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Management of Cucumber Mosaic Virus in Chilli

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ABSTRACT

Salicylic acid (SA) showed better performance in glasshouse studies as an SAR inducer against the CMV in chilli. Integrated disease management (IDM) experiment conducted in field, indicated that Seed treatment with Trisodium orthophosphate (TSOP) @ 150 g kg^{-1} followed by seed treatment with imidacloprid @ 8 g/kg + seedling dip in imidacloprid @ 1 ml/l at transplantation + spray application of imidacloprid 0.5 ml/l at 60, 75, 90 and 105 DAT was the best with low aphid population (3.20 aphids 10 p^{-1}), less disease incidence (28.51 %), increased yield attributes (16.10 branches plant $^{-1}$ and plant height of 111.20 cm) and maximum green chilli yield (9,403.9 kg ha^{-1}).

Key words : Chilli, Cucumber mosaic virus, Imidacloprid.

Chilli (*Capsicum annuum* L.) is one of the important cash crops among the spices and it is mainly used for culinary purpose adding flavour, colour, vitamins and pungency. India is the largest exporter of chilli occupying an area of 8.16-9.08 lakh hectares with an annual production of 6.18-9.70 lakh tonnes of dry chilli. Among the states, Andhra Pradesh has been leading both in area and production contributing 25 per cent of the total area with over 40-50 per cent total production followed by Karnataka, Maharashtra and Orissa.

In Southern Telangana Zone (STZ), the crop is cultivated in 2.97 lakh ha. with an annual production of 7.68 lakh tonnes (Statistical abstracts of Andhra Pradesh, 2005). In Southern Telangana zone the crop is cultivated in the districts of Nalgonda (8.72), Medak (7.45), Ranga Reddy (2.75) and Mahaboobnagar (1.12 thousand ha) during *kharif* for dry chilli, while in other seasons for green chilli, based on their utility. In Andhra Pradesh, natural occurrence of CMV was reported around Hyderabad and Warangal by Anjaneyulu and Appa Rao (1967), Vijayabhanu (1991) to an extent of 24-28 per cent.

MATERIAL AND METHODS

To determine the effect of different SAR inducers on mosaic disease in chilli crop, studies were carried out in glass house and main field during 2005. A pot culture study was conducted for selecting the best SAR inducer by using Completely Randomized Design (CRD), with five treatments (three concentrations each) replicated thrice. Control without any treatment but inoculated with CMV was also maintained. Seeds of chilli cv. Sindhur were sown in earthen pots and 10 plants per pot were maintained. Plants were sap inoculated with CMV at 3-4 leaf stage, 24 hrs prior, immediately after spraying and 24 hrs after spray application of the chemicals (SAR inducers)/extracts.

Treatments	Dosages
Benzothiadiazole (Bion)	5.0, 10.0 & 15.0 mM
Salicylic acid (SA)	5.0, 10.0 & 15.0 mM
Potassium Dihydrozen ortho phosphate	50.0, 75.0 & 100.0 mM'
Compost extract	4.0, 6.0 & 8.0%
Commercial formulation of Lantana extract (OVIS)	0.1, 0.2 & 0.3%
Control (inoculated with CMV alone)	

Observations pertaining to

1. Nature of symptom(s) upto 60 days at weekly intervals.
2. Days taken for symptom expression
3. Per cent disease incidence (PDI) using the formula.

$$\text{Per cent disease incidence (PDI)} = \frac{\text{No. of plants showing mosaic symptoms / pot}}{\text{Total No. of plants / pot}} \times 100$$

Per cent disease inhibition over control was calculated by using the formula given by Vincent (1927).

$$I = \frac{C - T}{C} \times 100$$

Where, I = per cent inhibition
C = Per cent disease in control
T = Per cent disease in treatment

The best chemical (SAR inducer)/extract which showed less mosaic disease incidence was further tested in the field for integrated disease management (IDM).

Integrated management of the disease (IDM)

The experiment was conducted in Randomized Block Design (RBD) using susceptible cv. Sindhur with 5 treatments including control and replicated 4 times. A plot size of 4.8 x 3.6 m with a spacing of 60 x 60 cm was adopted. The treatment details are as follows:

T_1: Seed treatment with Trisodium orthophosphate (TSOP) @ 150g/kg followed by seed treatment with imidacloprid @ 8 g/kg + seedling dip in imidacloprid @ 1 ml/l at transplantation + spray application of imidacloprid 0.5 ml/l at 60, 75, 90 and 105 DAT.

T_2: Seedling dip in imidacloprid @ 1ml/l at transplantation + spray of imidacloprid @ 0.5 ml/l at 60, 75, 90 & 105 DAT.

