Managing soil borne and virus diseases in cucurbits through eco-friendly approaches

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ABSTRACT
Among the cucurbits, Watermelon (*Citrullus lantus* Thunb) Matsum and Nakai, is grown in all parts of India up to an elevation of 1528 masl. Major limiting factors for successful cultivation are its susceptibility to soil-borne diseases such as fusarium wilt and mixed incidence of *Watermelon bud necrosis virus* (WBNV) transmitted by thrips and *Zucchini yellow mosaic virus* (ZYMV) transmitted by aphids causing yield loss ranging from 60-100%. Grafted seedlings of watermelon (bottle gourd used as rootstock) in combination with Agril Net cover in the open field until flowering gave protection against fusarium wilt as well as mixed infection of WBNV and ZYMV leading to economically successful production of watermelon in India. The benefit cost ratio was 3.21 and additional return:additional cost ratio was 11.3.

Keywords: Watermelon, virus diseases, Fusarium wilt, grafting, mature leaf resistance

INTRODUCTION
Among the soil-borne diseases of cucurbits, fusarium wilt of watermelon (*Fusarium oxysporum* f.sp. *niveum*) is the most damaging disease causing yield loss of 75% (Taylor et al. 2008). The pathogen can survive in the soil for more than a decade. The spread of the pathogen is via wind, water, mechanical tools and seeds. There is no cost-effective remedy for the disease control. Among the virus diseases of cucurbits, *Watermelon bud necrosis virus* (WMBNV) of watermelon transmitted by thrips (*Thrips palmi*) and *Zucchini yellow mosaic virus* (ZYMV) transmitted by aphids (*Aphis gossypii* and *Mysus persicae*) are endemic in southern states of India, causing yield loss ranging from 60-100% (Singh and Krishna Reddy 1995; Pandey and Pandey 2001; Rajasekharam 2010). Natural infection of WBNV has also been reported on muskmelon, ridge gourd, bottle gourd, and bitter gourd (Rajasekharam 2010). Chemical control of virus diseases is ineffective. Developing varieties for multiple resistance to soil-borne fungi and virus diseases is time consuming and capital intensive. For immediate short-term approaches, an experiment was conducted at the experimental farm of Namdhari Seeds Pvt. Ltd., Bangalore India during January to March 2013 using fusarium wilt resistant bottle gourd as rootstock and susceptible watermelon varieties as scion followed by Agril Net (unwoven polypropylene) cover until flowering (up to 55 days after sowing) to counter losses due to the mixed infection of WBNV and ZYMV and taking advantage of age related induced resistance to virus complex.

MATERIAL AND METHODS

Grafting and curing techniques

Four inbred lines of watermelon PI 175(V1), PI 5(V2), PI 55(V3), PI 1-10 (V4) and one watermelon hybrid NS 295(V5) susceptible to fusarium wilt as well as WBNV and ZYMV were sown in 99 plug trays 3 days prior to the sowing of fusarium wilt resistant bottle gourd (*Lagenaria siceraria*) used as rootstock. Sowing was done in an insect proof protected nursery. Side grafting operation was done on 13th day of sowing by giving an upward slanting cut with the help of a sharp blade to the hypocotyls region of the bottle gourd seedling, thereby removing one cotyledon leaf and 1st true leaf of the rootstock. The second cotyledon leaf and the root system of the rootstock were kept intact. A downward cut was given to the hypocotyl region of the scion keeping the two cotyledons and first true leaf intact. The cut region of the scion and rootstock was held together using a plastic clip. The grafted seedlings were kept in a curing chamber for a period of 7 days, maintaining 100% relative humidity at 25-30° C. During the first three days the grafted seedlings were ventilated for a period of 10 min each during morning (09:00) and evening (16:00). On the 4th and 5th days, the seedlings were ventilated for 30 min each during morning (09:00) and evening (16:00). On the 6th day, the ventilation was done for 45 min in the morning (09:00) and evening (16:00). On the 7th day, one hour of ventilation was done each during the morning (09:00) and evening (16:00). Thereafter, the grafted seedlings were transferred to the insect-proof nursery for further hardening. The non-grafted seedlings were raised in 99 plug trays in the protected nursery to be used as control treatment.

