Effect of plant extracts and derivatives, butter milk and virus inhibitory chemicals on pumpkin yellow vein mosaic virus transmission

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ABSTRACT: Among the ten plant extracts tested for their efficacy in controlling pumpkin yellow vein mosaic virus, Bougainvillea spectabilis showed maximum inhibition of the virus transmission followed by Boerhaavia diffusa. In the plant derivatives, neem oil and Thuja 30 inhibited the virus effectively. The only animal product tested was buttermilk which was also found to reduce virus transmission effectively. Acetyl salicylic acid recorded the least virus transmission among the virus inhibitory chemicals tested followed by barium chloride.

Key words: Plant extracts, plant derivatives, animal product, virus inhibitory chemical

Yellow vein mosaic is a very common and destructive disease of pumpkin transmitted by the vector, Bemisia tabaci Genn. The occurrence of the disease was first reported by Vasudeva and Lal(1943) round about Delhi. Later the disease was reported by Varma (1955) from New Delhi, Capoor and Ahmad (1975) from Poona, Ghosh and Mukhopadhyay (1979) from West Bengal and Latha and Gopalakrishnan (1993) from Kerala. In the present investigation, ten plant extracts, four each of plant derivatives and virus inhibitory chemicals and one animal product were tested for their efficacy in reducing the transmission of pumpkin yellow vein mosaic virus (PYVMV).

MATERIALS AND METHODS

*In vitro* screening of plant extracts for the presence of antiviral principles

All the leaf extracts from 10 plant species belonging to 7 different families significantly reduced the PYVMV infection in pumpkin plants to varying degrees. Among these, Bougainvillea spectabilis and Boerhaavia diffusa recorded maximum reduction in treatment, the per cent reduction in infection over control was calculated.

*In vitro* screening of plant derivatives and animal product against PYVMV

To evaluate the efficacy of neem products, neem oil (3 per cent aqueous solution) and neem seed kernel extract (5 per cent aqueous solution ) were sprayed on 6 day old pumpkin seedlings. Thuja 30, a homeopathic drug derived from Thuja orientalis was used at 2 per cent concentration. Butter milk which was fermented for 48 h was used at 10 per cent concentration.

*In vitro* screening of virus inhibitory chemicals against PYVMV

The efficacy of various virus inhibitory chemicals was tested by spraying the chemicals, viz., acetyl salicylic acid (200 ppm), ammonium molybdate (500 ppm) and barium chloride (500 ppm) on the primary leaves of 6 day old pumpkin (cv. Co 2) plants which were inoculated with viruliferous whiteflies at the rate of 15 insects per plant 24 h later.

RESULTS AND DISCUSSION

*In vitro* screening of plant extracts for the presence of antiviral principles

All the leaf extracts from 10 plant species belonging to 7 different families significantly reduced the PYVMV infection in pumpkin plants to varying degrees. Among these, Bougainvillea spectabilis and Boerhaavia diffusa recorded maximum reduction in
Table 1. Effect of plant extracts on PYVMV transmission under glasshouse condition

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Common name</th>
<th>Family</th>
<th>Transmission (%)</th>
<th>% reduction over control</th>
<th>Incubation period (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boerhaavia diffusa L.</td>
<td>Hogweed</td>
<td>Nyctaginaceae</td>
<td>8.3 (16.8)*</td>
<td>91.7</td>
<td>12.4</td>
</tr>
<tr>
<td>Bougainvillea spectabilis Wild.</td>
<td>Bougainvillea</td>
<td>Nyctaginaceae</td>
<td>6.7 (15.0)*</td>
<td>93.3</td>
<td>8.1</td>
</tr>
<tr>
<td>Croton bonplandianum Bail.</td>
<td>Croton</td>
<td>Euphorbiaceae</td>
<td>15.0 (22.8)*</td>
<td>85.0</td>
<td>12.1</td>
</tr>
<tr>
<td>Leucaena leucocephala (Lamk.) de Wit.</td>
<td>White popinac, Horse tamarind</td>
<td>Mimosaceae</td>
<td>33.3 (35.3)*</td>
<td>66.7</td>
<td>9.2</td>
</tr>
<tr>
<td>Mirabilis jalapa L.</td>
<td>Four O'clock plant</td>
<td>Nyctaginaceae</td>
<td>31.7 (34.3)*</td>
<td>68.3</td>
<td>9.5</td>
</tr>
<tr>
<td>Ocimum basilicum L.</td>
<td>Sweet basil</td>
<td>Labiatae</td>
<td>26.7 (31.1)*</td>
<td>73.3</td>
<td>10.4</td>
</tr>
<tr>
<td>Prosopis chilensis (Molina) Stuntz.</td>
<td>Algaraba, Mesquite</td>
<td>Mimosaceae</td>
<td>25.0 (30.0)*</td>
<td>75.0</td>
<td>11.1</td>
</tr>
<tr>
<td>Vitex negundo L.</td>
<td>Chinese chaste tree</td>
<td>Verbenaceae</td>
<td>43.3 (41.2)*</td>
<td>56.7</td>
<td>9.7</td>
</tr>
<tr>
<td>Catharanthus roseus (L.) G.Don.</td>
<td>Madagascar Periwinkle</td>
<td>Apocyanaceae</td>
<td>40.0 (39.2)*</td>
<td>60.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Azadirachta indica Juss.</td>
<td>Margosa tree, Neem tree</td>
<td>Meliaceae</td>
<td>21.7 (27.7)*</td>
<td>78.3</td>
<td>9.5A</td>
</tr>
<tr>
<td>Control (Water)</td>
<td>-</td>
<td>-</td>
<td>100.0 (90.0)*</td>
<td>0.0</td>
<td>6.7</td>
</tr>
</tbody>
</table>

