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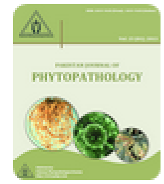

RESISTANT GENOTYPES AND BOTANICALS ARE THE POTENTIAL HINDRANCES AGAINST VECTOR TRANSMITTED OKRA YELLOW VEIN MOSAIC VIRUS

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ABSTRACT

Okra yellow vein mosaic virus (OYVMV) is vectored by sucking insect whitefly into the phloem of the okra plant. It devastates the fruit quality and production if infection started at early growth stages. Commonly, the virus is tried to manage by using chemical insecticides against the whitefly that has adverse effects on the ecosystem. The present study was to explore the non-chemical ways to fight against OYVMV and it vector. The available germplasm consisting of 11 cultivars was sown in augmented design to estimate the level of resistance under natural conditions where no whitefly control measure was used. Based upon the results of disease severity, the cultivars were categorized into highly resistant, resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible; the most effective and highly resistant variety was "Sabz Pari" that showed minimum disease incidence (60.58%) and disease severity (7.72%). A separate experiment was conducted to evaluate the role of plant extracts in the management of OYVMV disease and whitefly where 5 moderately resistant to moderately susceptible varieties were sown. Among the plant extracts applied garlic extract showed more significant results in controlling whitefly (79.23%) and OYVMV transmission (88.27%). It could be concluded that "Sabz Pari" is the most suitable and high yielding okra variety against OYVMV disease and garlic extract could be applied as a best bio-pesticide against sucking insects.

Keywords: Evaluation, Begomoviruses, Defense mechanism, Bio-pesticide.

INTRODUCTION

Okra yellow vein mosaic virus (OYVMV) is a prominent member of *Geminiviridae* family that is commonly known as geminiviruses which have ssDNA genome with geminate shape and may contain 1 or 2 particles. OYVMV occupies a significant position in genus *begomovirus* although it is not the type member of this genus; means the name of group has not been derived from it rather it was derived from bean golden mosaic virus (Murilo-Zerbini *et al*., 2017). As viruses are deficient in the character of active entry into their host and these need some sort of aid for host penetration; OYVMV is acquired from and released into the phloem of okra plants during whitefly feeding from lower side of the leaves (Kumar and Shivaprasad, 2020).

OYVMV impairs the photosynthetic activity and frequency by disturbing light harvesting capacity of the chlorophyll that ultimately results in reduced energy for driving the metabolic activities. The reduced metabolism leads to disturbance in physiology of plants and lower growth, development and yield of okra plant (Emmanuel *et al*., 2020). The yield decrease and quality deterioration of okra fruits depend upon the virulence of the viral pathogen, susceptibility of the cultivar and

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duration of feasible weather conditions that prevail during OYVMV and okra interaction. However, if all the epidemic development conditions prevail for appropriate duration that may lead more than 70 % yield losses when compared with healthy okra fields apart from quality of the fruits (Naresh *et al*., 2019).

Once the okra plant is infected with virus, it is difficult to avoid from damages caused by the virus replication inside the host. The most common method used against the vector transmitted OYVMV is to control or avoid from the whitefly infestation in the field that is accomplished more efficiently by using different insecticides (Siddique *et al*., 2014). Although the use of insecticides is a quick and efficient way to control the insects, but its extended usage develop resistance in the insects and they remain no longer vulnerable to chemical insecticides leading to transmission of virus and severe disease progression (Hayd *et al*., 2020). The development of resistance in insects diminishes the prime objective of insecticide application that is focused upon breaking the virus transmission mechanism. The insecticides application has also other harmful effects on the environment like deterioration of soil and water; targeting the beneficial insects and microbes (Tudi *et al*., 2021). Insecticides application exacerbates the factors affecting climate change as it emits greenhouse gases leading to increased temperature and unusual pattern of rainfall (Davidson, 2018). The increased temperature causes serious hindrances in the management of plant viruses (Jones and Naidu, 2019). This is because of the fact that virus replication speeds up at elevated temperatures and its cell to cell movement along with ability to hijack host machinery increased (Amari *et al*., 2021). The plant varieties having resistance genes recognize pathogen elicitors and initiate a signal cascade that activates plant defense system to fend off the pathogen (Andersen *et al*., 2018). The R genes occupy dominant position in the plants which easily recognize the elicitors released by the pathogens while genes of susceptibility are unable to receive the elicitor signals and thus pathogen remain unchecked and can easily invade the host (Gust *et al*., 2017).

