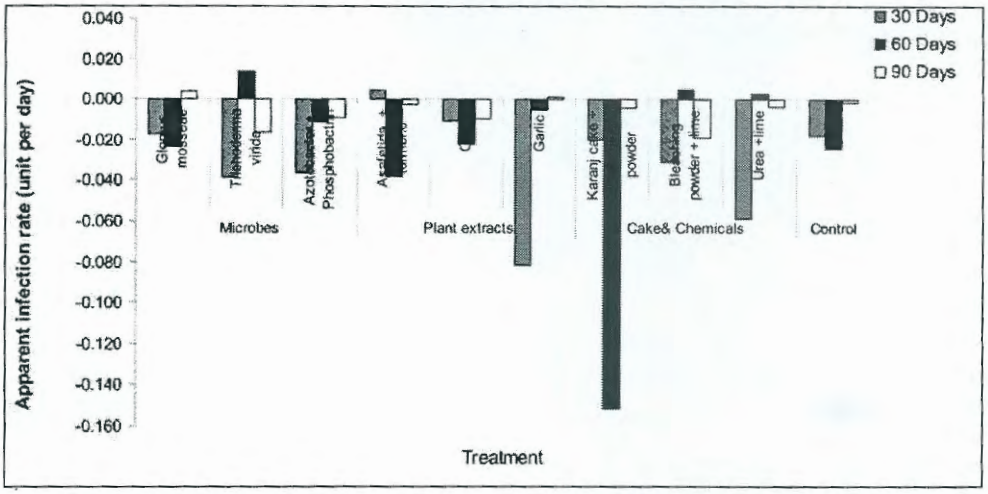

Fig. 1. Effect of microbes, plant extract cake and chemical on apparent infection rate of Ralstonia solanacearum in tomato cv Pusa Ruby

logarithmic reduction in apparent infection rate (Fig.2). This effect of reduction may be due to colonization by the *G. mosseae* in the root. It has been reported that Vascular Arbuscular Mycorrhiza (VAM) and charcoal compost caused reduction in the bacterial wilt in tomato (Kobayashi, 1990). The extract of tomato roots colonized with *G. fasciculatum* reduced population of *P.solanacearum* in nutrient broth *in vitro* (Suresh and Rai, 1991). Several reports on VAM fungi are available that these fungi decrease the severity of many soil borne fungal diseases as well as that caused by *P. solanacearum,* however; further studies are required to establish the mechanism of active principle of VAM fungi inhibiting *P.solanacearum*. (Bagyaraj, 2002).

The yield was found maximum 760.18 kg/ha which was 141.1% increase over control (310.6 kg/ ha). The increase in yield may be due to mycorrhizal symbiosis which might have colonized with roots resulting into higher plant uptake of water and minerals from the soil thereby the yield has increased. It has been reported that mycorrhizal fungus colonies the cortical cell of the roots, therby form a symbiotic relationship with the host (Read *et al.*, 1992).

Trichoderma viride

Trichoderma viride supplemented soil resulted in 29.0% reduction at 90 days the in R. *solanacearum* population. The apparent infection rate was (r = - 0.039 unit /day) at 30 days and (r = -0.016 unit/day) at 90 days (Fig.1). The trend resulted in logarithmic reduction in apparent infection rate (Fig.2). It has been reported that *Trichoderma* spp. have ability to adopt extreme soil conditions viz, soil temperature, moisture and fungicidal load added in soil and that is why, it is importance in the field of biological control of plant diseases. The *T. viride* produced antibiotics viz, viridin, dermadine and gliotoxis (Sawant, 2002). A vast number of plant pathogens, particularly soil borne fungi controlled by *Trichoderma* spp. (Anahosur, 1999).Singh *et al.* (2004) has reviewed and reported that *Trichoderma, a* microbe has multifaceted activity.

Application of *T.viride* in soil resulted 760.5 kg/ ha which was 142.1% increase over control (Fig.2). *T. viride* used as bioagent for control of various fungal pathogens viz; *Macrophomina* stem rot and root rot in Sesame (Rajpurohit, 2004), white rot in Pea (Kapoor and Kumar, 2004), Seed borne pathogen in Okra (Gurjar *et al.*, 2004) and bacterial wilt in chilli (Yan Sihuang *et al.*, 2005).

