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RESEARCH ARTICLE

ZUCCHINI YELLOW MOSAIC VIRUS: CHARACTERIZATION AND MANAGEMENT IN IRAQ

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Chenopodium amaranticolor .

ABSTRACT

A disease characterized by severe yellow mosaic, plant stunting, leaf reduction and distortion, and knobbed fruits, suspected to be of *Zucchini Yellow Mosaic Virus* (ZYMV) was observed on summer squash growing areas at different sites in Iraq. Samples of symptomatic plants were collected and tested by bioassay and by serological DAS-ELISA, using anti-ZYMV polyclonal antibodies. Results showed that the virus infects; *Cucurbita pepo*, *C. moschata*, *Cucumis melo*, and *Citrullus lanatus* giving the same symptoms observed on squash plants. Mild mottling with slight deformation of fruit was observed on *Cucumis sativus* sap-inoculated by the virus. *Chenopodium amaranticolor* developed chlorotic local lesions on sap-inoculated leaves. Necrotic local lesions were formed on *Euphorbia peplus* leaves sap-inoculated by the virus. The susceptibility of these hosts to the virus was confirmed by serological DAS-ELISA test. Based on these results, it can be concluded that the virus could be a strain of *Zucchini yellow mosaic virus* (ZYMV). The foliar application of chemical product on squash plants at 50 ppm one week before virus inoculation confer a protection period against ZYMV infection for up to 30 days. No symptoms were developed on the treated plants, as well as no virus was detected by ELISA test. Meanwhile, when the product applicated after 2 weeks of virus inoculation at 75 ppm, the virus was detected in the old leaves only for 3 days of inoculation, then it disappeared for up to 30 days. These results indicate that the product may induce the synthesis of compounds in the plants leading to inactivate the virus and inhibit its replication locally at the penetration site or systemically throughout the plant.

INTRODUCTION

Zucchini yellow mosaic virus (ZYMV), member of the genus potyvirus is one of the most economically important viruses on cucurbit crops worldwide (Lisa and Lecoq, 1984). The virus inducing, on squash, cucumber, watermelon, and melon, severe mosaic, severe stunting, yellowing, necrosis, leaf reduction, leaf and fruit malformations with heavy yield losses (Davis, 1986, Hseu *et al.,* 1987, Lisa and Lecoq, 1984, Provvidenti 1984, Wu *et al.,* 2009, Yuki *et al.,* 2000, Kwon *et al.,* 2005). Various isolates of ZYMV with different biological characteristics as determined on different hosts including cucurbit crops as well as other indicator plants have been reported (Kwon *et al.,* 2005, Lecoq and Purcifull, 1992, Lee and Wong, 1998, Wisler *et al.,* 1995, Yoon and Choi, 1998). ZYMV is transmitted by several species of aphids in non-persistent manner, among them, the species *Aphis gossypii* reported to be the most important and more distributed in the fields (Al-Shahwan, 1990, Perring *et al,* 1992, Sutic *et al.,* 1999). The virus (ZYMV) is composed of filamentous particles of 750 nm long, containing a positive-sens single strand RNA genome with viral protein covalently linked on 5´-terminal and poly (A) tail on 3´-terminal ends (Desbiez and Lecoq 1997, Dougherty and Semler 1993, Lisa *et al.,* 1981).

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Several methods were adopted to identify and characterize ZYMV in squash and other cucurbit crops. Bioassay, on test plants belong to Cucurbitaceae and other families, as well as double antibody sandwich using ZYMV specific antibodies were widely used for this objective (Bananej and Vahdat, 2008, Choi *et al.,* 2002, Oukie *et al.,* 2002). Control measures of plant virus disease were mostly restricted to early eradication of infected plants and vector control using specific insecticides, but these measures revealed ineffective for the viruses transmitted by aphids in non-persistent manner (Hull 2002). So the research was oriented toward searching of compounds more effective to manage plant virus diseases. It was reported that treating *Eggplant blister mottled potyvirus*-infected eggplant with Vit-org nutrient (0.5 ml/L) and the chemical product (2-nitromethyl phenol N,N- Diphenyl-1,1-biphenyl-4,4,4-Nitroethoxy phenol, diamine) 1 ml/L caused a reduction in virus concentration and suppression of disease symptoms development compared with non-treated plants infected with same virus (Al-Ani *et al.,* 2011). Various species of cucurbits especially watermelon, melon, cucumber and squash were widely cultivated in Iraq. Very destructive disease characterized by severe mosaic, stunting and deformation, which is a widely spread on squash in farmer fields causing heavy yield losses and very difficult to manage was observed. The objectives of this study were to identify the causal virus by biological and serological methodologies, and

