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Antiviral Activity of Plant Extracts and other Inducers against Tobamoviruses Infection in Bell Pepper and Tomato Plants

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ABSTRACT

The present study was aimed to investigate the effect of inducers viz., plant extracts, plant seed oils and salicylic acid on tobamoviruses in both indicator and host plant. Tobamoviruses are major hurdles in the production of tomato and bell pepper. Currently, different inducers derived from various origins are being used to reduce the virus concentration. Preliminary screening of the inducers against tobamoviruses was conducted by using the local lesion assay (*Nicotiana glutinosa*). The results showed that all the inducers used for screening were effective in reducing the number of local lesions formed by the challenge inoculation of tobamoviruses. Both spray and seed treatment of inducers against the tobamoviruses reduced the concentration of viruses in the seedlings as evident from the results of Indirect Enzyme linked immunosorbent assay. Among the inducers used for the induction of resistance against tobamoviruses, *Bougainvillea spectabilis* extract was found to be most effective. The inducer-treated seeds also showed enhancement of seed germination and seedling vigor.

Key words: Tobamoviruses, antiviral agents, plant oils, salicylic acid, enzyme linked immuno sorbent assay

INTRODUCTION

Plant viruses are of great concern to farmers, researches and policy makers because of enormous loss they cause in different crops like cereal, vegetable, fruit, legume and cash crops. Chemical control of plant virus diseases is difficult to control. Viral pathogens have become major constraints to the production of tomato and pepper (Waterworth and Hadidi, 1998). Among them seed transmitted tobamoviruses cause considerable damages. *Tobacco mosaic tobamovirus* (TMV) and *Tomato mosaic tobamovirus* (ToMV) are the important viral pathogens of pepper and tomato, respectively. Seed-borne ToMV and TMV were transmitted to the seedlings at the rates of 1-13% and 1-10%, respectively (Chitra *et al*., 1999). The survey of tomato and bell pepper fields showed 3-95 and 1-90% of ToMV and TMV infection, respectively (Chitra *et al*., 2002).

The plants are important sources of antimicrobial compounds. Several efforts have been made to control the plant viruses infecting different plant species. Botanicals are being extensively used in the management of plant viruses. Plant extracts/products have also been found effective against a wide range of pathogens (Manickam and Rajappan, 1999; Srivasata *et al.,* 2010; Chakraborty and Chakraborty, 2010).

Effective viricides are lacking for the control of plant viral diseases. However, in recent years, safer botanical extracts are gaining importance in crop protection against pests and diseases. Higher plants posses endogenous virus inhibitors, of which proteinaceous antiviral substances are of particular interest (Balasubramanyam *et al*., 2000). Very little information is available on the effect of plant extracts and chemicals on plant viruses. Plant seed oils were mainly used to control plant pathogens viz., fungal, bacterial and viral pathogens (Jayashree *et al*., 1999; Mandal and Singh, 2001; Kulkarni and Byadagi, 2004; Madhusudhan *et al*., 2005). Scanty reports are available on induction of resistance against plant viruses by using chemicals (Murphy and Carr, 2002). The aqueous extracts showed different mechanism inhibiting the plant viruses.

In this study, the effect of inducers viz., plant extracts, plant seed oils and SA on tobamoviruses using an indicator plant (*Nicotiana glutinosa*) was examined. The effect of spray and seed treatment by inducers on the viral concentration in tomato and bell pepper plants challenge inoculated with ToMV and TMV, respectively was evaluated. Finally, the effect of inducers on the seed quality parameters of tomato and bell pepper was also clarified.

MATERIALS AND METHODS

The experiments were conducted at Seed Virology Laboratory, Department of Studies in Applied Botany and Biotechnology, University of Mysore, Mysore, India during the period July, 2006 to July, 2007.

Extracts of plants (10% w/v) neem (*Azadirachta indica*), pongamia (*Pongamia glabra*), bougainvillea (*Bougainvillea spectabilis*), plant seed oils, neem oil (5%) and phyllanthus oil (5%) and SA (25 and 50 mM) were used as inducers. The effect of inducers was assessed in terms of virus concentration and the change in defense-related enzymes, peroxidase and catalase in the seedlings obtained from treated seeds and in spray-treated seedlings.

Preparation of inducers for seed and seedling treatment: Mature leaves (100 g) of neem, pongamia and bougainvillea were homogenized in a pre-chilled pestle and mortar using chilled distilled water. The extract was filtered through four layers of moistened muslin cloth. The final volume was adjusted to 100 mL of distilled water. The filtrate was centrifuged at 8,000 rpm for 15 min at 4°C. The supernatant was diluted to 10% (v/v).

Commercially available phyllanthus oil was procured from the local market and neem oil was obtained from Medinova Chemicals, Bangalore from which five percent (v/v) solution was prepared by using chilled distilled water.

The SA solutions (25 and 50 mM) were prepared by dissolving 0.03453 and 0.06906 g in 100 mL of chilled distilled water, respectively. Polyethylene glycol (PEG-4,000) (25% w/v) was added as carrier for seed treatment.

Preparation of standard virus inoculum: The leaves showing mosaic symptoms were harvested from the virus-infected plants. The leaves were homogenized in the phosphate buffer (pH 7.2, 100 mM) in a pre-chilled pestle and mortar. After homogenization, the extract was filtered through muslin cloth and the supernatant was used as the source of inoculum.

Efficacy of inducers on indicator plant (*N. glutinosa*) against tobamoviruses: The inducers were screened for their efficacy against ToMV and TMV on *N. glutinosa*. The inducers were sprayed using a hand sprayer (Misty, Varun Industries) on the primary leaves of two month-

old *N. glutinosa* plants. The control plants were sprayed with distilled water. The treated and control *N. glutinosa* leaves were inoculated with the standard inoculum, 1 h after (pre-inoculation) application of inducers. Observations on development of local lesions were recorded on the third day (72 h) after inoculation. Each treatment having five plants was replicated three times.

The percentage of inhibition of local lesion formation by each treatment over the control was calculated based on the number of local lesions produced using the formula:

$$I = (C - T) \times 100/C$$

Where:
I = Percent inhibition of lesion formation over control
C = No. of local lesions in control
T = No. of local lesions in plants treated with AVP

Seed treatment: The seeds (200) of tomato and pepper were soaked in the leaf extracts, plant oils and SA solutions and kept on the Magnetic Shaker (KS 250, Janke and Kunkel) for 24 h. The treated seeds were sown in earthen pots containing sand, soil and manure in the ratio of 1:2:1, respectively. After 15 days of sowing, the seedlings were transplanted to new pots and maintained in the screen house.

Seedling treatment: The inducers were prepared as described above. Inducers were sprayed onto twenty-day-old tomato and pepper seedlings and after 24 h they were challenge-inoculated with ToMV and TMV, respectively.

Quantification of virus concentration by using Enzyme Linked Immunosorbent Assay (ELISA): The leaves (100 mg) harvested from untreated or treated plants showing viral symptoms were crushed in a mortar and pestle in antigen buffer. The homogenate was centrifuged at 10,000 rpm for 5 min at 4°C. The supernatant extracted from bell pepper and tomato was subjected to indirect-ELISA against anti-TMV and anti-ToMV, respectively (Hobbs *et al.*, 1987).

Effect of inducers on seed germination and seedling vigor of tomato and bell pepper seeds: Four hundred seeds of tomato and bell pepper were placed in conical flasks containing the plant leaf extracts (10% w/v). The flasks were kept on a rotary shaken at room temperature at 100 rpm for 24 h. Flasks with distilled water served as the control. The treated seeds were subjected to germination test using the between paper method (ISTA, 2003). Vigor index was calculated using the formula:

$$\text{Vigor Index} = (\text{Mean Shoot Length} + \text{Mean Root Length}) \times \% \text{ germination}$$

Data analysis: For data analysis the statistical computer application package SPSS 10.0 was employed. The data generated were average of three independent experiments. Data were subjected to analysis of variance (ANOVA) and the means were compared for significance using Duncan's Multiple Range Test (DMRT; p = 0.05) (Duncan, 1955).

