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DISCOVERY

Efficacy testing of eco-friendly and commercial antiviral products for the management of Zucchini Yellow Mosaic Virus

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ABSTRACT

An experiment was conducted to test the efficacy of eco-friendly and commercial antiviral products for the management of Zucchini yellow mosaic virus of zucchini squash at Plant Pathology Division Khumaltar, NARC. The experiment was conducted using a randomized complete block design with eight treatments namely Garlic clove extracts@ 50ml/lit, Victovirus@3ml/lit, Bougainvillea leaf extracts@200ml//lit, Viricon-H@3ml/lit, Cow–urine@200ml/lit, Anti-Vs@1gm/lit, Control and Yoghurt extracts@25ml/lit, each

replicated for three times. Seedlings were transplanted with 100cm x 75cm spacing in 2.25m2 plots, accommodating 4 plants in each plot. The plots were inoculated with ZYMV before the application of treatments. The first spray of treatments was done after 15-20 days after transplanting followed by second, third and fourth and fifth sprayings at 7 days intervals. In control plots, water sprayed with equal volume instead of plant extracts. Observations were recorded weekly on first appearance of disease, disease incidence, disease severity and yield of fruit. Symptoms were recorded at regular interval in all the treatments on the basis of visual observations. The scale used for scoring diseases was 1-5. During 2016, highest severity was found in Control plot (55%) and least in cow urine treated plot (27%). In 2017, highest severity was found in Control plot (65%) and least in yoghurt treated plot (30%). In both the year cow urine 200ml/litre was found most effective to minimize the zucchini yellow mosaic virus and increased the yield. The highest percent disease control was found in cow urine treated plot in both years.

Keywords: Antiviral products, Eco-friendly, Severity, Zucchini, ZYMV

1. INTRODUCTION

Zucchini squash is a summer squash, a member of a cucurbitaceous family, grown for its tender fruits used as a vegetable. Mainly, zucchini squash is planted through direct seeding or transplanting seedlings and matures within 40-65 days. It has both male and female flowers on the same plant and therefore requires a pollination agent for fertilization to take place [1].

One of the most economically important viruses on cucurbit crops worldwide is zucchini yellow mosaic virus causing severe mosaic, necrosis and malformation. *Zucchini yellow mosaic virus* (ZYMV) has emerged as one of the most economically important virus infecting cucurbits in many of the important cucurbit-growing regions of the world and is considered to be an example of an emerging plant virus [2]. The virus was first identified on squash in northern Italy [3] and has spread to major cucurbit-producing regions around the world [4]. ZYMV has also been reported to infect 52 species in 27 genera of 11 families [5].

Zucchini yellow mosaic virus is transmitted by aphids in non persistent manner. It is also transmitted by mechanical inoculation methods but not seed transmitted [6]. Frequently it occurs in combination with other viruses like Watermelon mosaic virus (WMV 1 & 2), Cucumber mosaic virus on cucurbits [7]. ZYMV belongs to the genus potyvirus and family potyviridae, a family of viruses characterized by a mono or bipartite, positive sense, single-stranded RNA genome encapsidated in flexeous, filamentous particles. Zucchini yellow mosaic virus is considered one of the serious threats to cucurbit production worldwide, because of its virulence and aggressiveness. Serologically, ZYMV is related to watermelon mosaic virus-2 and causes symptoms resembling those caused by papaya rings potvirus.

Due to increased awareness about the risks involved in use of pesticides, much attention is being focused on the alternative methods of pathogen control. The continuous use of chemicals causes residual effects and develops resistant races to the chemicals. So now it is necessary to pay attention for the methods which are ecologically, friendly, safe and specific for pathogens. Most of the chemical have posed a serious threat to human health and some of them already been proved to be either mutagenic or carcinogenic. Keeping in view the drawback of chemical management of plant diseases, the use of products of plant and animal origin in the management of plant diseases is gaining importance. So in the present investigation, different products of plant and animal origin like bougainvillea leaf , garlic cloves , cow urine, yoghurt extract and commercial antiviral products like viricon-h and anti-vs were tested to manage Zucchini yellow mosaic virus of Zucchini.