* Figures within the parentheses are transformed values

PYVMV infection by 93.3 and 91.7 per cent over control respectively whereas Vitex negundo showed minimum reduction of 56.7 per cent over control (Table 1).

All the plant extracts tested were found to increase the incubation period of the virus when compared to control. Incubation period in the plants treated with the leaf extracts of B. diffusa and C. bonplandianum were 12.4 d and 12.1 d respectively against 6.7 d in control plants. The presence of AVPs in non-host plant species effective against different viruses has been reported by several workers (Okuyama et al., 1978).

AVPs from Basella alba and Bougainvillea spectabilis (Murthy et al., 1981), Mirabilis jalapa (Verma and Kumar, 1980; Murthy, 1982) were found to be inhibitory to TMV. Narayanasamy and Ramiah (1983c) reported that AVPs from coconut, sorghum, finger millet were effective against TSWV. Pre-inoculation sprays of rice plants with leaf extracts of M. jalapa, Cocos nucifera and Sorghum vulgare reduced the transmission of rice tungro virus (RTV) and increased the incubation period of the virus (Srinivasulu and Jeyarajan, 1986).

The AVPs may act on the host in different ways to reduce virus infection. The AVPs might act as competitive inhibitor to virus infection by occupying the infective sites which become unavailable for virus particles to initiate infection (Van Kammen et al., 1901; Zaitlin and Siege 1963). Narayanasamy (1984) and Verma and Khan (1985) viewed that the AVP might induce the resistance by inactivating the host defense system.

All the plants have their own defense mechanisms against viruses. Treatment of plants with particular agent could accelerate the process involved in the production of protective substances associated with the defense mechanism of plants (Verma, 1982). Also the plant extracts contain certain virus inhibitory substances such as proteins, glycoproteins, flavones, phenols, polysaccharides and glycoalkaloids which, when applied on to other plant species make them resistant to viruses either by inhibiting infection or by interfering with replication of viruses. (Verma, 1985; Narayanasamy, 1990).

In vitro screening of plant derivatives and butter milk against PYVMV

From the results shown in Table 2, it is evident that all these plant products and butter milk reduced PYVMV infection significantly over control. Neem oil was more effective than neem seed kernel extract as evidenced by 78.3 and 66.7 per cent reduction over control respectively. The effectiveness of neem products in reducing PYVMV infection can be explained in terms of their direct interference with the vector behaviour rather than their interaction with the viral
Table 2. Effect of plant derivatives and butter milk on PYVMV transmission under glasshouse condition

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Transmission (%)</th>
<th>% reduction over control</th>
<th>Incubation period (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neem oil (3.0)</td>
<td>21.7 (27.7)**</td>
<td>78.3</td>
<td>14.3</td>
</tr>
<tr>
<td>Neem seed (5.0) kernel extract</td>
<td>33.3 (35.3)b</td>
<td>66.7</td>
<td>9.2</td>
</tr>
<tr>
<td>Tea waste (2.0) extract</td>
<td>23.3 (29.0)*</td>
<td>76.7</td>
<td>11.1</td>
</tr>
<tr>
<td>Thuja 30 (2.0)</td>
<td>21.7 (27.7)*</td>
<td>78.3</td>
<td>13.2</td>
</tr>
<tr>
<td>Butter milk (10.0) (48h fermented)</td>
<td>20.0 (26.6)*</td>
<td>80.0</td>
<td>13.0</td>
</tr>
<tr>
<td>Control (Water)</td>
<td>100.0 (90.0)§</td>
<td>0.0</td>
<td>6.7</td>
</tr>
</tbody>
</table>

*Figures within the parentheses are transformed values.