Experimental design and statistical analysis

Yield data pertaining to 15 treatment combinations was analyzed in a 5 × 3 factorial RBD design: A total of 3 treatment combinations were given each to the 5 watermelon lines/varieties as follows:

Treatment 1 (T1): Grafted seedlings + Agril Net cover in the wilt sick open field till flowering (55 days of sowing)
Treatment 2 (T2): Grafted seedlings without Agril Net cover in the wilt sick open field.
Treatment 3 (T3): Non-grafted seedlings without Agril Net cover in the wilt sick open field.

Twenty-three-day-old grafted and non-grafted seedlings were transplanted in the wilt sick open field. Ten seedlings in each treatment combination were transplanted on raised bunds in a 5 x 3 factorial RBD with three replications in a wilt sick plot keeping row to row distance of 2.5 m and plant to plant distance of 0.7 m. Reflective plastic mulch was used to cover the raised bunds. Fertigation was done at the rate of 150 kg N+ 100 kg P2O5 and 75 kg K2O using water soluble fertilizers. Yield observations were recorded on 5 plants. Average yield (kg/plant) for each treatment combination was recorded replication wise. Once flowering commenced, the Agril Net cover was removed gradually from the seedlings to facilitate pollination by bees.

The Benefit Cost Ratio for each treatment combination was calculated by dividing the gross return by gross expenditure. Additional Return: Additional Cost ratio for T1 or T2 was calculated by subtracting the gross return of T3 from gross income of T1 or T2 and dividing the resultant by additional expenditure on T1 or T2.

Observations pertaining to fusarium wilt were recorded on 55, 75 and 90 days of sowing (Table 8, Fig. 3). Visual observations were confirmed by lab test. Infected samples were collected from freshly wilted plants rinsed under running water to remove the soil and debris. Stem section of 1 to 2 mm in length were surface infected in 1.2% of active sodium hypochlorite for 5 minutes followed by 1 to 2 rinses in sterile water. Sections were plated on to Kodama's medium, which is selective for fusarium oxysporum. Plates were incubated at room temperature under 12 hours of fluorescent lighting. Fusarium colonies that grow on Kodama's selective medium were identified as *Fusarium oxysporum* on the basis of morphological characteristics. Pathogencity test was conducted by preparing a suspension of *Fusarium oxysporum* microconidia using Esposito and Fletcher broth and adjusted to a concentration of 1 x 10^5 spores per ml. The root system of 10 replicated seedlings of susceptible watermelon variety Sugar Baby were dipped into the spore suspension and transplanted into the pots containing 4:1:1 sand: vermiculite: peat medium by volume maintaining soil temperature at 24-24° C. Replicated seedlings of Sugar Baby dipped in Esposito and Fletcher broth without fungus isolate when used as negative control plants, started wilting at onset of flowering.

Observations pertaining to WBNV and ZYMV were recorded on 5 plants randomly selected in each treatment combination replication wise on 75 and 95 days of sowing. For ELISA test young expanded leaves with typical symptoms of WBNV and ZYMV were collected from the terminal portion of secondary branches whereas mature leaves were collected towards the base of secondary branches (Fig. 4). Typical WBNV produced chlorotic and necrotic spots on leaves, necrosis, elongated dark brown streaks and die back of young growing shoots and buds (Fig. 1), whereas, that of ZYMV caused upward growth of the growing shoot tips, yellowing of the apical leaves, short internodes, and discrete mottled spots and discoloration of the leaves (Fig. 2). Visual symptoms of WBNV were confirmed by Direct Antibody Coating-Enzyme Linked Immuno assay (DAC-ELISA) procedure, as suggested by Rajasekaram (2010). The polyclonal antiserum directed against nucleocapsid (N) protein of PBNV purchased from ICRISAT, Hyderabad was used (Rajasekaram 2010). Double antibody sandwich ELISA using polyclonal antiserum virus (Bioreba AG Reinach Switzerland) was used for confirmation of ZYMV.