2. MATERIALS AND METHODS

The study was conducted for two consecutive year during 2016 and 2017at Plant Pathology Division Khumaltar, NARC. Experiment layout was randomized block design with seven treatments and three replications. Around 30 days old seedlings were transplanted with 100cm x 75cm spacing in 2.25m2 plot, accommodating 4 plants in each plot. Fertilizer was applied in18 ton/ha compost, and DAP, potash and urea@of 1.7, 0.8, 0.8 ton/ ha respectively. The plots were inoculated with ZYMV before the application of treatments. Virus inoculum was prepared in 0.02 M phosphate-buffer by grinding virus infected leaves samples (1:5 w/v) in pestle and mortar. At two leaf stage, carborundum powder was dusted on young seedlings and inoculum was applied with the help of cotton swab on the leaves. All inoculated leaves were washed with water. There were eight treatments including control, such as Garlic clove extracts@50ml/lit, Victo-virus@3ml/lit, Bougainvillea leaf extracts@200ml//lit, Viricon-H@3ml/lit, Cow –urine@200ml/lit, Anti-Vs@ 1gm/lit, Yoghurt extracts@25ml/lit and Control. The first spray of treatments was done after 15-20 days after transplanting followed by second, third and fourth and fifth sprayings at 7 days intervals. In control plots, water sprayed with equal volume. Observations were recorded on first appearance of disease, disease incidence, disease severity and yield of fruit.

Observations were recorded at a week intervals for the first appearance of disease symptoms in each plot separately. Symptoms were recorded at regular interval in all the treatments on the basis of visual observations. Field diagnoses based upon visual symptoms were confirmed by taking leaf samples from up to 1 symptomatic and 1 asymptotic plant per plot and leaves were tested by DAS-ELISA for the presence of ZYMV using a commercial kit (Agdia, Co.) and ZYMV-specific immunostrips (Agdia, USA). ZYMV was detected only in symptomatic plants. The scale given below was used for scoring disease.

1=No symptom
2=mild symptoms
3=moderate symptoms
4=severe symptoms
5= very severe symptoms
(Source: AVRDC described for Mung bean Yellow Mosaic Virus)

Disease severity can be calculated by using following formula given by [8]

$$\text{Disease severity (\%)} = \frac{\text{Sum of all numerical ratings}}{\text{Highest rate} \times \text{Number of plants}} \times 100$$

Percent disease control was calculated on the basis of the formula developed by [9].
Percent disease control =(C-T/C)*100 where,
C= Disease in control plot
T= Disease in treated plot

Table 1 The meteorological data recorded at the experimental area in 2016 and 2017.

Trial	Month	Mean temperature(℃)		RH%	Rainfall(mm)
		Max. Temp.	Min. Temp.		
Trial: 2016	March	25.0	9.7	66.4	3.3
	April	29.7	13.6	57.4	5.3
	May	27.6	16.3	71.8	11.1
	June	24.2	19.8	68.5	4.9
Trial: 2017	March	22.4	9.0	74.0	5.2
	April	26.9	13.1	87.6	7.9
	May	27.6	16.3	73.1	9.3
	June	29.3	20.1	80.4	12

Source: AGD, 2016/2017. Annual Report 2072/73-2073/74. Agronomy Division, NARC, Khumaltar, Lalitpur, Nepal.

Statistical analysis

Statistical Tool for Agricultural Research (STAR) version 2.01 was used for the analysis of variance (ANOVA) to test the significance of treatment effect on severity of Zucchini yellow mosaic virus. Least significant difference (LSD) test was used to compare the values of significant treatment means at 1% and 5% level of significance.

3. RESULTS

Environmental observation

During the research period, total rainfall was 24.61mm during 2016 and 34.4mm during 2017. The numbers of rainy days were 45 during 2016 and 50 during 2017 (Table 1). There was high rainfall 11mm in month of May during 2016 and 12mm in month of June during 2017. Similarly high relative humidity was found during 2017 but in case of temperature, high minimum and maximum temperature was found during 2016. The present study revealed that higher the temperature lower was the disease severity during 2016 whereas lower the temperature, higher was the disease severity during 2017. Likewise, higher the relative humidity higher was the disease severity during 2017 whereas lower the relative humidity lower was the disease severity during 2016.