Table 3. Effect of virus inhibitory chemicals on PYVMV transmission under glasshouse condition

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Transmission (%)</th>
<th>% reduction over control</th>
<th>Incubation period (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetyl salicylic acid</td>
<td>6.7 (15.0)**</td>
<td>93.3</td>
<td>13.3</td>
</tr>
<tr>
<td>Salicylic acid (0.02)</td>
<td>16.7 (24.1)</td>
<td>83.3</td>
<td>11.4</td>
</tr>
<tr>
<td>Ammonium molybdate (0.05)</td>
<td>23.3 (28.9)*</td>
<td>76.7</td>
<td>0.0</td>
</tr>
<tr>
<td>Barium chloride (0.05)</td>
<td>10.0 (18.4)b</td>
<td>90.0</td>
<td>8.2</td>
</tr>
<tr>
<td>Control (Water)</td>
<td>100.0 (90.0)§</td>
<td>0.0</td>
<td>6.7</td>
</tr>
</tbody>
</table>

*Figures within the parentheses are transformed values.

It is well-known that neem derivatives possess repellent, antifeedant and insecticidal properties against vectors and thus, indirectly interfere with the virus infection (Mariappan and Saxena, 1983). Neem oil was highly effective in reducing the survival of leafhoppers, *Nephotettix virescens* (Distant) and transmission of RTV (Mariappan and Saxena, 1983) and also the incidence of rice ragged stunt virus disease transmitted by the brown plant hopper, *Nilaparvata lugens* (Stal) (Saxena et al., 1981). Thus, antifeedant and repellent action of neem derivatives that retard or disrupt the feeding activities of whitefly vector by rendering the treated plants unattractive or unpalatable, offers a novel approach for the management of PYVMV disease.

Tea waste extract, Thuja 30 and butter milk also reduced the virus infection by 76.7, 78.3 and 80.00 per cent over control respectively and were on par statistically. The effect of milk in reducing viral infection was reported by many workers (Anzalone, 1962; Hughes, 1967). Singh *et al.* (1985) reported the effect of goat milk, buffalo milk and cow’s milk in suppressing urdbean mosaic virus *in vitro*.

All these products significantly increased the incubation period of the virus. Spraying with neem oil increased the incubation period of the virus to maximum extent (14.3 d) followed by Thuja 30 (13.2 d) and butter milk (13 d) as against 6.7 d in control plants.

**In vitro screening of virus inhibitory chemicals against PYVMV infection**

Among the chemicals, acetyl salicylic acid (0.02 per cent) was found to be highly effective which caused maximum reduction in PYVMV infection over control (93.3 per cent) and the least reduction in infection was recorded in the case of ammonium molybdate (0.05 per cent) which registered 76.7 per cent reduction over control (Table 3). In general, the chemicals were more effective in reducing the per cent infection under glasshouse condition. Acetyl salicylic acid was also effective in increasing the incubation period of the virus (13.3 d) followed by salicylic acid (11.4 d) as against 6.7 d in control plants.

The inhibition of virus infection by chemicals has been reported by many workers (Dumas and Gianinazzi, 1984; Hooft Van Huijsduijnen *et al.*, 1986). Treatment of plants with chemicals as well as pathogen attack induce a variety of defense mechanisms including the production of new proteins. White (1979) observed the formation of several new proteins after injecting acetyl salicylic acid and benzoic acid into tobacco leaves before inoculating TMV. Hansen (1988) observed that several chemicals induced systemic acquired resistance. Concomittant induction of resistance and the synthesis of PR- proteins have led to speculation about the role of these proteins in inducing resistance to the pathogen (Fritg *et al.*, 1989; Van Loon, 1989; Bol *et al.*, 1990). The fact that salicylic acid treatment also induces virus resistance suggests a function of these proteins in inducing resistance against the virus.

The results obtained in the present investigation have indicated the potential of various products for the management of this whitefly-borne disease in pumpkin. Further work to identify the active component involved, its purification and its formulation and its effect on the vector *B. tabaci* will offer a practical method for the management of this virus disease.
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REFERENCES


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