RESULTS

Efficacy of inducers on indicator plant (*N. glutinosa*) against tobamoviruses: Treatment with inducers, showed reduced number of local lesions formed per 100 cm^2 after challenge inoculation with tobamoviruses. Particularly, plant extracts showed maximum inhibition (%) in number of local lesions formed on inoculated leaves. *Bougainvillea spectabilis* extract treatment showed 62% and 59% inhibition in number of local lesions formed per 100 cm^2 after challenge inoculation with TMV and ToMV, respectively in comparison with control leaves and other botanicals (Table 1).

Comparison of virus concentration in tomato and bell pepper seedlings treated with different inducers by indirect DAC-ELISA: In general, irrespective of the application methods, treatment with inducers showed very low concentration of viruses in comparison to the control. In tomato seedlings, seed/seedling treatment with inducers the concentration of virus was low when compared to untreated ones. Seed treatment with bougainvillea leaf extract reduced the ToMV concentration (0.114) followed by 25 mM SA (0.165) and neem oil (0.165) when compared to the control. Seedling treatment with bougainvillea leaf extract (0.148) reduced the ToMV concentration as evident from absorbance at 410 nm, followed by 50 mM SA (0.156) (Table 2).

Table 1: Efficacy of inducers on tobamoviruses in *Nicotiana glutinosa*.

	TMV		ToMV	
Inducers used	No. of local lesions/100 cm^2	% inhibition	No. of local lesions/100 cm^2	% inhibition
25 mM SA	88±0.27^{d}	52	105±0.54^{d}	47
50 mM SA	87±0.54^{d}	53	97±0.29^{e}	51
Neem oil	106±0.57^{c}	43	125±0.33^{c}	37
Phyllanthus oil	155±0.2^{b}	16	139±0.27^{b}	29
Bougainvillea leaf extract (10%)	70±0.17^{e}	62	81±0.51^{g}	59
Neem leaf extract (10%)	71±0.57^{e}	61	87±0.28^{f}	55
Pongamia leaf extract (10%)	87±0.25^{c}	53	89±0.37^{f}	54
Control	185±0.33^{a}	-	197±0.45^{a}	-

Every Value represents the mean of three replicates±SE followed by the same letter in a column do not differ significantly according to Duncan's multiple range test at p = 0.05

Table 2: Reduction in virus concentration in inducer treated bell pepper and tomato seedlings challenge inoculated with TMV and ToMV, respectively (indirect ELISA) (Absorbance at 410 nm).

	TMV		ToMV	
Inducers used	Seed	Spray	Seed	Spray
Control	0.318±0.57^{a}	0.417±0.66^{a}	0.478±0.57^{a}	0.416±0.67^{a}
Phyllanthus oil	0.227±0.43^{b}	0.284±0.34^{b}	0.165±0.33^{f}	0.297±0.33^{b}
Neem oil	0.139±0.33^{g}	0.234±0.67^{c}	0.209±0.37^{d}	0.284±0.45^{b}
25 mM SA	0.151±0.67^{e}	0.136±0.33^{e}	0.165±0.67^{f}	0.167±0.56^{e}
50 mM SA	0.146±0.33^{f}	0.186±0.47^{d}	0.189±0.67^{e}	0.156±0.74^{f}
Bougainvillea leaf extract	0.118±0.49^{h}	0.117±0.33^{f}	0.114±0.74^{g}	0.148±0.37^{g}
Neem leaf extract	0.215±0.47^{c}	0.189±0.47^{d}	0.301±0.29^{b}	0.203±0.45^{c}
Pongamia leaf extract	0.172±0.37^{d}	0.106±0.37^{g}	0.274±0.37^{c}	0.177±0.29^{d}

Every Value represents the mean of three replicates±SE followed by the same letter in a column do not differ significantly according to Duncan's multiple range test at p = 0.05

Seed and seedling treatment of bell pepper with inducers reduced the concentration of virus after challenge-inoculation with TMV when compared to control. In seed treatment with inducers, bougainvillea leaf extract reduced the virus concentration to lower level (0.118) followed by neem oil (0.139) treatment. In case of seedling treatment, pongamia leaf extract (0.106) was more effective in reducing the concentration of TMV followed by bougainvillea leaf extract (0.117) (Table 2).