The direct and indirect harmful and disastrous effects of the insecticide application for the management of OYVMV and other vector transmitted viruses necessitates to explore the more appropriate and durable way of OYVMV disease management. As the susceptible host plant is an important factor for disease development; the use of resistant cultivars may hinder the onset of OYVMV infection. This study is also hypothesized to find the possible way of whitefly control through using the plant based solutions rather than synthetic chemicals.

MATERIALS AND METHODS

Experimental details: Assessment of resistance potential in okra germplasm against OYVMV disease: The experiment for assessing the resistance potential of okra cultivars was conducted at research area Department of Plant Pathology, College of Agriculture, University of Sargodha, Pakistan. The seed of 11 cultivars was obtained from Vegetable Research Institute (VRI), Ayub Agricultural Research Institute (AARI), Faisalabad (Pakistan). The details of the germplasm are provided in Table. 1, okra seed was sown on ridges by maintaining 60 cm row to row and 30 cm plant to plant distance, respectively. The experiment was conducted in augmented design and a row of susceptible check (Ok-1314) was sown after every 3 rows of the germplasm to be tested against OYVMV disease. The trial was conducted near tomato and mungbean fields in order to ensure maximum whitefly infestation and subsequent OYVMV transmission. The requirements for raising successful crop like fertilizers, irrigation and sanitation were appropriately implemented.

Table 1. Germplasm screened against OYVMV under natural field conditions

Serial Number	Details of Germplasm
1	Sultan 121
2	Sultan 77
3	TOK 1203
4	Sabz Pari
5	Diamond 2626
6	Diamond 2525
7	OK 31
8	OK 1900
9	OK 669
10	Bs 782
11	Sh 8225
12	OK 1314

Data recording from screening trial: The field having above mentioned cultivars for estimation of resistance was visited daily for observance of the whitefly infestation and characteristic virus symptoms i.e. mosaic, puckering of veins etc. As the symptoms appear data for disease incidence was recorded from each cultivar on weekly basis and up to 5 times. When the disease incidence was noted in all the cultivars; data for disease severity was started recording from each cultivar; for disease severity 3 plants were randomly selected from each row and average was taken for data collected. The data was recorded by using following formulae:

$$\text{Disease incidence (\%)} = \frac{\text{Number of symptomatic plants in a row}}{\text{Total number of plants}} \times 100$$

$$\text{Disease severity (\%)} = \frac{\text{Number of symptomatic leaves}}{\text{Total number of leaves}} \times 100$$

The varieties were categorized according by using following modified disease rating scale (Ali *et al*., 2005) (Table. 2).

Table 2. Disease rating scale for scoring the okra cultivars for level of resistance

Rating Number	Category	Disease severity (%)
0	Immune	0
1	Highly resistant	1-10
2	Resistant	11-20
3	Moderately resistant	21-30
4	Moderately susceptible	31-40
5	Susceptible	41-50
6	Highly susceptible	51-100

Confirmation of the virus: As the symptoms of OYVMV disease appeared in the field, a branch of the symptomatic plant was detached from the field and grafted on to the plants grown under screen house for the confirmation of virus. The grafted plants were covered with polythene bags to avoid from whitefly infestation and kept under proper care for observation of symptoms (Kashina *et al*., 2007).

The confirmation of OYVMV was also done by adopting whitefly inoculation method; for this whiteflies were collected from the symptomatic fields through aspirator early in the morning and released on to the already covered plants grown in the pots under screen house. The appearance of the characteristics symptoms in both experiments indicated the presence of the OYVMV in the field and then data recording was started (Lapidot *et al*., 2001).

Survey of okra fields for assessment of OYVMV disease in Tehsil Sahiwal, District Sargodha: In order to assess the disease incidence at different locations; 4 fields in different areas of Tehsil Sahiwal, District Sargodha were marked and data for disease incidence was recorded on weekly basis for 5 times according to above mentioned formula.

Application of botanicals for the management of whitefly and OYVMV disease: After screening the available germplasm according to the given disease rating scale, moderately resistant to moderately susceptible germplasm i.e. 5 varieties were sown in randomized complete block design with 3 replications. This trial was planned to assess the effect of different plant extracts on whitefly infestation and subsequent virus inoculation. The details of plant extracts application are provided below in Table 3.