to study the possibility to manage the virus through inducing systemic resistance in the plant by using the chemical product.

MATERIALS AND METHODS

Sample collection: Survey was undertaken in squash growing areas at many sites of Iraq (Baghdad, Diyala, Babylon, and Waset) during 2009 – 2010 growing seasons. Samples of squash plants showing, yellow mosaic, leaf curling and deformation, and fruit abnormalities were collected (approximately 100 samples). The samples were stored at -20˚C until use.

Test and host plants: Plants used were, *Cucurbita pepo*, *C. moschata*, *Cucumis sativus*, *C. melo*, *Citrullus lanatus*, *Luffa acutangula*, *Euphorbia peplus*, *Chenopodium amaranticolor*, and other belonging to Leguminaceae, Crucifereae, Compositae, Malvaceae, Polygonaceae, and Plantaginaceae. Seeds of these plants were sown in mix soil and peatmoss (2:1) in pots (20 cm diameter). The pots were placed in a glasshouse at 24-30˚C. The plants were watered daily and fertilized weekly with NPK fertilizer.

Virus inoculation: Leaves from systemically infected squash plants were homogenized in 0.02 M phosphatase buffer pH=7.2 (1 g/4 ml). The homogenate was filtered through double layer of muslin and the filtrate was used as virus inoculum. The inoculum was gently rubbed on the upper leaf surface of test and host plants previously dusted by carborandum (600 mesh) at 3-4 leaf stage. Additionally, a traverse cut was made in an infected fruit by a razor blade, the cut fruit surface was dusted with carborandum and smeared gently on leaf surfaces. The inoculated plants were maintained in the glasshouse and the plants were monitored weekly for symptoms development.

Insect transmission: Aphids *Myzus persicae* were collected from eggplants and passed successively for several times onto healthy squash plants. Non-viruliferous aphids were given an acquisition access periods of 15, 30, 60 min. on infected squash plants, then transferred to healthy squash plants (5 insects/plant) for inoculation access for 15, 30, 60 min in insect proof-cages under glasshouse conditions. The inoculated plants were monitored daily for symptoms developments.

Serological test: The virus was initially identified by serological double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA), by using anti-ZYMV polyclonal antibodies (Agdia, Elkhart, India) according to Clark and Adams (1977). Leaves from infected and healthy squash plants were grounded in phosphate buffer saline (PBS), (0.2 M Na_2HPO_4, 0.13 M NaCl, 0.003 M Kcl, ph 7.5)(1 g/ 10 ml buffer). The homogenate was filtered by double layer of muslin and used for ELISA reaction. The absorbances of ELISA reactions at 405 nm were measured, and twice of the absorbance values of healthy plants were considered positive.

Effect of the chemical product on virus multiplication

Determination of the effective concentration: A preliminary experiment was carried out to determine the more effective concentration of the product 2-nitromethyl phenol N,N-Diphenyl-1,1-biphenyl-4,4,4-Nitroethoxy phenol, diamine (obtained from Tariq Company for Chemicals, Iraq) against ZYMV replication among 4 concentrations 25, 50, 75, 100 ppm used. Three squash plants were sprayed by each concentration one week before virus inoculation, three plants were sprayed with water before inoculation as control. Three other plants were sprayed by the product after 2 weeks of virus inoculation. The treated plants were maintained in insect-proof cages in glasshouse.