T_3: Seed treatment with TSOP @ 150g/kg + nursery protection with single spray of the best SAR inducer + seedling dip in the SAR inducer at transplantation + spraying 4 times at 60, 75, 90 & 105 DAT.

T_4: Seed treatment with TSOP @ 150 g/kg + application of Neem cake @ 150 kg/ha in nursery + Spray of Neem seed kernal extract (NSKE) 15 DAS in nursery @ 5% and application of neem cake @ 225 kg / ha in 3 splits + spray of NSKE @ 5% 60, 75, 90 and 105 DAT.

T_5: Control (No plant protection, only water spray).

In each treatment, observations on population of aphids, incidence of mosaic, yield attributing characters *viz.,* plant height, number of branches per plant, and green chilli yield per plot were recorded at 3rd, 9th and 15th days after spraying for the sprays given at 60, 75, 90 and 105 DAT and was statistically analyzed. The performance of each treatment was assessed by recording number of aphids (Two each from top, middle and bottom leaves) per plant on ten randomly selected plants. Mosaic disease incidence was recorded on total plant stand in all the treatments. Per cent disease incidence was calculated by using the formula given for pot experiment.

Observations were recorded at the time of harvest from 10 randomly selected plants, replication wise, in each treatment. Height(in cm) of the ten randomly selected plants was measured in each treatment from soil level upto the base of the last terminal in each treatment. Total number of branches per plant was recorded on ten randomly selected plants in each treatment, replication wise. Green chilli yield per treatment was expressed in kg/ha replication wise. Yield data of three pickings at 75, 105 and 135 DAT was pooled and expressed. The trial was terminated at 135 DAT, after harvest of green chillies.

RESULTS AND DISCUSSION

In the present investigation, all the SAR inducers at all the concentrations tried have significantly increased the incubation period of the virus in plants. Among them, salicylic acid was significantly superior with maximum mean incubation period and reduced disease incidence at all concentrations compared to control. There were no earlier reports with regard to use of SA as SAR inducer against CMV in chilli. Bion treated plants showed significant increase in the mean incubation period with all concentrations and reduced the symptom severity also, thereby inhibiting the disease (Table 1).

It is suggestive that salicylic acid analogue-bion, delayed the symptom development. It is due to inhibition of virus multiplication resulted by the activation of host defense mechanism through the peroxidase activity which means the systemic acquired resistance as reported by Anfoka (2000), was operative here who used BTH against CMV – yellow strain on tomato 7 days before sap inoculation, with reduced disease incidence over control. It was reported that exogenous application of salicylic acid analogue BTH, activates the plant defense through callose, peroxidase and PR proteins, phenyle alanine, ammonia lyase (PAL), phenolics and lignins against a wide spectrum of pathogens.(Table 2)

Foliar application of monopotassium phosphate (KH_2PO_4) at different concentrations has increased the mean incubation period ranging from 27.9 to 35.5 days after inoculation. The symptoms expressed were milder in comparison to control. Foliar application of potassium fertilizers enhances the systemic protection from spectrum of diseases. In addition to the disease suppression through stress and tolerance, it offers good agronomic traits. Potassium can strengthen the plant system with a cumulative effect on cell wall, stalks, stems and

Table 1. Effect of SAR inducers on CMV in chilli under glass house conditions.

Treatment / Compound	Concentration	Incubation period *		Symptoms**
		Range	Mean#	
Salicylic acid (SA)	15 mM	40-50	45.5	Vein clearing, chlorosis, mosaic and mosaic mottling
	10 mM	35-45	40.5	
	5 mM	30-35	32.9	
Benzothiadiazole (Bion)	15 mM	35-45	40.5	Vein clearing, chlorosis, mosaic and mosaic mottling
	10 mM	25-35	30.5	
	5 mM	25-30	28.0	
Mono potassium phosphate ($KH_2.PO_4$)	100 mM	30-40	35.5	Vein clearing, chlorosis, mosaic and mosaic mottling
	75 mM	25-35	30.5	
	50 mM	25-30	27.9	
Compost extract	8%	20-25	24.0	Vein clearing, chlorosis mosaic, mosaic mottling, irregular leaf lamina, fili- form leaf and rat tailing
	6%	20-25	23.0	
	4%	15-25	20.0	
Lantana extract (Ovis)	0.30%	20-25	23.0	Vein clearing, chlorosis, mosaic, mosaic mottling, irregular leaf lamina, fili- form leaf and rat tailing
	0.20%	20-25	22.6	
	0.10%	15-25	20.5	
Control	Only water spray	12-15	13.5	Vein clearing, chlorosis mosaic, mosaic mottling, irregular leaf lamina, fili- form leaf and rat tailing
C.D. (P = 0.05)			2.22	
S.Em ±			1.09	

***** Days taken for symptom appearance
** Symptoms observed upto 90 days after sap inoculation
Mean of 10 plants

Table 2. Effect of SAR inducers on PDI and per cent reduction of CMV under glass house conditions.