Azotobacter chroococcum

Azotobacter chroococcum and *Phosphobactrin* resulted in reduction (37.3%) at 90 days in the primary inoculum. The apparent bacterial growth rate was r = -0.018 unit/ day at 30 days and at flowering stage (60 days) was r = -0.011 unit /day (Fig.1) and remained ineffective in reducing (r =

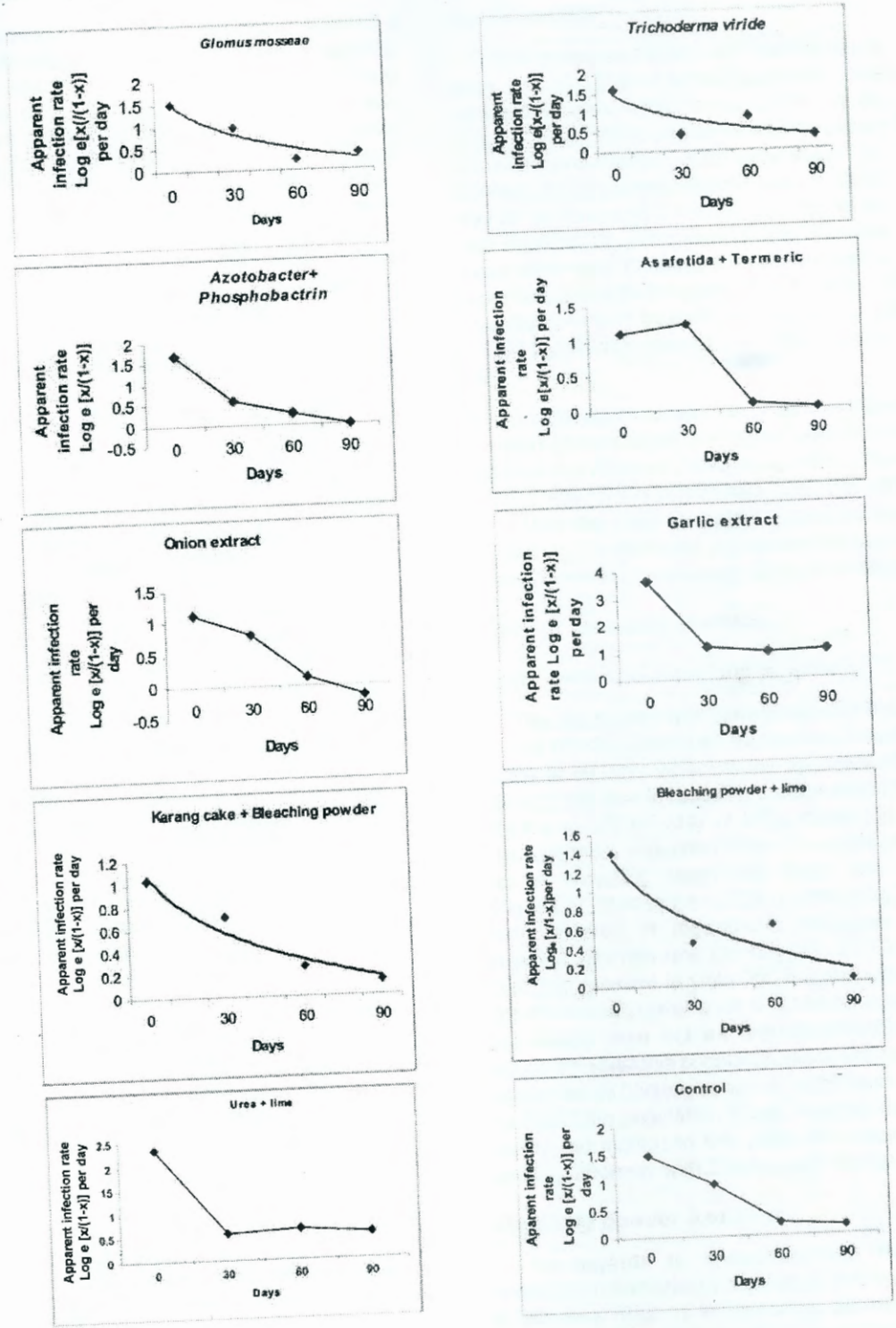

Fig. 2. Apparent infection rate (unit /day) under various treatments

0.004 unit/day) at 90 days. The trend showed a logarithmic reduction in apparent infection rate (Fig. 2). It has been reported that extract of tomato roots colonized with N-fixing bacteria *Azotobacter* was ineffective (Suresh and Rai, 1991). It is reported that the *Azotobacter* has ability to produce anti-fungal antibiotics and also to fix nitrogen, which improve the fertility of soil (Laxmi – Kumari *et al.*, 1975) and also act as bioagents (Wani, 2002). Thus the results indicated that the use of microbes will be effective for longer period in managing the *R.solanacearum* population in soil. *Azotobacter chroococcum* treated plot resulted in yield of 600.0kg/ha which was 89.9% increase over control.

Effect of plant extract: asafoetida and turmeric

The asafoetida + turmeric powder treated plot also reduced the primary inocula to 32.7% at 90 days. In the early stage (30 days) the effectiveness was slow. The apparent infection rate was r = -0.038 unit/day at flowering stage (60 days) and it remain effective in reducing at 90 days (r = -0.002 unit /day). The trend indicated moving trend which was different from microbes (Fig.2). It has been reported that seedling treatment with mixture of asafoetida (*Ferula foetida* Ritz.), turmeric powder (*Curcuma longa* Linn.) and water (ATW) in ratio of 1g: 5g: 10 liters controlled bacterial wilt or by spraying 5% ATW solution or soil drenching at 15, 30 and 45 days after transplanting (Bora, 1995, Pun and Das, 1997). In the present investigation asafetida + turmeric powder treated plot resulted 540.1 kg/ha which was 71.2% increase over control. The result was in conformity with Mazumder (1998) reported from Assam.

