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Original Article

Impact of okra yellow vein mosaic virus on the physiology of okra crop and its management

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Abstract

Okra plants infected with okra yellow vein mosaic virus (OYVMV) were observed in the field for assessment of disease incidence and disease severity. Virus confirmation was done by whitefly inoculation, chip and leaf patch grafting. Electrolyte leakage and membrane stability index was calculated by recommended methods. The disease was managed by applying the mixture of buprofezin (0.001%), neem extract (0.005%) and citric acid (0.0003%). Parbhani kranti variety was most infected while Green wonder was less infected. More electrolytes leakage was recorded in infected plants while minimum in healthy plants. Neem extract was the more significant in reducing disease incidence by deterring the whiteflies from the okra crop. Citric also gave good results by repairing the plants cell damage and boosting the defense mechanism.

Keywords: Electrolyte, Geminivirus, Management, Okra, OYVMV, SAR, Whitefly

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Introduction

Okra (*Abelmoschus esculentus* L.) is the key member in Malvaceae family. It is cultivated on larger area all over the world with significant production (Wammanda et al., 2010). In Pakistan average yield (800-1000 Kg/ha) of okra is lower than other countries (Alam and Hossain, 2008). The reason for low yield is the epidemic incidence of okra yellow vein mosaic virus disease (OYVMVD) (Venkataravanappa et al., 2013). This disease is caused by Okra yellow vein mosaic virus (OYVMV) and its infection may reduce the yield almost 50% (Khan et al., 2005). OYVMV is vectored by whitefly (*Bemisia tabaci* Genn.) persistently and circulative way (Sattar, 2012). Extent of damage depends upon the stage of infection. Early infected plants are more prone to more damage as compared to late infection (Ali et al., 2010). Infected plants have cleared veins, yellow foliage with reduced size and smaller fruits as the salient symptoms (Pun and Doraiswamy, 1999). Symptomatic plants undergo extensive physiological changes that induce stress on plant defense (Gupta and Paul, 2001). The virus belongs to Geminiviridae family that mutates rapidly and therefore it bypasses the resistance of many cultivars (Raja, 2010).

The objective of the following study was to assess the disease prevalence and to record the physiological changes in affected plants. Furthermore, disease was managed by using resistance inducing chemicals and indirectly by controlling the insect vector.

Material and Methods

Planning of experiment

The experiment was conducted in University College of Agriculture, University of Sargodha (Pakistan). Pots (6 inches diameter) were collected and filled with sand, clay and farm yard manure in equal proportion. Okra seeds were sown in different pots. In some pots indicator plants were planted. Five varieties (Laxmy, PMS-Beauty, Parbhani Kranti, Green Wonder, Selection-31) were sown in completely randomized design (CRD). Chenopodium and mungbean was used as indicator plants.

Observation of symptomatic plants

Okra fields were visited in University College of Agriculture for assessment of OYVMVD in different areas surrounding to the University College of Agriculture, University of Sargodha, Sargodha (Pakistan).

Confirmation of virus through whitefly

Suspected viruliferous whiteflies were collected from symptomatic plants through aspirator. Whiteflies were released on the covered plants in pots and given 48 hours inoculation access period. After that whiteflies were killed by spraying insecticides and symptoms were observed.

Leaf patch grafting

Symptomatic leaf was detached from the infected plant in the surveyed field. A cut was made on the healthy plants sown in the pots and infected leaf inserted followed by wrapping with parafilm. The plant was covered with polythene sheet and symptoms were recorded.

Chip grafting

A small chip was excised from the stem of infected plant and inserted into a similar cut on the healthy plant, plant was covered and symptoms observed.

Data recording

Data were recorded at weekly intervals and up to three times.

After confirmation of the virus by above mentioned methods, disease incidence data were recorded from the surveyed field by using following formula.

Disease incidence = No. infected plants/Total no. of plants *100

Disease severity = No. of infected leaves/Total no. of leaves*100

Measurement of electrolyte leakage and membrane stability index

To assess the effect of OYVMVD on the physiology of okra plants, infected leaves were collected and subjected to further processing for measurement of electrolyte leakage and membrane stability index.

For electrolyte leakage 20 mg of healthy and infected leaves were collected separately. Leaves were cut in small pieces of small disc 20mm. 10ml deionized water were in tube containing leaves sample were water bathed at 45°C temperature and EC was measured it was ECa. And then water bathed at 55c temperature this was ECb and after this these were mixed in third tube and water bath at 100c temperature and EC was measured it was ECc.

Formula= (ECb- ECa/ ECc) X1OO

For membrane stability index 200mg leaves were collected infected and healthy both and was cute in small pieces of 20 cm disc. 10 ml double distilled water was taken in tube and water bathed at 40c temperature and Ec was measured this was EC1 and then water bathed at 100c temperature and EC was measured this was Ec2. MSI was measured by Formula.

Formula: (1-(C1/C2) x 100

Management

For the management of OYVMVD buprofezin, citric acid and neem extract was applied at weekly intervals. Data were recorded before and after the application of treatments.

Statistical analysis

Data was analyzed statistically by using software statistix 8.1. All possible interactions was determined through ANOVA and the treatment was compared by LSD test at 5% level of probability (Steel et al., 1997).

Results

Least infected variety was Green wonder followed by PMS-Beauty. The highly infected variety was Laxmy followed by Parbhani karanti.