Treatment / Compound	Concentration	Per cent disease incidence	Per cent disease reduction over control
Salicylic acid (SA)	15 mM	8.89 (17.12)*	87.00 (68.90)
	10 mM	11.11 (15.35)	83.87 (66.34)
	05 mM	17.78 (24.85)	74.19 (59.47)
Benzothiadiazole (Bion)	15 mM	20.67 (31.09)	69.99 (56.78)
	10 mM	31.11 (33.87)	54.84 (47.77)
	05 mM	33.33 (35.26)	51.61 (45.92)
Mono potassium phosphate ($KH_2.PO_4$)	100 mM	24.45 (29.85)	64.50 (53.43)
	75 mM	26.67 (30.97)	61.28 (51.52)
	50 mM	33.33 (35.26)	51.61 (45.92)
Compost extract	0.30%	55.56 (48.21)	19.34 (26.31)
	0.20%	55.56 (48.21)	19.34 (26.31)
	0.10%	55.55 (48.20)	19.36 (26.33)
Lantana extract (Ovis)	8.0 %	60.00 (50.81	12.90 (21.01)
	6.0 %	60.00 (50.81)	12.90 (21.01)
	4.0 %	60.00 (50.91)	12.90 (21.01)
Control	Only water spray	68.89 (56.13)	0.00 (0.00)
C.D. (P = 0.05)		9.34	2.19
S.Em ±		3.24	1.07

* Figures in parentheses are angular transformed values

roots. It also reduces total sugars and nitrogen accumulation, hence it makes plant resistant to diseases caused by spectrum of organisms (Gottestein and Joseph, 1989).

In the present study commercial formulation of Lantana extract (Ovis) spray offered reduction of disease over control and increased the incubation period which ranged from 20.5 to 23.0 days which was 13.5 days in control. However, disease incidence was on par with similar to that of untreated control. (Table 2)

Antiviral agents from plants, act locally and are mostly proteins, though carbohydrates, polysaccharides, tannins and alkaloids are also present in few plants. The active products present in these extracts have no direct effect on viruses. Their antiviral activity is mediated by host cells in which they induce the antiviral state. The active products in plant extracts inducing resistance are mostly small molecular weight proteins which sometimes may be glycosylated. They exert their antiviral effect by rendering host cells incapable of supporting viral replication. In the present experimentation also similar mechanism might have operated and hence reduction in disease and increase in incubation period was observed.

Compost extract treated plants showed slight increase in incubation period after sap inoculation. However, plants exhibited severe symptoms which were similar to the untreated ones. These observations suggested that compost extracts have the ability to induce systemic resistance in treated plants. In this regard there were no previous reports on compost extract mediated viral disease management via systemic acquired resistance. However, the reasons for disease resistance might be SAR and ISR as it was proved in other host pathogen combinations.

Integrated Disease Management

Aphids play a major role in transmission of mosaic viruses. At present, effective chemicals are not available to control mosaic disease or its vector. Continuous use of chemicals lead to residual toxicity and human health problems, increase cost of cultivation due to increase in input costs. Hence, in the present study, attempts were made to manage the disease by using select insecticide, botanicals and SAR inducers, under field conditions.

In the present experimentation, four rounds of imidacloprid spray scheduled in T_1 (seed treatment with TSOP @ 150 g/kg + seed treatment

Table 3. Cumulative effect of insecticide and salicylic acid on aphid count, disease incidence, yield attributing characters and yield of chilli cv Sindhur during *kharif* 2005.

	Observations after fourth spray						
	Plant height (cm)	Number of branches	Aphid* count	Percent disease incidence	Green chilli yield # (kg ha^{-1})	Cocci-nellid mean	Syr-phids mean
T_1	111.20	16.10	2.20 (1.90)**	28.51 (32.27)	9,403.90	0.80 (1.22)	0.32 (1.01)
T_2	108.50	15.75	4.51 (2.31)	37.34 (37.67)	7,523.14	1.04 (1.24)	0.30 (1.68)
T_3	81.21	17.61	10.62 (3.30)	47.37 (43.49)	1,446.75	2.35 (1.68)	3.23 (1.91)
T_4	86.50	12.10	7.60 (2.60)	48.75 (44.28)	1,994.98	2.45 (1.71)	1.64 (1.51)
T_5(Control)	65.15	10.12	16.7 (3.90)	58.75 (50.04)	1,109.18	2.71 (1.79)	4.98 (2.88)
Mean	90.51	13.73	8.19 (2.85)	44.14 (41.55)	4295.58	1.87 (1.51)	2.0 (1.35)
CD (P=0.05)	5.55	0.51	0.29	1.9	111.01	0.96	0.84
SEm ±	1.75	0.13	0.09	0.6	36.02	0.04	0.03