Onion extract

The seedling treated with onion extracts (5%) a significant reduction in primary inoculum was also found to be 39.3% at 90 days. The apparent infection rate was r = - 0.0106 unit/day at 30 days whereas r = -0.023 unit/day at flowering stage (60 days) and r = -0.01 unit/day at 90 days (Fig.1). The trend was also different from microbes at 30 days and reduction was more at 60 days Fig 2. Thus the plant extracts can also be used for minimizing the *Ralstonia solanacearum* population in soil, which will be cheaper than other treatment. The onion extract treated plot resulted in yield of 540.1 kg/ha which was 71.2% increase over control. Onion extract was better than garlic extract.

Garlic extract

The seedlings treated with garlic extracts (5%) were less effective in reducing primary inoculums which was also found to be 9.97% at 90 days (Fig.1).The seedling treated with garlic extract (5%) showed more effective at the initial stage (30 days) in which the apparent infection rate r = -0.082 unit/day at 30 days and r = -0.01 unit/day at 60 days and later on its effectiveness was reduced. The trend was also different from microbes but the reduction rate was constant after 30 days. (Fig.2). The garlic extract treated plot resulted in yield of 510.95 kg/ha which was 64.4%increase over control only.

Several workers have reported that the extract of onion (*Allium cepa*) and garlic (*Allium sativum*) reduced the disease (Hutaglung, 1988; Hanudin, 1987; Komarova and Korunets, 1997) *in vitro* and *vivo*. Thus the plant extracts can also be used for minimizing the *Ralstonia solanacearum* population in soil, which will be cheaper than other treatment.

Effect of cake and chemical

Karanj cake and bleaching powder

The soil treated with cake along with bleaching powder ($CaOCl_2$) reduced the primary inoculum to 32.5% at 90 days whereas the apparent infection rate was effective in reducing the apparent infection rate r = -0.021 unit/day at initial stage (30 days). The maximum reduction rate r = -0.152 unit/day was at flowering stage (60 days) and remain effective at 90 days r = -0.004 unit/day (Fig.1). The trend resulted in logarithmic reduction in the apparent infection rate per day (Fig.2). Cake and Chemical resulted in yield 700.6 kg/ha which was 123.4% increase over control. It has been reported that karanj cake act as organic amendment to reduce the *Ralstonia* population in soil and bleaching powder act as bactericide which might have reduced the *Ralstonia* population in soil resulting into good health and increased the yield. The above result was in conformity with Sharma and Kumar (2000).

Bleaching powder and lime

As regards to chemicals, the maximum reduction in bacterial population in soil was 36.2% at 90 days (Fig. 1) in bleaching powder + lime treated plot. The apparent BGR r = -0.031 unit/day was at initial stage (30 days) and at final stage r

= -0.019 unit/day at 90 days. The trend resulted in logic reduction in the apparent infection rate per day (Fig.2). The yield obtained was 650.58 kg/ha which was 107.5% increase over control. It has been reported that application of CaO alone significantly reduced the population of *Ralstonia* (Michel *et al.*, 1997). Pre plant application of lime 200 lb/acre in form of $CaCO_3$ augmented Ca accumulation in leaf tissue and soil and reduced the rate of bacterial development on susceptible cultivars soil but had little effect on fruit yield (Locscio *et al.*, 1998). Yamazaki *et al.* (1998) have reported that the resistance was negated at low concentration in Hawaii 7998 (highly resistant) and pathogen population in stem decreased with increasing Ca concentration. On the contrary to above Jaworski and Morton (1964) did not found any difference in plant survival in a manorial experiment with 4 tomato varieties, and the plants receiving different levels of Ca and Mg.

Urea and lime

Urea (200 Kg/ha N) and $CaCO_3$ (5000 Kg/ha) treated plot resulted reduction in primary inoculums 30.8% at 90 day which was significant reduction in BGR. The apparent BGR(r) = -0.0 59 unit/day at 30 days and r = -0.004 unit/day at 90 days. The apparent infection rate was different from other two combined above treatments (Fig.2). The yield was increased to 52% over control.

The relative impact of microbes, plant extracts, cake and chemical with respect to *Ralstonia solanacearum* population and yield were presented above clearly indicated that the yield increased maximum to 142.1% and 141.1%, respectively with use of *Trichoderma viride* and Mycorrhyza i.e. *Glomus mosseae* than control. However, karanj cake along with bleaching powder was more effective in reducing the apparent bacterial growth rate at flowering stage, which is the most critical stage of bacterial wilt, in which 123.2%more yield was obtained which was at par with that of microbes than control whereas the plant extract resulted more effective in reducing the *Ralstonia solanacearum* population in soil. Thus microbes, Karanj cake and bleaching powder resulted in logistic reduction in primary inocula which are sustainable in management of primary inocula of *Ralstonia solanacearum* in soil and increased the yield.

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