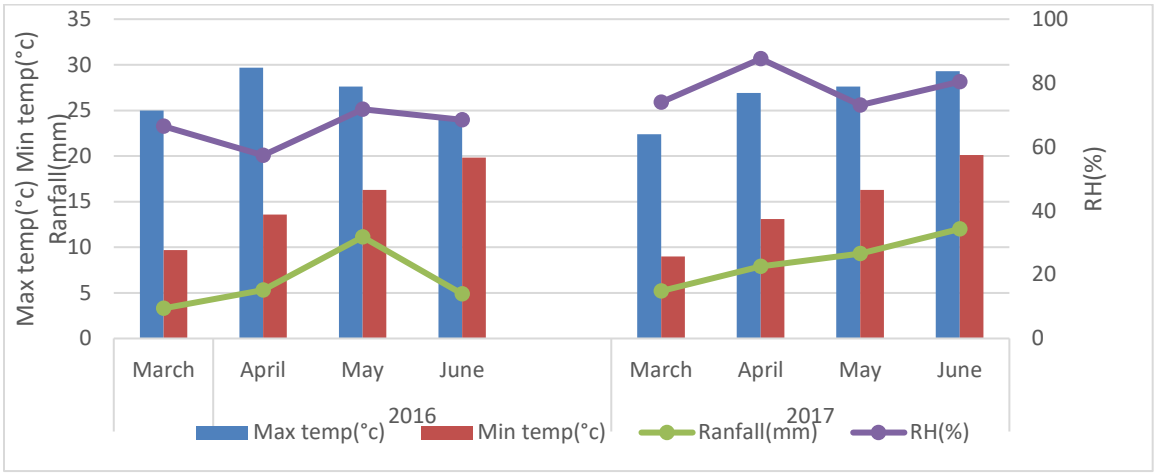

Figure 1 Max. temp., Min. temp., Rainfall and Relative humidity during experiment period (2016 and 2017) at Khumaltar

Typical symptoms of ZYMV were observed on inoculated Zucchini plants as compared to non inoculated. The effect of antiviral products on disease severity of Zucchini is presented (Table 2 & 3). A higher significant effect of the virus was found in control infected compared with treatments. In year 2016, disease incidence (100%) was found in case of bougainvillea leaf extracts, Victo virus and control whereas low incidence (75%) was found in cow urine. Similarly in case of disease severity, highest severity was found in Control (55%) followed by Victo virus (44%), Anti-Vs(43%), Bougainvillea leaf extracts (36%), Garlic extracts and Viricon-H (35%),Yoghurt extracts (34%) and Cow urine (27%) as shown in (Table 2). The highest disease control (51%) was found in cow urine sprayed treatments whereas lowest disease control (19%) was found in Victo virus sprayed treatments as compared to untreated control. The results also revealed that zucchini plants suffered less damage when sprayed with cow urine and more damage when sprayed with victo virus.

Table 2 Effect of treatment on disease incidence, disease severity and yield of Zucchini squash at Khumaltar during 2016

Treatment	Disease incidence	Disease severity (%)	Yield(ton/ha)	Percent disease control
Garlic extracts	83.33	35.33bc	17.70	36.43ab
Victovirus	100	44.67ab	16.89	19.85bc
Bougainvillea leaf extracts	100	36.33bc	18.96	33.87ab
Viricon-H	91.67	35.67bc	16.63	34.35ab
Cow-urine	75	27.00c	20.41	51.31a
Anti-Vs	83.33	43.00ab	16.30	23.36bc
Control	100	55.33a	17.82	0c
Yoghurt extracts	83.33	34.33bc	17.07	36.18ab
Mean	89.58	38.96	17.72	29.42
P-value (0.05)	Ns	*	Ns	*
LSD(0.05)		14.13		25.13
CV%	15.07	20.72	8.44	48.80

Treatments mean followed by common letter within column are not significantly different among the treatments (NS, Non significant).