Table 3. Details of the plant extracts used

Treatments	Common name	Botanical name	Dose
1	Garlic	*Allium sativum*	5ml/liter
2	Ginger	*Zingiber officinale*	5ml/liter
3	Neem	*Azadirachta indica*	5ml/liter
4	Gum arabic tree	*Vachellia nilotica*	5ml/liter

Disease and whitefly data recording: The data for disease severity was recorded according to above formula 1 day before and 2 days after the application of plant extracts. The data for whitefly infestation was recorded by selecting 3 plants randomly from each row and counting number of whiteflies from lower, middle and upper portion of the plant and average was taken.

STATISTICAL ANALYSIS

The collected data was analyzed using statistical software Statistix 8.1. All means were compared using least significant difference test at 5% probability level (Steel and Torrie, 1997).

RESULTS

Screening of okra cultivars against OYVMV disease under field conditions: Above mentioned 11 genotypes were sown under natural field conditions to assess the level of resistance against OYVMV disease. The data was recording weekly after the confirmation of virus under screen house experiment through grafting and whitefly inoculation. The salient symptoms of mosaic and vein puckering were kept as a criterion to record the data and categorized according to scale given above. There was a positive correlation between disease incidence and severity; maximum disease incidence was 97.03% while minimum was 60.58% that represented the highly susceptible and highly resistant cultivars, respectively (Table 4). None of the cultivars was immune against OYVMV disease, while 2 cultivars Sabz Pari and Diamond 2626 were highly resistant having minimum disease severity. OYVMV disease was observed with moderately resistant reaction in 3 genotypes i.e. TOK 1203, OK 1900 and Sh 8225. Bs 782 showed highly susceptible reaction with maximum disease severity (53.11%) followed by 39.04 and 31.95% disease severity in moderately susceptible cultivars i.e. OK 669 and OK 31.

Table 4. Screening of okra cultivars against OYVMV disease under field conditions

Okra cultivars	Disease incidence (%)	Disease severity (%)	Category
Sultan 121	95.82ab	42.07b	S
Sultan 77	74.81e	18.02g	R
TOK 1203	81.38d	23.62f	MR
Sabz Pari	60.58g	7.72h	HR
Diamond 2626	60.83g	9.05h	HR
Diamond 2525	70.03f	15.87gh	R
OK 31	85.05c	31.95d	MS
OK 1900	84.16cd	27.81e	MR
OK 669	92.34b	39.04c	MS
Bs 782	97.03a	53.11a	HS
Sh 8225	79.06de	25.67ef	MR

Means with different letters in a column indicate the significantly different values at 5% probability
HR = Highly resistant, R = Resistant, MR = Moderately resistant, MS = Moderately susceptible, S = Susceptible, HS = Highly susceptible

Correlation of OYVMV disease incidence and severity: The data of disease incidence and disease severity was correlated and it was observed that disease incidence recorded in the cultivars sown for screening is directly proportional to the disease severity. The germplasm indicating high disease incidence has more disease severity and less disease severity was recorded where incidence was low. According to disease rating scale scoring, the cultivars which are highly resistant have low disease incidence and severity which showed a positive and increasing trend in susceptible and highly susceptible varieties (Figure 1a & 1b).