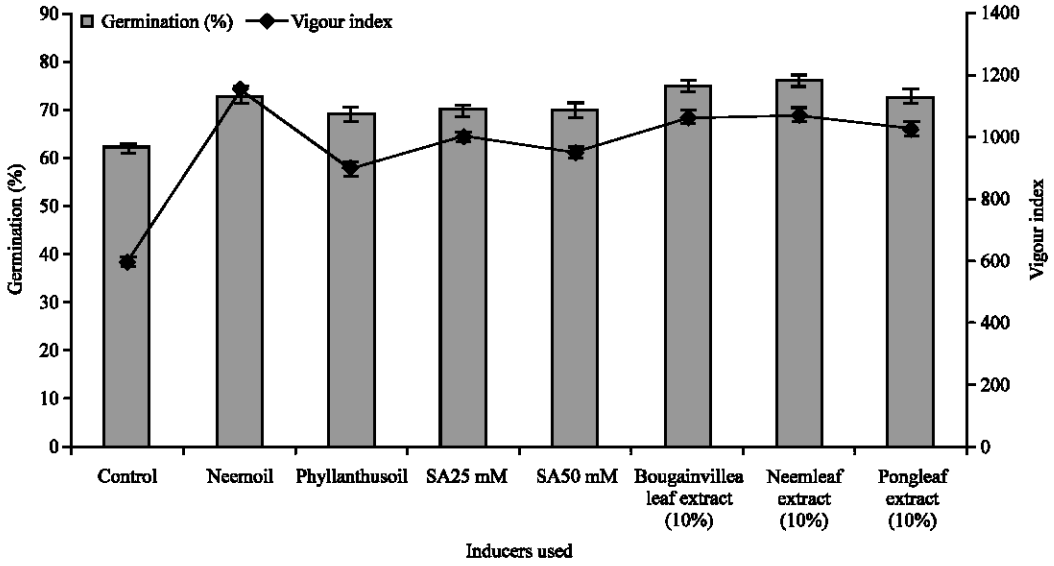

Fig. 1: Effect of seed treatment with inducers on tomato seed germination and seedling vigour 7 days under *in vitro* conditions. Vertical bars represents SE of mean

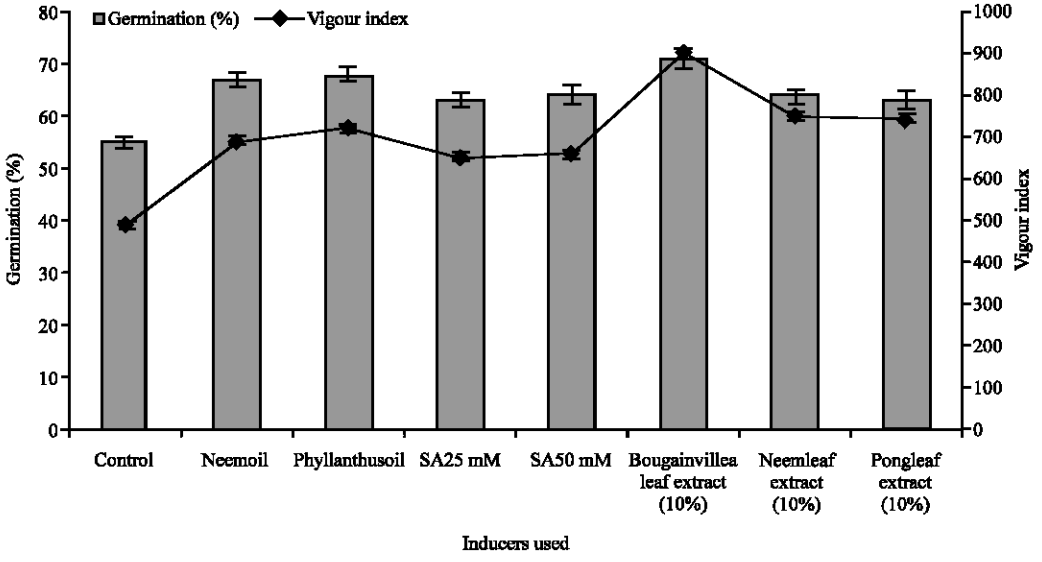

Fig. 2: Effect of seed treatment with inducers on bell pepper seed germination and seedling vigour 7 days under *in vitro* conditions. Vertical bars represents SE of mean