* Mean of four rounds of spray

Mean of three pickings, **Figures in parentheses are angular transformed values

with imidacloprid @ 8 g kg^{-1} + seedling dip with imidacloprid @ 1 ml/l + spray application of imidacloprid @ 0.5 ml/l in main field at 60, 75, 90 and 105 DAT) recorded minimum aphid population (2.20 aphids 10 p^{-1}) and also minimum disease incidence (28.51 %), better yield attributes (plant height 111.20 cm and 16.10 branches) with maximum green chilli yield of 9,403.90 kg ha^{-1}.

Seed treatment with tri-sodium orthophosphate (TSOP) in combination with other seed / root dip treatments consisting imidacloprid had an additive effect in reducing the mosaic incidence and increasing the yield. This could be attributed to the fact that seed treatment with tri-sodium orthophosphate might have eliminated external seed contaminants such as TMV, which is an important virus in chilli. This chemical was found effective in reducing the disease incidence and enhancing plant height, number of branches and green chillies yield indicating phyto-tonic effect on treated plants (Jagadeeshwar, 2004).

Imidacloprid shows quick knock down effect on aphids, due to interference with transmission of impulses to the nervous system of insects, by altering the naturally occurring signal transmitting acetylcholine. Further, it acts by inactivating certain nerve cells through masking receptor protein (Mastsuo *et al*., 1998). The chemical acts on the nicotinic acetylcholine receptor, causing the insect to reduce or stop feeding and reduces mobility. Other mode of action of the chemical was non flight movement of aphid and thus checking the virus spread also (Gourmet *et al*., 1994).

The observations from overall efficacy of neem products consisted in T_4 (seed treatment with TSOP @ 150 g kg^{-1} + soil application of neem cake @ 150 kg ha^{-1} + spray of neem seed kernel extract @ 5 per cent at 15 days after sowing followed by application of neem cake @ 225 kg ha^{-1} in main field 3 splits + spray application of NSKE @ 5 per cent at 60, 75, 90 and 105 DAT) recorded low aphid population of 7.60 aphids 10 p^{-1} thereby, reducing mosaic incidence (48.75 per cent), increase in plant height (86.50 cm), number of branches (14.10) and yield (1,994.98 kg ha^{-1}) compared to untreated ones.

Neem based treatments were superior to salicylic acid treated plots and are also ecofriendly to predatory population of coccinellids and syrphids. However, NSKE was inferior to imidacloprid with respect to the aphid control, reduction of mosaic disease, yield and yield attributes of chilli. It is suggestive that, neem products possess insecticidal properties and they are relatively safe bio-pesticides to an array of beneficial organisms (Men *et al*., 2002)

In the present study, the results obtained from plants treated with SA in glasshouse and plots applied with SA in field studies were contrary. In *vitro* study, plants treated with salicyclic acid @ 15 mM concentration 24 hr prior, simultaneous and 24 hr after sap inoculation offered better protection over control. However, when the same was tried as a component of integrated disease management in the main field, the results obtained were not superior compared to insecticide treated plots, inspite of recording more predator population (T_1 and T_2).

In the present study, T_3 (seed treatment with TSOP @ 150 g kg^{-1} + nursery protection with single spray application of salicylic acid 15 mM as nursery protection + application of salicylic acid 15 mM in main field at 60, 75, 90 and 105 DAT) recorded comparatively high aphid population, diseases incidence and low yield attributing characters and yield, suggesting that the exogenous application of salicylic acid was inferior to imidacloprid and neem products based management. Here it is interesting to note that exogenous application of salicylic acid @ 15 mM concentration though did not influence mosaic disease incidence directly, but recorded more predator population than in control and other treatments. This indicates that aphid number was more in the treatment as revealed by increase in predator population (Table 3).

Conclusion

SA does not translocate efficiently throughout the plants when applied exogenously. Moreover, SA deposited upon a leaf surface, is rapidly broken down, resulting in only a short term response. Finally, only a narrow safety margin separates the rate at which the compound is effective. Because of these limitations SA has not been considered a practical solution to disease control based on the present experience. A number of reports are available claiming induction of resistance by use of chemicals or plant extracts (Enyedi and Raskin, 1993) indicated that Although these compounds have failed to fulfil the criteria established for SAR activators, they still could be considered as part of an integrated disease management (Percival, 2001). In addition to this, the physiological relations between SAR and anti-herbivore defense might be the reason for the failure of defense against mosaic disease through exogenous application of salicylic acid in the present study (Martin Heil, 1999).

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