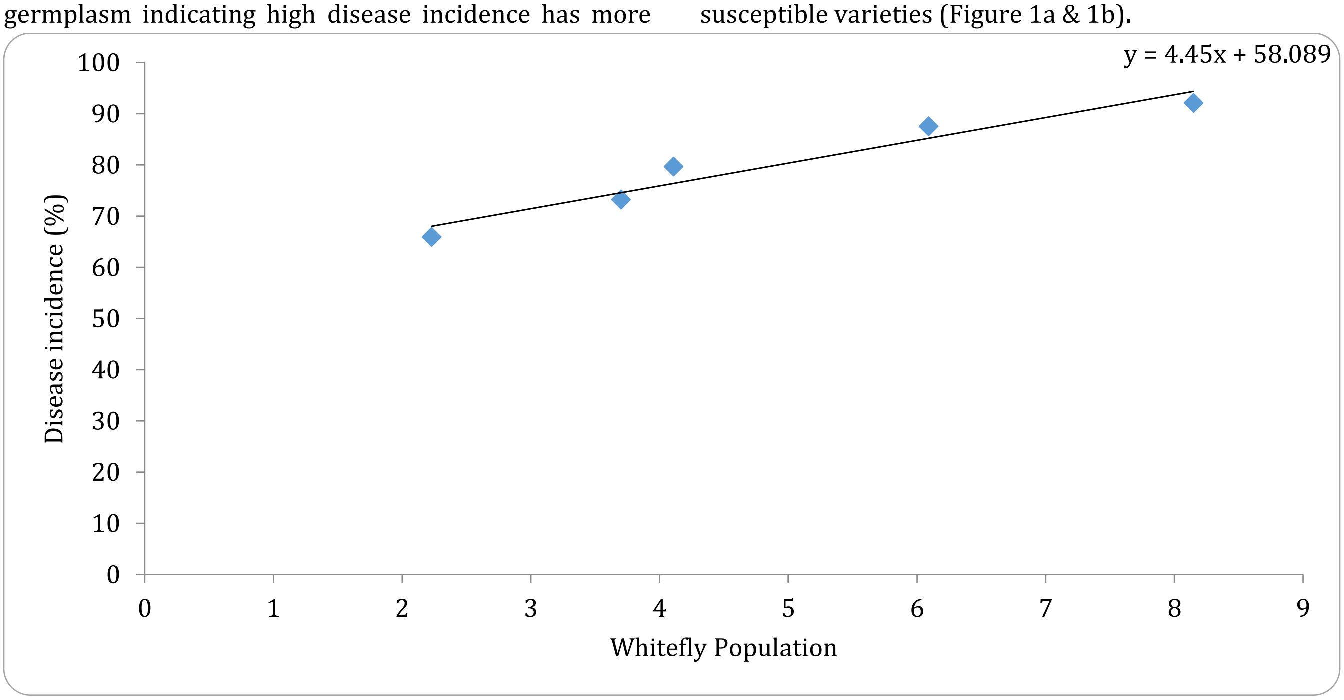

Figure 1a. Correlation of whitefly and disease incidence

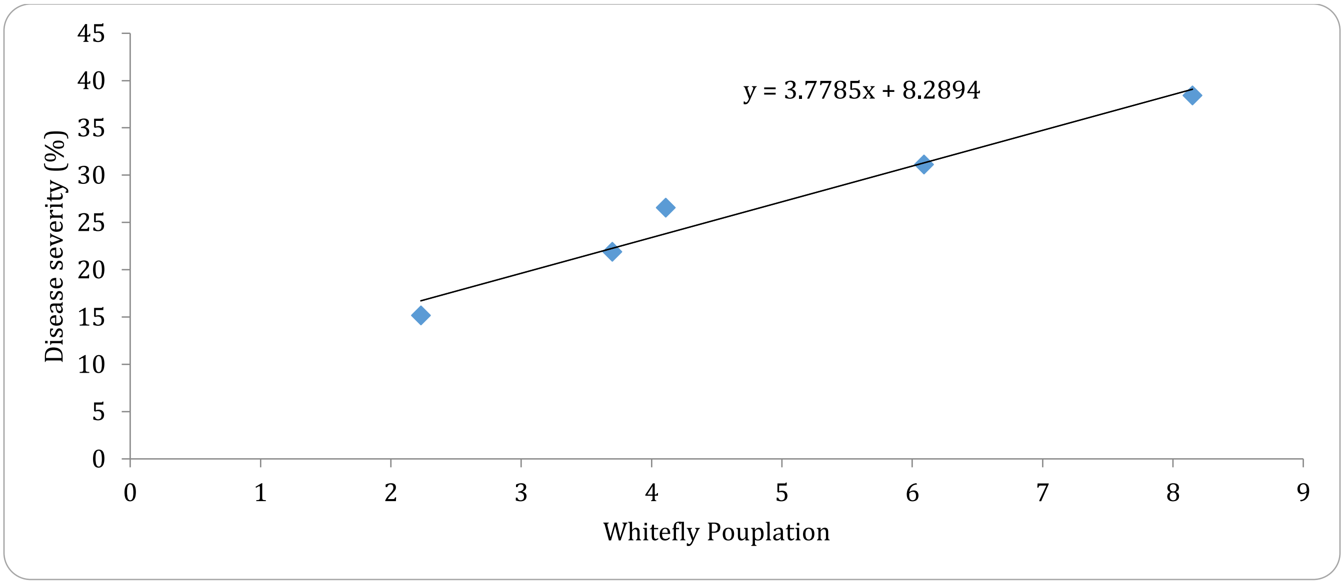

Figure 1b. Correlation of whitefly and severity

Assessment of OYVMV disease potential at different locations of Tehsil Sahiwal, District Sargodha: Okra fields were randomly selected at 4 locations i.e. Thatthi Raju, Mubarary Khan, Dhero Sial and Sheikh Jaleel in Tehsil Sahiwal, District Sargodha (Pakistan). On the basis of symptomology, the data of disease incidence and severity was recorded on weekly basis. It was observed that fields with high disease incidence have more severity of the disease and vice versa. The maximum disease incidence and severity was recorded in the fields of "Sheikh Jaleel" followed by Thatthi Raju, Mubarary Khan and Dhero Sial (Figure 2).

Figure 2. Survey of OYVMV disease in different locations of Tehsil Sahiwal, District Sargodha

Role of plant extracts in the management of whitefly infestation and OYVMV disease: The germplasm for the management OYVMV disease through control of whitefly was selected after screening of okra cultivars, 5 moderately resistant to moderately susceptible varieties i.e. TOK 1203, OK 1900, Sh 8225, OK 31 and OK 669 were used. Minimum disease severity was recorded in plants where garlic extract was applied followed by gum arabic tree, neem and ginger in that order (Table 7). The disease severity tends to decrease with each spray as it was maximum after 1st spray and minimum after 5th spray (Table 5). There was minimum whitefly infestation observed in garlic treated plants and subsequent OYVMV disease severity and maximum was