Effect of inducer treatment on seed germination and seedling vigor in tomato and bell pepper seeds: The seeds of tomato and bell pepper treated with inducers had influenced the seed germination and seedling vigor. In tomato seeds treated with inducers, the maximum germination was found in seeds treated with neem leaf extract (79%) followed by bougainvillea and pongamia leaf extract (78%) in comparison with the control (61%) and other treatments. However, seeds treated with neem oil showed maximum increase in seedling vigor (1142). Along with neem oil treated tomato seeds, botanicals treated seeds showed maximum increase in germination and seedling vigor (Fig. 1). In bell pepper seeds treated with inducers, maximum germination (76%) and seedling vigor (879) were noticed in bougainvillea leaf extract treatment in comparison to control and other treatments (Fig. 2).

DISCUSSION

Present results clearly demonstrated the effectiveness of inducers in promoting growth and induction of resistance against tobamoviruses infecting tomato and bell pepper plants. Impressive results were achieved with inducer application for enhancement of seed germination and seedling vigor.

Moreover, various plant extracts were screened for the antiviral activity against tobamoviruses. The results suggested that viral concentration was reduced in treated plants when compared to untreated ones. Spray treatment was more effective than seed treatment in reducing the virus concentration. Along with lower virus concentration, the higher peroxidase activity and lower catalase activity was observed in spray-treated plants. Higher plants posses endogenous virus inhibitors, of which proteinaceous antiviral substances are of particular interest (Balasubramanyam *et al*., 2000). Induction of resistance against virus infection by plant extracts has not been fully understood. Botanical resistance inducers themselves do not act on virus directly. Antiviral resistance by plant extracts could be due to one of the following mechanisms i.e., de novo synthesis of antiviral substances, production of virus inhibiting agents and production of mobile inducing signal that bind to host plant surface, which produces virus inhibiting agents (Verma *et al*., 1998). The plant oils such as, neem and phyllanthus oil (5%) showed lowest catalase and highest peroxidase activity and lower virus concentration in treated when compared to control ones. Similar results were noticed by Madhusudhan *et al*. (2005) in reducing the tobamoviruses by spray treating neem oil (5%). Similarly, Deepthi *et al*. (2007) used different plant extracts and acetone precipitated proteins from six medicinal plants against tobamovirus infection. Ramesh *et al*. (2009) studied the effect of latex of *Euphorbia Tirucalli* Against Tobamoviruses. All the results supported that, plant extracts reduces the concentration of tobamoviruses.

The results obtained by Zida *et al*. (2008), aqueous extracts of two plants enhanced the seedling vigour and health of the sorghum and pearl millet seeds. Similarly, the plant extracts and other inducer treatment enhanced the growth promotion and other seed quality parameters in tomato and bell pepper.

The results of the present experiments revealed that both seed and seedling treatment by inducer induced resistance against tobamoviruses. The defense-related enzyme assay also supports the induced resistance against plant viruses. The ELISA results showed lower concentration of viruses in both tomato and pepper seedlings treated with inducers compared to control seedlings. This research also demonstrated that inducers used in our experiment are highly effective in growth promotion of tomato and bell pepper. Bougainvillea leaf extract had more antiviral activity than other leaf extracts, SA and plant seed oils.

CONCLUSION

The tobamovirus concentration can be reduced by the treatment of inducers. The inducers were effective in reducing concentration of viruses in both indicator and host plants. Both spray and seed treatment of inducers against the tobamoviruses reduced the concentration of viruses in the seedlings as evident from the results of ELISA. *Bougainvillea spectabilis* extract was found to be most effective in reducing virus concentration. The inducer-treated seeds also showed enhancement of seed germination and seedling vigor.

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