Total number of symptomatic and asymptomatic leaves were counted on 3 infected plants selected randomly from each variety in treatment #1 and #2 replication wise at an interval of 55, 65, 75, 85 & 95 days after sowing. In treatment #3 the leaf count could not be taken because of the onset of wilt during 65-75 days after sowing. The symptomatic and asymptomatic leaf count in treatment #1 and #2 for 5 varieties and 3 replications at different intervals were averaged separately (Table 5).

RESULTS AND DISCUSSION

Yield analysis in RBD factorial experiment

Yield data was analysed in 5 × 3 factorial design with 3 replications in RBD. Considering the 15 treatment combinations together in a randomized block design with 3 replications, the total variation between the plot yields was partitioned into the components for replications, treatments and experimental error (Table 1 & 2).

The F-test indicated that there were significant differences among the treatment combinations. For finding significant differences between the means, the CD @ 5% was calculated (Table 2).

The data indicated that there was a definite decreasing trend in varietal yield when treatment #1 was changed to treatment #2 and treatment #3. Treatment combination V5T1 was the best and it differed significantly from rest of the treatment combinations (Table 1). Further the significance of the variance for the main effect of varieties (V) and treatments (T) and the interaction (VT) was tested against the error variance by F-test. A table of complete analysis of variance pertaining to 5 x 3 factorial experiment was prepared (Table 3).

The F-test indicated that the main effects of variety (V) and treatment (T) were significant at 1% level of significance; however, the interaction (VT) was not significant at 5% level of significance (Table 3).

All the 3 treatments differed significantly from each other as indicated by CD @ 5%. The best treatment combination was V5T1 giving the highest yield of 74.4 mt/ha. The nonsignificance of interaction (VT) proved that different varieties did not alter the effect of a particular treatment. Irrespective of the varieties used, the treatment #1 (60.3 mt/ha) remained significantly superior (10.2 mt/ha, CD 5%) to treatment #2 (mean 24.6 mt/ha) and treatment #3 (mean 13.2 mt/ha) (Tables 2 & 3). Fifty-nine percent yield reduction was noticed when T1 was changed to T2 and 78% yield reduction was observed when T1 was changed to T3 (Table 4).

Benefit cost ratio was analyzed as 3.21 in T1, 1.51 in T2 and 0.90 in T3. Additional Return: Additional Cost ratio was analyzed as 11.3 in T1 and 6.84 in T2 indicating that for each additional expenditure of Rupee 1 there was an additional return of Rupees 11.3 in T1 and Rupees 6.84 in T2. In T3 for every one rupee of investment there was a loss and just a return of Rupee 0.905.

Grafting and quality parameters

In grafted plants the fruit harvesting was delayed for one week, the fruit texture was firm to medium crispy and the Total Soluble Solids (TSS) was comparable to fruits from non-grafted plants (Table 7).

Viral disease management during different stages of plant growth

The induction of resistance to disease during plant development is widespread in the plant kingdom. Resistance appears at different stages of host development and varies with the plant age or tissue maturity. Above the critical leaf stage and physiological maturity when the cells are not dividing, the disease symptoms do not develop in the infected leaves (Atkinson and Matthews 1970). In Treatment #1, where grafted seedlings were given a protection against virus infection for a period of 55 days, they produced on an average 46 asymptomatic leaves as compared to symptomatic leaf count which was zero; however, the ratio between asymptomatic and symptomatic leaf count gradually decreased from 29:1 to 4.8:1 during 65 to 95 days of sowing when the protection was taken off at 55 days of sowing (Table 5). At the time of harvest between 85 to 95 days the number of asymptomatic leaf count ranged from 335 to 367 per plant as compared to symptomatic leaf count which ranged from 38 to 69, yielding on an average 60.3 mt/ha in treatment #1.

