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Full Length Research Paper

Antiviral activity of Vit-org, 2-nitromethyl phenol and Thuja extract against eggplant blister mottled virus (EBMV)

Rakib A. Al-Ani, Mustafa A. Adhab* and Kareem A. Hassan

Plant Protection Department, College of Agriculture, University of Baghdad, Iraq.

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Eggplant (*Solanum melongena*) is an important vegetable and widely cultivated in polyethylene covered houses in Iraq. A new virus, Eggplant blister mottled virus (EBMV), was isolated in previous study, from naturally infected eggplant and was characterized as potyvirus. The virus is considered now as the most important virus infecting eggplants and causing heavy damage to yields. This study was conducted to evaluate the effect of three products, Vit-org nutrient, 2-nitromethyl phenol, and Thuja extract on the multiplication of EBMV in eggplants. Results of the study showed that the application of the three products on EBMV-inoculated eggplants at 2.5 and 1 ml/L, and 6 g/L caused a reduction in ELISA reactions absorbance to 0.073, 0.091, and 0.092, respectively, which reflect a reduction in virus concentration. The application of the products on eggplants at the same concentrations followed by virus inoculation, after 24 h of protecting the plants against virus infection for 18, 10 and 12 days, respectively accompanied by retardation of symptoms development in 26, 16 and 20 days for the three products, respectively. The treated eggplants neither developed visible symptoms nor contained detectable concentrations of virus during these periods. Vit-org nutrient was found to be more efficient in protecting the plants against EBMV in both post and pre-applications.

Key words: Eggplant blister mottled virus (EBMV), eggplant, Thuja extract, Vit-org, 2-nitromethyl phenol.

INTRODUCTION

Eggplant (*Solanum melongena*) is an important vegetable and widely cultivated in green and plastic houses during winter months in Iraq. Several viruses are reported to infect eggplants naturally from different parts of the world (Sastry et al., 1974, Naqvi and Mahmood 1976, Igwegbe and Waterwooth 1982, Ladipo et al., 1988, Rajamannar and varma 1988, Aramburu et al., 2006, Dombrovsky and Pearlsman 2009).

A new virus Eggplant blister mottled virus (EBMV), was isolated from natural infected Eggplant in polyethylene covered cultivation and characterized as potyvirus based on its fluxuous particles morphology of 720 nm length in Iraq (Al-Ani et al., 2011).

EBMV is considered now as the most important virus of eggplants that is causing heavy damage in both quantity and quality of eggplant yields. It has been found that the virus is transmitted from infected to healthy plants mechanically by sap and by aphids *Myzus persicae* in non-persistent mode. Systemic symptoms characterized by leaf mottling, severe curling, blistering accompanied by stunting were manifested on eggplants upon inoculation by sap of symptomatic leaves. The virus induced necrotic local lesion on the inoculated leaves of *Zinnia elegans* and *Gomphrena globosa* (Al-Ani et al., 2011).

Several trials were undertaken to manage virus diseases by use of chemicals without significant success. The main obstacle to the development of effective chemotherapy, is the nature of virus multiplication in the host cells (Yarmolinsky et al., 2009), In addition to that some viruses persist in a latent infection in the host (Horvath 1983, Hull, 2002).

It was recently shown that the treatment of plants with plant extracts and synthetic chemicals can lead to the

*Corresponding author. E-mail: maa_adhab@hotmail.com.

induction of resistance agents, that characterized by restricting of virus multiplication and suppression of disease symptoms compared with untreated plants (Hammerschmidt, 1999; Al-Ani and Hassan, 2002; Al-Ani et al., 2002; Walters et al., 2005; Al-Ani et al., 2010).

This study was undertaken to evaluate the effect of *Thuja orientalis* extract, the 2-nitromethyl phenol N,N-Diphenyl-1,1-biphenyl-4,4,4-Nitroethoxy phenol, diamine, chemical compound, and the nutrient Vit-org (Green Co. Italy), on virus multiplication and disease development.

MATERIALS AND METHODS

The virus source

Leaves of EBMV-infected eggplants were collected and homogenized with 0.01 M phosphate buffer pH = 7.0 containing 0.2% of Na-Diethyl Dithio Carbamate (Na-DIECA) (1 g/ 4 ml) in a mortar with pestle. The homogenate was filtered through two layers of muslin and the filtrate was mechanically inoculated on to eggplant leaves (Al-Ani et al., 2011). The inoculated plants were maintained in insect-proof greenhouse until symptoms development.

Efficiency evaluation of some chemical products on EBMV

Thuja extract at 3, 4 and 6 g/L, a chemical product, 2-nitromethyl phenol N,N-Diphenyl-1,1-biphenyl-4,4,4-Nitroethoxy phenol, diamine (from Tariq Co., Iraq), at 0.5 and 1 ml/L, and the nutrient product, Vit-org (from Green Co., