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Effect of Inducers Against Tobamovirus Infection in Tomato and Bell Pepper

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Abstract: Tomato and bell pepper seeds were treated with salicylic acid (50 mM), neem oil (5%) and *Pseudomonas fluorescens* (slurry). The seedlings were sprayed with salicylic acid (50 mM) and neem oil (5%). The concentration of *Tomato mosaic tobamovirus* (ToMV) and *Tobacco mosaic tobamovirus* (TMV) was assessed based on the number of local lesions on *Nicotiana glutinosa*. The results showed that the seed/seedling treatment with inducers reduced the number of local lesions when compared to untreated ones. Salicylic acid was an effective inducer.

Key words: Tobamoviruses, inducers, salicylic acid, neem oil, *Pseudomonas fluorescens*

INTRODUCTION

Tomato and bell pepper are important vegetable crops grown all over the world. *Tobacco mosaic tobamovirus* (TMV) and *Tomato mosaic tobamovirus* (ToMV) are the important seed borne viruses of pepper and tomato[1]. TMV along with other viruses reduce the yield up to 90% in pepper[2] and ToMV reduces yield upto 25% in tomato plants[3]. Seed-borne ToMV and TMV were transmitted to the seedlings at the rates of 1-13% and 1-10%, respectively[4]. The survey of tomato and bell pepper fields showed 3-95 and 1-90% of ToMV and TMV infection, respectively[5].

Since viral diseases can be devastating, cross protection strategies can be employed to minimize the infection, but due to breakdown of cross protection[6], in recent years induction of resistance is being implicated to manage the plant diseases. Various mechanisms are involved in resistance against plant viruses including cross protection, localization, Local Acquired Resistance (LAR), Systemic Acquired Resistance (SAR), green islands and chemically-induced resistance[7].

Botanical extracts have gained importance in modern days for crop protection against pest and diseases because of their safety and target specificity. Plant extracts/products have also been found effective against a wide range of pathogens[8]. Plant seed oils were mainly used to control viral pathogens[9].Several reports are available on induction of resistance against plant viruses by using chemicals, one among them is salicylic acid which is a natural messenger used to control TMV[10]. Soil or seed applications with Plant Growth Promoting Rhizobacteria (PGPR) have been used to enhance growth of several crops as well as to suppress the growth of plant pathogens[11].

We report here the effect of inducers such as salicylic acid (50 mM), *Pseudomonas fluorescens* and neem oil (5%) on the concentration of tobamoviruses (ToMV and TMV) in tomato and bell pepper.

MATERIALS AND METHODS

Seed treatment

Preparation of inducers: Salicylic Acid (SA) solution (0.05 M) was prepared by dissolving 0.069 g of powder in carrier solution (25% PEG-4000). Neem oil was obtained from Medinova chemicals, Bangalore and 5% (V/V) was prepared by using distilled water.

Pseudomonas fluorescens strain, from the culture collection of Department of Applied Botany, was sub-cultured on King's B medium. After two days of incubation, the bacteria were inoculated to King's B broth. After 48 h of incubation at 26±2°C, the content was centrifuged at 10,000 rpm for 5 min at 4°C. Supernatant was discarded and the pellet was suspended in sterile saline (0.85% of NaCl). Once again saline was centrifuged at 10,000 rpm for 5 min at 4°C. Supernatant was discarded. The process was repeated four times. The spore load in the pellet was adjusted to $1x10^8$ cfu at 610 nm. The suspension was mixed with the talcum powder and slurry was prepared by using distilled water.

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Seed treatment, sowing of seed and transplantation of seedlings: Four replicates of 40 seeds each of tomato Cv. Plant gene and pepper Cv. California wonder were mixed thoroughly with freshly prepared solutions and were kept on the magnetic shaker for 24 h. The seeds were sown in earthen pots containing soil, sand and manure in the ratio of 2:1:1. Fifteen-day-old seedlings were transplanted to small plastic pots and maintained in the screenhouse condition.

Seedling treatment: The seedlings of tomato and pepper were sprayed with salicylic acid (0.05 M) and 5% (V/V) neem oil. For each treatment, four replicates each of 20 seedlings were used.

Challenge inoculation of virus: The seedlings obtained from treated seeds/spray-treated ones were challenge-inoculated with the virus. TMV and ToMV inoculum were obtained from the infected *Nicotiana sylvestris* and tomato plants, respectively. The leaves were homogenized in phosphate buffer (0.1 M, pH 7.2) in a pre-chilled pestle and mortar. After homogenization, the extract was filtered through muslin cloth and the supernatant was used as a source of inoculum. Cotton swab was dipped in virus inoculum and swabbed over carborundum pre-dusted leaves. After 10 min, inoculated leaves were washed with distilled water.

Indexing of virus: Leaves from infected plants were harvested 15 days post-inoculation and extracted using phosphate buffer (0.1 M, pH 7.2) in a pre-chilled pestle and mortar. The carborundum was dusted to leaves of *Nicotiana glutinosa*. Four replicates, each of four leaves were maintained for each treatment.

The number of local lesions formed on inoculated leaf/ 100 cm^2 was calculated by using the formula:

$$\frac{\text{Number of local lesions on inoculated leaf}}{\text{Area of inoculated leaf}} \times 100$$

Statistical analysis: The data generated was average of four independent experiments having three replicates each. Data was subjected to analysis of variance (ANOVA) and the means were compared for significance using Duncan's Multiple Range Test (DMRT, P = 0.05).

RESULTS AND DISCUSSION

Seed treatment: The viruses recovered from tomato and bell pepper seedlings raised from treated seeds produced lesser number of local lesions in *N. glutinosa* when compared to control. The tomato seedlings obtained from SA treated seeds produced only 2.84 local lesions per sq. cm^2 when compared to other treatments. In case of bell pepper seedlings, *P. fluorescens*-treatment reduced the number of local lesions to 2.4 per sq. cm^2 when compared to 4.5 per sq. cm^2 in control (Table 1 and 2).

Table 1: Efficacy of seed treatment with salicylic acid, *Pseudomonas fluorescens* and neem oil on ToMV infection in tomato, indexed on *Nicotiana glutinosa*

Treatments	No. of local lesions ± SE
Control	4.76±0.8[a]
Salicylic acid (0.05 M)	2.84±0.09[d]
Pseudomonas fluorescens	3.21±0.07[b]
Neem oil (5%)	3.09±0.5[c]

Every value represents the mean of four experiments with Standard Error and Values with the different letter are significantly different according to Duncan Multiple Range Test (P=0.05)

Table 2: Efficacy of seed treatment with salicylic acid, *Pseudomonas fluorescens* and neem oil on TMV infection in bell paper, indexed on *Nicotiana glutinosa*

Treatments	No. of local lesions±SE
Control	4.53±0.09[a]
Salicylic acid (0.05 M)	2.86±0.1[c]
Pseudomonas fluorescens	2.4±0.07[d]
Neem oil (5%)	3.14±0.06[b]

Every value represents the mean of four experiments with Standard Error and Values with the different letter are significantly different according to Duncan Multiple Range Test (P=0.05)

Seedling treatment: The concentration of the viruses was less in SA and neem oil treated tomato and bell pepper seedlings when compared to control. The virus particles recovered from tomato seedlings treated with SA produced minimum number of local lesions (1.84 per sq. cm^2) when compared to neem oil and control. In bell pepper seedlings, both SA and neem oil treatment were effective (2.3 per sq. cm^2) (Table 3).

The use of inducers to control pathogen infection is well known. SA as a natural signal molecule plays an important role in plant defence responses against pathogen invasion. SA and its biologically active analogues increased pathogenesis-related protein (PR) gene expression, disease resistance and inhibited catalase and induced accumulation of lipid peroxidation products[12]. SA can induce resistance to a wide range of pathogens. In the present study, SA (50 mM) was used as seed as well as spray treatment. The virus concentration reduced to the lowest level. It has been proved that SA can inhibit TMV replication and cell-to-cell movement[10]. Salicylic acid activates multiple antiviral defense mechanisms against plant viruses. SA stimulates inhibition of all the three stages of virus infection: replication, cell-to-cell movement and long distance movement[10]. However, evidence has recently emerged that SA may stimulate a separate downstream pathway, leading to the induction of an additional mechanism of resistance based on RNA interference[13].

Table 3: Efficacy of seedling treatment with salicylic acid and neem oil on ToMV infection in tomato and TMV in bell pepper, indexed on *Nicotiana glutinosa*

	No. of local lesions±SE	
Treatments	ToMV	TMV
Control	3.85±0.05[a]	3.92±0.03[a]
Salicylic acid (0.05 M)	1.82±0.01[c]	2.31±0.03[b]
Neem oil (5%)	2.33±0.07[b]	2.34±0.07[b]

Every value represents the mean of four experiments with Standard Error and Values with the different letter are significantly different according to Duncan Multiple Range Test (P=0.05)

Plant seed oils have been used to control various plant pathogens[9]. In our experiment, neem oil (5%) was used to reduce the concentration of tobamoviruses infecting tomato and bell pepper. The results showed that, seed/spray treatment with neem oil reduced the virus concentration when compared to control.

Soil or seed applications of PGPR have been used to enhance growth of several crops as well to suppress the growth of plant pathogens[14]. PGPR also elicits Induced Systemic Resistance (ISR) in the treated plant. ISR occurs when the plants defense mechanisms are stimulated and it resists infection by pathogens[10]. Present results showed that *P. fluorescens* seed treatment reduced the concentration of TMV/ToMV when compared to control seedlings.

Seed treatment with SA was found to be effective in reducing tobamovirus concentration followed by *P. fluorescens* and neem oil treatment. SA was also effective as seedling treatment. From the results SA was found to be effective in reducing the concentration of tobamoviruses.

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