In year 2017, final disease incidence (100%) was found in case of cow urine, viricon-H, victovirus and garlic extracts, however in control it was (91.67%) whereas, lowest disease incidence (75%) was found in case of yoghurt extracts. Similarly, in case of disease severity, highest disease severity (65%) was found in control plot followed by victo-virus (56%), viricon-H (53%), garlic extracts (51%), Anti-Vs (47%), bougainvillea leaf extracts (41%), cow urine (39%) and yoghurt (30%). The highest disease control was found 50% by cow urine followed by yoghurt extracts 47%, bougainvillea 31%, Anti-Vs 29%, Garlic extracts (20%), Viricon-H (16%), Victovirus (13%) as shown in table 3.

Table 3 Effect of treatment on disease incidence, disease severity and yield of Zucchini squash at Khumaltar during 2017.

Treatment	Disease incidence	Disease severity (%)	Yield(ton/ha)	Percent disease control
Garlic extracts	100	51.67abc	19.04	20.43bc
Victovirus	100	56.33ab	16.89	12.97bc
Bougainvillea leaf extracts	83.33	41.00bcd	14.96	31.98ab
Viricon-H	100	53.67abc	18.63	16.79bc
Cow-urine	100	39.33cd	21.41	50.30a
Anti-Vs	91.67	47.33bc	18.63	29.79ab
Control	91.67	65.00a	18.48	0c
Yoghurt extracts	75	30.00d	14.74	47.46a
Mean	92.71	48.04	17.85	26.22
Pvalue(0.05)	Ns	**	Ns	*
LSD(0.05)		16.15		22.71
CV%	15.15	19.20	14.15	49.48

Treatments mean followed by common letter within column are not significantly different among the treatments (NS, Non-significant).

Relationship between disease severity and yield

Treatment of the some eco friendly or commercial products reduced disease severity while increasing the fruit yield. Maximum fruit yield 20 (ton/ha) was recorded in cow-urine treated plot whereas the least 16 (ton/ha) was found in anti-vs treated plot during 2016. Similarly in case of 2017, maximum fruit yield 21 (ton/ha) was recorded in cow urine treated plot whereas the least 15 (ton/ha) was found on yoghurt extract treated plot. In both years there was negative correlation between disease severity and yield in cow urine treated plot suggesting that cow urine treatment increased crop yield by reducing disease.

4. DISCUSSION

We tested the efficacy of eco-friendly and commercial antiviral products for the management of Zucchini yellow mosaic virus of zucchini squash. In all treatments, incidence level was found statistically nonsignificant compared to control. In case of disease severity during 2016, treatments like Anti-Vs and Victovirus were found nonsignificant. Similarly treatments like viricon-H, bougainvillea leaf extracts, garlic extract and yoghurt extract were found nonsignificant among each other however significant with respect to control but cow urine was found significant with respect to control the disease. In second year, treatments like viricon-H, victo virus and garlic extracts were found nonsignificant but treatments like yoghurt, cow urine, bougainvillea leaf extracts and anti-Vs were found significant over the disease management.

It is clear from the study that Zucchini crop may be protected against infection and spread of infection of Zucchini yellow mosaic virus by the multiple sprays of cow urine and bougainvillea leaf extracts. The study also revealed that spraying of antiviral products of animal origin (cow urine) reduced the disease severity and increased the yield in both years.

Attempts were made earlier for the management of viral diseases in the zucchini crop through insecticides to prevent the movement of insect vectors. But all the insecticides caused environmental pollution, health hazards and phytotoxicity. Use of insecticides cannot be eliminated but avoided by some preventive measures including antiviral agents of plant and animal origin. The antiviral agents used in this study are non-chemical, non-hazardous, easily bio-degradable, eco-friendly and did not have a residual effect.

5. CONCLUSION

In both year, out of seven treatments, cow urine, bougainvillea leaf extracts, and yoghurt extracts were found effective than other four treatments. Garlic extracts was found significant in first year but found nonsignificant in second year. In both years, cow urine was found significant in reducing disease severity suggesting it be the best among all the tested products. Anti-Vs was found nonsignificant in first year but was found significant in second year. The use of cow urine is a cost effective and eco-friendly approach to control viral diseases of vegetable crops. Further, field studies are to be carried out to justify the possible utilization of cow urine against Zucchini yellow mosaic virus of Zucchini squash.

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