Determination of protective period against ZYMV: Two groups of squash plants, each contain 24 plants of 2 weeks old were used in this experiment. Plants of the first group were sprayed by the product (3 plants/time) at 50 ppm and mechanically inoculated by the virus after 0, 1, 2, 4, 7, 14, 21 and 30 days of product application. Plants of the second group were inoculated by the virus and sprayed by the product (75 ppm) at the same periods used before. Plants sprayed by distilled water and inoculated by the virus were served as control.

Virus detection: The titers of ZYMV were determined by symptoms on indicator plants (*Euphorbia peplus*, *Ch. amaranticolor*) and by serological DAS-ELISA test as described previously. The upper leaves of treated plants were sampled, extracted in phosphate buffer saline (0.02 M, pH 7.5)(1 g/4 ml) and used for virus detection.

RESULTS

Host range: Of the 22 plant species tested for susceptibility to ZYMV by sap-inoculation, most species belonging to the family cucurbitaceae, namely *Cucurbita pepo*, *C. moschata*, *Cucumis melo*, *Citrullus lanatus*, and *Luffa acutangula*, one species to Chenopodiaceae, *Ch. amaranticolor*, and one to Euphorbiaceae, *Euphorbia peplus*, were found susceptible (Table 1). The virus caused severe yellow mosaic, blistering of leaf lamina, leaf reduction and deformation with deformed fruit covered with knobs on, *Cucurbita pepu*, *C. moschata*, *Cucumis melo*, *Citrullus lanatus*. Symptoms of veinbanding and yellowing with leaf reduction were developed on *Luffa acutangula* sap-inoculated by virus extract without deformation on leaves or fruits after one month of inoculation. Mild mottling with slight fruit deformation was observed on *Cucumis sativus*. *Ch. amaranticolor* developed chlorotic local lesions on the leaves sap-inoculated by crude extract from squash infected plants after 10 days of inoculation (Fig.1), which turned to necrotic after plant decays. Sap-inoculated leaves of *Euphorbia peplus* developed necrotic local lesions after 20 days of inoculation.

Fig. 1. Symptoms of chlorotic local lesions on *Ch. amaranticolor* leaf, sap inoculated by ZYMV

No symptoms were observed on plants of, *Lagenaria siceraria*, *Pisum sativum*, *Phaseolus vulgaris*, *Vicia faba*, *Vigna sinensis*, *Glycine max*, *Phaseolus mungo*, *Brassica napus*, *Helianthus annuus*, *Lactuca serriola*, *Chenopodium quinoa*, *Malva rotundifolia*, *Rumex dentatus*, *Plantago lanceolata*, which mechanically inoculated by virus extract. No reactions of extracts from these hosts with anti-ZYMV polyclonal antibody by ELISA were observed.

in the first 3 days then disappeared for up to 30 days when the product was used as curative agent (Table 3, 4).

Effect of the chemical product on virus replication and squash yield under field conditions: Results showed that the virus caused a yield reduction of 90 – 100% within 2-3 weeks in naturally infected squash plants (8th week old) (Fig 2, 3). Meanwhile the yield of treated plants was not affected and no virus was detected in these plants.

Table 1. Response of plant species to Zucchini yellow mosaic virus under glasshouse conditions

Family	Species	Infectious reaction
Cucurbitaceae	*Cucurbita pepo* L.	Systemic mosaic
	Cucurbita moschata Duchesne	Systemic mosaic
	Lagenaria siceraria (Mol.) Standl.	No infection
	Cucumis sativus L.	Systemic mosaic
	Cucumis melo L.	Systemic mosaic
	Citrullus lanatus (Thunb.) Matsum. & Nakai	Systemic mosaic
	Luffa acutangula (L.) Roxb	Systemic mosaic
Leguminaceae	*Phaseolus vulgaris* L.	No infection
	Pisum sativum L.	No infection
	Vicia faba L.	No infection
	Vigna sinensis L.	No infection
	Glycine max L.	No infection
	Phaseolus mungo L.	No infection
Cruciferae	*Brassica napus* L.	No infection
Compositae	*Helianthus annuus* L.	No infection
	Lactuca serriola L.	No infection
Euphorbiaceae	*Euphorbia peplus* L.	Necrotic local lesions
Chenopodiaceae	*Chenopodium quinoa* Willd.	No infection
	Ch. amaranticolor Coste & Ryen.	Chlorotic local lesions
Malvaceae	*Malva rotundifolia* L.	No infection
Polygonaceae	*Rumex dentatus* L.	No infection
Plantaginaceae	*Plantago lanceolata* L.	No infection