In Treatment #2 where the protection to the grafted seedlings against virus infection was provided only up to 23 days in nursery and thereafter no protection was provided in the field after transplanting, the ratio of asymptomatic and symptomatic leaf count decreased from 3.1:1 to 1.7:1 during 55 to 65 days of sowing. Thereafter the asymptomatic to symptomatic leaf count ratio increased from 1:1.2 to 1:2.75 during 75 to 95 days of sowing (Table 5). Thus in Treatment #2 there was a reduction

of 82% in mature asymptomatic leaf count as compared to Treatment #1. This might be the reason for 59% yield reduction in Treatment#2 as compared to Treatment #1.

ELISA Test

The ELISA test was used to confirm presence of both WBNV and ZYMV qualitatively in diseased plants (Table 6). Young expanded leaves with typical symptoms of WBNV collected during 75 days and 95 days of sowing showed positive reaction, whereas, mature asymtomatic or mild symtomatic leaves collected during 75 days of sowing showed negative reaction and at 95 days of sowing it showed positive reaction. This might be due to the rate of virus replication and or long distance transport of virus infectivity, which varies with physiological maturity of the leaves (Mathews 1995). Age related response in tobacco was assoicated with five-fold increase in endogenous salicylic acid known to have antiviral properties (Yalpani et al. 1993). In the case of ZYMV, the young expanded leaves collected with typical symptoms of ZYMV showed positive reaction during 75 and 95 days of sowing; however, in mature asymptomatic leaves or leaves with mild symptoms, it showed positive reaction at 75 days of sowing, whereas some of them showed negetive reaction at 95 days of sowing. This might be due to the strong interaction of plant age and environmental conditions with host genotype to produce the final response. Many metabolic changes occur as leaves mature. Variations in physiological maturity of leaves within the plant may give different response to the infection (Mathews 1995). Grafted watermelon seedlings using fusarium wilt resistant bottle gourd rootstock provided sure protection from fusarium wilt. Further protection of the grafted seedlings with Agril Net for a period of 55 days provided ample oppurtunity to develop 337 mature leaves with mild symptoms or no symptom of virus infection in Treatment #1, leading to the production of a healthy economic crop.

Occurance of mature tissue and mature plant resistance irrespective of the susceptible level of genotype of *Peanut bud necrosis virus* has been reported by Buiel and Parlevliet (1996). Bell and banana pepper also exhibited mature plant resistance to *Tomato spotted wilt virus* transmitted by Thrips (Beaudoin 2009). Overall, it can be concluded that grafted watermelon seedlings with Agril Net cover until flowering (55 days of sowing) gives suffcient protection against fusarium wilt and virus complex of WMBN and ZYMV, leading to the production of an economically sucessful crop.

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Table 1. Average yield (kg/plant) in RBD

Treatment combination	R-1	R-2	R-3	Total	Mean	mt/ha	Yield (mt/ha) descending order	
V1T1	8.8	9.5	10.3	28.6	9.5	63.5	**V5T1**	74.4
V2T1	7.0	8.5	6.5	22.0	7.3	48.9	**V1T1**	63.5
V3T1	8.5	9.5	10.3	28.3	9.4	62.9	**V3T1**	62.9
V4T1	6.0	9.1	8.1	23.2	7.7	51.6	**V4T1**	51.6
V5T1	10.0	11.0	12.5	33.5	11.2	74.4	**V2T1**	48.9
V1T2	5.0	3.5	2.7	11.2	3.7	24.9	**V5T2**	31.1
V2T2	4.5	2.5	3.4	10.4	3.5	23.1	**V1T2**	24.9
V3T2	3.5	2.2	3.0	8.7	2.9	19.3	**V4T2**	24.4
V4T2	4.0	3.0	4.0	11.0	3.7	24.4	**V5T3**	23.3
V5T2	5.0	3.5	5.5	14.0	4.7	31.1	**V2T2**	23.1
V1T3	2.0	1.0	1.5	4.5	1.5	10.0	**V3T2**	19.3
V2T3	2.0	1.5	2.5	6.0	2.0	13.3	**V2T3**	13.3
V3T3	1.0	2.0	1.3	4.3	1.4	9.6	**V1T3**	10.0
V4T3	2.0	1.4	1.0	4.4	1.5	9.8	**V4T3**	9.8
V5T3	4.0	3.5	3.0	10.5	3.5	23.3	**V3T3**	9.6