in ginger treated plants (Table 6). The effectiveness of all the botanicals was significant as compared to control where no treatment was applied. The individual response of all the plant extracts was similar as overall in all the varieties and replications.

Table 5. Effect of botanicals on OYVMV disease severity after 4 sprays

Botanicals	OYVMV disease severity (%)			
	S_1	S_2	S_3	S_4
Neem extract	46.15a	37.39b	29.95c	22.23d
Garlic extract	24.06a	17.13b	13.44c	10.09d
Ginger extract	51.45a	42.16b	33.22c	22.65d
Gum arabic tree	31.14a	25.23b	18.49c	14.08d
Untreated check	74.42a	77.56b	81.09c	85.99d
LSD		1.43		

* Mean values with different letters in a row are significantly different as described by the LSD test at 5% level of probability

Table 6. Effect of botanicals on whitefly infestation after 4 sprays

Botanicals	Whitefly infestation			
	S_1	S_2	S_3	S_4
Neem extract	17.11a	14.03b	11.14c	08.05d
Garlic extract	12.15a	10.05b	08.12c	05.19d
Ginger extract	21.25a	18.55b	13.05c	12.88c
Gum arabic tree	15.29a	12.35b	10.13c	07.18d
Untreated check	24.06a	25.11a	24.07a	25.11a
LSD		1.56		

* Mean values with different letters in a row are significantly different as described by the LSD test at 5% level of probability

Table 7. Overall performance of plant extracts in controlling whitefly and OYVMV disease

Plant extracts	OYVMV disease control (%)	Whitefly control (%)
Neem extract	74.15c	67.94c
Garlic extract	88.27a	79.33a
Ginger extract	68.66d	48.71d
Gum arabic tree	83.63b	71.41b
LSD	0.921	

* Mean values with different letters in a column are significantly different as described by the LSD test at 5% level of probability