Table 2. Effect of product concentrations used as preventive or curative agent on ZYMV replication in squash plants cv. Opal type

Before virus inoculation Concentrations ppm				After virus inoculation Concentrations ppm			
25	50	75	100	25	50	75	100
+	-	-	-	+	+	-	-

(-) = No virus , (+) = Virus detected

Table 3. Protective period by the products to squash plants against ZYMV infection

Protective period (days)	Treated Plants									Untreated Plants								
Virus detective method	0	1	2	3	4	7	14	21	30	0	1	2	3	4	7	14	21	30
DAS-ELISA	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
Test plants	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+

(-) = No virus , (+) = Virus detected

Table 4. Effect of the product used as curative agent against ZYMV infection on squash plants cv. Opal type under glasshouse conditions

Effective period (days)	New developing leaves									Old leaves									Control								
	0	1	2	3	4	7	14	21	30	0	1	2	3	4	7	14	21	30	0	1	2	3	4	7	14	21	30
Virus detective method																											
DAS-ELISA	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	+	+	+	+	+	+	+	+	+
Test plants	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	+	+	+	+	+	+	+	+	+

(-) = No virus , (+) = Virus detected

Effect of the chemical product concentrations on virus replication under glasshouse conditions: No symptoms were developed on squash plants for up to 30 days when sprayed by the product at 50 ppm one week before virus inoculation. While a concentration of 75 ppm of the product was needed to inhibit virus replication when applied two weeks after virus inoculation (Table 2). No virus was detected in the new developing leaves of the treated plants for up to 30 days of applications, whereas the virus was detected in the old leaves

Spraying of plants suffered from severe deformation by the virus resulted in gradually disappearance of virus symptoms accompanied with increasing in yields to approximately 50% after 1 week of treatment, then reached to the same level of yield of the protected plants after 3 weeks of treatment.

DISCUSSION

A virus naturally infecting summer squash *Cucurbita pepo*, inducing severe yellow mosaic, prominent plant stunting, leaf

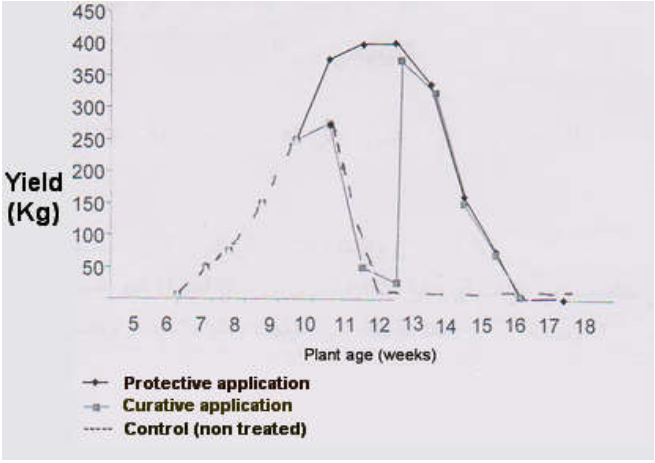

Fig. 2. Effect of chemical product applications on squash plant as protective and curative treatments on the yield