Table 2. RBD Analysis of Variance

SOV	df	SS	MSS	F	
Replication	2	0.51	0.26	0.304755791	NS
Treatment	14	449.90	32.14	38.22255367	**
Error	28	23.54	0.84		
Total	44	473.95			
CD 5%	1.533 kg/pl	10.2mt/ha			

Table 3. Factorial Analysis of Variance

Source of Variation	SS	df	MS	F	P-value	
Replication	0.51	2	0.26	0.305		NS
V (Columns)	29.31	4	7.33	9.140	**5.94E-05**	**
T (Rows)	407.07	2	203.54	253.856	**1.58E-19**	***
Interaction	13.51	8	1.69	2.106	**0.066832**	NS
Error	**23.54**	**28**	**0.84**			
Total	473.95	44				
CD 5% for Variety	0.885 kg/Pl	5.8 mt/ha				
CD 5% for Treatments	0.685 kg/Pl	4.5 mt/ha				

Table 4. Percent reduction in yield

	T1 Vs T2	T1 Vs T3	T2 Vs T3
V1	60.8	84.3	59.8
V2	52.7	72.7	42.4
V3	69.3	84.8	50.2
V4	52.6	81.0	59.8
V5	58.2	68.7	25.0
Mean	**59.0**	**78.0**	**47.5**

Table 5. Asymptomatic and symptomatic leaf count ratio during different developmental stages of plant growth

	Treatment 1			Treatment 2		
Days	**Asymptomatic : Leaves (no)**	**Symptomatic Leaves (no)**	**Ratio**	**Asymptomatic Leaves (no)**	**Symptomatic Leaves (no)**	**Ratio**
55	46	0	46:0	31	10	3.1:1
65	174	6	29:1	85	50	1.7:1
75	325	17	19:1	73	90	1:1.2
85	367	38	9.6:1	65	150	1:2.3
95	335	69	4.8:1	58	160	1:2.75

Table 6. WBNV and ZYMV ELISA test

Variety	Sample No.	WBNV		ZYMV	
		75 days	95 days	75 days	95 days
		Reaction	Reaction	Reaction	Reaction
Pi 175	Sample 1	+	+	+	+
	Sample 2	-	+	+	-
NS 295	Sample 1	+	+	+	+
	Sample 2	-	+	+	+
Pi 5	Sample 1	+	+	+	+
	Sample 2	-	+	+	-
Pi 55	Sample 1	+	+	+	+
	Sample 2	-	+	+	-
Pi 1-10	Sample 1	+	+	+	+
	Sample 2	-	+	+	-

Samle 1: Young expanded leaves collected from terminal portion of growing secondary branches showing typical symptoms of WBNV and ZYMV. Sample 2: Mature leaves with mild symptoms or no symptoms collected from the base of infected secondary branches.

Table 7. Quality parameters

Treatment	**Maturity (days)**	**TSS (%)**	**Texture**
T1	85-95	10-12	Medium crisp
T2	85-95	10-12	Medium crisp
T3	78-90	10-12	Granular

Table 8. Incidence of fusarium wilt (%) in different treatment combinations (average of 3 replications)

Treatment combination	Fusarium wilt (%)		
	55 days	75 days	90 days
V1T1	0	0	0
V2T1	0	0	0
V3T1	0	0	0
V4T1	0	0	0
V5T1	0	0	0
V1T2	0	0	0
V2T2	0	0	0
V3T2	0	0	0
V4T2	0	0	0
V5T2	0	0	0
V1T3	35	71.5	84.2
V2T3	28.5	53.2	72.6
V3T3	33	65.8	85
V4T3	36	68.7	86
V5T3	29.1	48.6	65.7

Figure 1. Watermelon bud necrosis

Figure 2. Zucchini yellow mosaic virus

Figure 3. Fusarium wilt in non- grafted watermelon