DISCUSSIONS

The resistance potential in the okra cultivars is the more suitable way to fend off the attack of whitefly infestation and OYVMV disease. Okra varieties were screened to find out the level of resistance so that injudicious usage of insecticides and environment degrading chemicals be avoided. The available germplasm was screened and none was immune against OYVMV disease, only 2 varieties were highly resistant, 2 were categorized as resistant, 3 varieties were moderately resistant, 2 moderately susceptible, susceptible and highly susceptible 1 each. The varied level of resistance is attributed to different gene pool in the germplasm. There are reports for significant yield and quality losses if the virus attacks at the initial growth stages (Das *et al*., 2012). Although no variety was found immune, highly resistant and resistant varieties could be implemented into breeding programs for development immunity into okra cultivar. There had been attempts to evaluate the resistance potential against OYVMV disease and it was observed that no variety was free from OYVMV infection (Jamil *et al*., 2020). OYVMV has innate potential to hijack the host machinery and it was observed that most of the germplasm was vulnerable against this virus and very few cultivars were found resistant (Bhagat *et al*., 2001). OYVMV caused significant yield reduction in susceptible and highly susceptible varieties, high disease incidence and severity has positive correlation with yield and quality deterioration (Benchasri, 2012). In present study, “Sabz Pari” variety showed highly resistant reaction against OYVMV and whitefly and such finding was found in a previous study marinating the fact that it may be recommended to the farming community as this cultivar proved resistant in varied environment (Rehman *et al*., 2017). The reason for high resistance in “Sabz Pari” variety seems due to the present high density trichomes because these hairy structures create obstacle against whitefly piercing in the phloem from lower side of the leaves (Hasanuzzaman *et al*., 2016). The difference in resistance potential in various germplasm have many baseline factors apart from gene pool; these include texture, lignin production and structural barriers. The presence of resistance gene (R-gene) that encode for different defense mechanism hinders the attack of begomoviruses and it is present in "Sabz pari" (Jatav *et al*., 2018). Some of the workers on molecular characterization of the presence of resistance gene in host plant and found that these genes opt the way of epistatic gene action against begomoviruses (Devi *et al*., 2020). In many screening experiments, it was observed that maximum germplasm is susceptible against OYVMV and whitefly infestation; this is because of the fact that begomoviruses inoculum dissolve in the saliva of whitefly during transmission (Ghanim *et al*., 2001).

There was varied disease incidence and severity in different locations of the Tehsil Sahiwal District Sargodha. Although the environmental conditions within a similar locality are not different; the difference in the disease potential may be due to the germplasm (Namrata *et al*., 2012). The other reason for difference in disease occurrence in similar environment may be the field locality i.e. an okra field near other hosts of whitefly would have more infestation and subsequent virus transmission (Reddy *et al*., 2022). The difference in OYVMV disease occurrence in various fields of similar environment may also be attributed the micro climatic conditions and surrounding that break insect efficiency or unfavorable conditions for its activities (Pankaj *et al*., 2022).

The plant extracts were used to keep away the whitefly from okra fields so that OYVMV transmission may be disrupted. All the extracts showed best results and subsequent reduction in disease incidence and severity was also noted in all the varieties. These plant extracts not only repel the whitefly from okra fields but also boost up the plant defense mechanism to repair the damages caused by viral infection (Sharma *et al*., 2018). Among the treatment used in present experiment, maximum whitefly disease management was recorded in garlic extract treated plants. Garlic extract contains allicin, lectin, alliin and dialyl sulfide as the major component; the lectin disrupts the food detection ability of the sucking insects (Mondal *et al*., 2011). Garlic lectin inhibit nutrients absorbing ability of the sucking insects particularly in order *Hemiptera* after entering into the gut (Karley *et al*., 2005). OYVMV disturbance the formation of chlorophyll and structure of chloroplast cells leading to the development mosaic symptoms that impairs photosynthetic activity. Allicin present in garlic extract take part in the development of chlorophyll and carotenoid thus intends to repair the damages caused by OYVMV. These significant findings have been approved by many extensive studies on biochemistry and physiology of allicin treated plants (Martins *et al*., 2016). Garlic extracts contains enzymatic antioxidants that overcome the harmful effects of toxic reactive oxygen species during biotic stress caused by the infection of OYVMV (Gokul *et al*., 2016). The plant extract significantly repelled the landing of whitefly insect on okra plants that resultantly deter the disease occurrence (Malik *et al*., 2018).

Apart from deterring the insects from invading the plants; the plant extracts initiate the defense signals by triggering induced mechanism; stimulate the production of antimicrobial compounds; capable the plant to overcome harmful effects of reactive oxygen species and strengthen the plant cell walls.

CONCLUSIONS

Based upon the experiments, it is concluded that "Sabz Pari" is the highly resistant variety against OYVMV disease and whitefly infestation; therefore it should be recommended for cultivation to the growers. The garlic extract is a safe and sustainable treatment against whitefly infestation and to stop virus spread.

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Muhammad U. Ghani	:	Statistical Analysis
Rana Binyamin	:	Refined and Finalized the Manuscript
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