Table. 1. Assessment of OYVMVD in the field

Variety	Disease incidence (%)	Disease severity
Laxmy	50	41.2
PMS-Beauty	30	19.6
Parbhani Kranti	47	41.8
Green Wonder	28	16.4
Selection-31	41	31.9

Data is the average of four values

More electrolytes leaked from the membranes in infected plants as compared to healthy plants. Due to electrolyte leakage, the membranes of the infected plants were less stable as compared to the membranes of the healthy plants.

Table. 2. Effect of OYVMVD on plant physiology

Sample	Electrolyte leakage (%)	Membrane stability (%)
Healthy	8.84	57.3
Infected	29.1	33.47

Table. 3. Management of OYVM disease through different treatments

Serial No.	Treatment	Disease incidence %	Disease severity %
1	Citric acid	34.74 b	48.46 b
2	Neem Extract	27.18 d	37.31 d
3	Buprofezin	30.33 c	42.85 c
4	Control	55.62 a	57.14 a

*Different letters are significantly different at 5% level of probability, LSD value 1.2

Plants treated with neem extract showed minimum disease incidence (27.18%) and disease severity (37.31%) as compared to control (Table. 3). Buprofezin caused significant whitefly mortality and consequently disease incidence and severity also reduced as 30.33% and 42.85%, respectively. The use of citric acid also gave considerable reduction in disease incidence and severity by stimulating plant defense systems. The disease incidence and severity in citric acid treated plants were recorded as 34.74% and 48.46%, respectively.

Discussion

Under diseased conditions, the survival of plant depends upon the strengthening of cell membrane (Blum and Ebercon, 1981). Lipid peroxidation indicates the extent of cell membrane damage by free radicals (Smirnoff, 1995). In diseased tissues high electrolyte leakage reduced the membrane stability index was recorded. Exogenous application of citric acid decreased the EL and increased MSI thus managing the oxidative stress damage (Hu et al., 2016). In affected plants, the activity of SOD (Superoxide dismutase) is reduced (Gong et al., 1997). SOD serves as protection against oxidative stress (Ostrovskaya et al., 2009). The foliar spray of citric acid enhance the SOD role in reducing the oxidative damage (Hu et al., 2016) by converting hydrogen peroxide into water (Sharma et al., 2013). Citric acid application also increase peroxidase (POD) and catalase (CAT) enzyme activities. These enzymes reduce membrane leakage by decreasing hydrogen peroxide production. Citric acid application improves plant defense by increasing phytohormones synthesis and nutrient uptake functions of the roots (Liu et al., 2002). Citric acid boosts up chlorophyll production that mitigates the photosynthetic losses caused by OYVMV disease (Kyle, 1987).

The reduction in OYVMV disease prevalence in buprofezin treated plants was recorded. Buprofezin deteriorates the development of whitefly young ones (Yasui et al., 1987). It enters into the insects through inhaling process and its high vapour pressure interrupts with insect physiology (De Cock et al., 1990). Buprofezin may reduce the egg hatching ability of whitefly when contacted with the sprayed foliage (Ishaaya et al., 1988). Buprofezin inhibits chitin synthesis and effectively control development of nymph (Palumbo et al., 2001). Use of insect growth regulator at early stages of the plant would be more effective against whitefly in Arizona because do not harm the natural enemies (Ellsworth et al., 2001). Zidan-Lobna et al., 2013 stated that buprofezin has translaminar activity and good in controlling the whitefly. Das and Islam, 2014 evaluated different insecticides against whiteflies and concluded that buprofezin caused maximum mortality of the insects.

Although chemical control is easy, direct and rapid action to solve pest and disease problems but continuous dependence on pesticides has contributed towards environmental pollution and degradation (Singh and Bhat, 2003). Chemical control is expensive

(Palumbo et al., 2001) and has become less effective due to the development of resistance against insecticide in insects (Siebert et al., 2012). Plant based pesticides are safe to use and also prevent the development of resistance in insects (Abou-Yousef et al., 2010). The azadirachtin in neem aqueous extract interrupt with defense system of whitefly. Plant extract also deter the insects from the fields (Pun et al., 2003). In current experiment, the extract of *A. indica* (neem) was very effective against the *B. tabaci* population and OYVMV disease incidence. The constituents of neem extract affect the physiology of insects (Schaaf et al., 2000). The active ingredient of neem extract (Azadirachtin) disturbs the corpus cardiacum and blocks the activity of the molting hormone that reduces fecundity, molting, pupation and adult emergence (Ascher, 1993). Neem based pesticides azadirachtin, neema (liquid type) and neema-plus (pellet type) caused significant reduction in the rates of female oviposition, subsequent egg hatch and adult formation (Lynn et al., 2010). Neem extract significantly reduced the TLCVD incidence and severity (Bhyan et al., 2007). Neem and eucalyptus extracts controlled the *B. tabaci* as well as CLCuVD most effectively as compared to other plant extracts (Ali et al., 2010).

Conclusion

Citric acid improves the plant cells integrity by reducing the electrolyte leakage and boosts up the defense system. Buprofezin and neem extract are effective against the whitefly population and thus minimizes the spread of OYVMV disease.

Contribution of Authors

Zeshan MA: Conducted research and write up
Iftikhar Y: Planned lay out of the experiment and provided germplasm
Ali S: Confirmation of OYVMV through whitefly and grafting
Yousaf M: Conducted management experiment
Ahmed N: Statistical analysis
Ghani MU: Evaluated Mean stability index and electrolyte leakage

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