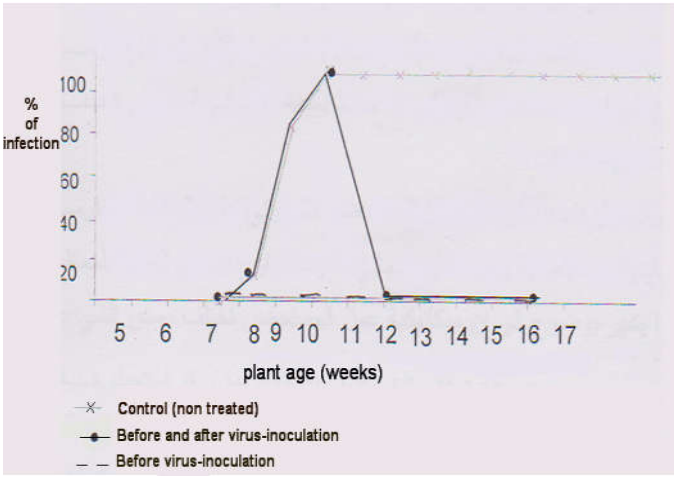

Fig. 3. ZYMV-disease development on squash plants in the field.

reduction, leaf and fruit deformations with heavy yield losses, was isolated from all areas that cultivated squash in Iraq. The virus was characterized at biological and serological levels. The host range of the virus was found restricted mainly to Cucurbitaceae, squash, melon, watermelon, luffa and cucumber. Similar results concerning the response of these hosts to ZYMV, were reported previously (Al-Shahwan 1990, Lisa *et al.,* 1981, Lisa and Lecoq 1984, Provvidenti, 1984, Stobbs *et al.,* 1990, Wong, 1994).

Unlike to squash, melon, watermelon, the virus induced veinbanding on *Luffa actangula* with slight leaf reduction, and mild mottling with slight fruit abnormalities on *Cucumis sativus*. The infectivity of ZYMV on *L. actangula* was reported by many researches (Lisa *et al.,* 1981, Lisa and Lecoq, 1984, Prieto *et al.,* 2001, Provvidenti *et al.,* 1983). The virus induced chlorotic local lesions on *Ch. amaranticolor* leaves. Similar response of this hosts to ZYMV was reported by other researches (Lisa *et al.,* 1981, Nameth *et al.,* 1986, Provvidenti 1984, Purcifull *et al.,* 1984). *Euphorbia peplus* developed necrotic local lesions on the leaves sap-inoculated by crude extract from virus-infected squash leaves. This host may represent a new host for this virus, which not reported by other researches in the literature. Based on symptomatology on test plants which confirmed by serological DAS-ELISA reaction, it is concluded that this virus could be a strain of Zucchini yellow mosaic virus (ZYMV). The restriction of ZYMV replication and suppression of disease symptoms on squash plants treated by the chemical product may be due to its contents of phenolic groups which may acts directly on the virus by linking to the viral proteins which leads to inactivate the virus and inhibit its replication. This idea was supported by the observation of no infection on sap-inoculated test plants when the product was added to the inoculums (results not shown). Other possible mechanism of slow down the virus replication is that the product acts as inducer of systemic resistance through activation of endogenous squash defense system. This resistance could be localized at the site of virus penetration by causing cell collapse around the site of infection known as Hypersensitive reaction (HR), as well as synthesis of small molecules, (e.g. phytoalexins) deposition of callus around the penetration site, and reactive oxygen species, which lead to localize the virus.

Modification of virus cell receptors at plasmic membrane level that inhibit the attachment of virus to these receptors is also possible. Several previous studies indicated that plant resist pathogen attack or slow down its growth by variety of biochemical and molecular defense at the penetration site of the pathogen (Baker and Orland, 1995, Karthikeyan *et al.,* 2005, Mehdi, 1994). The induced resistance may be systemic through synthesis proteins which may acts directly on the virus or indirectly through induced other proteins able to inactivate the virus. This ideas was supported by the observation of no infection when extract from new developing leaves of treated plant was mixed with virus inoculums. Various chemicals have been described that induce systemic resistance in the plants effective against broad spectrum of pathogens (Kessmann *et al.,* 1994, Morris *et al.,* 1998, Uknes *et al.,* 1992, Vallad and Goodman, 2005, Ward *et al.,* 1991). Since no effective methods to control and manage plant virus disease especially those transmitted by aphids in non-persistent manner, so, induce systemic resistance could be a promising way in plant virus management strategies.

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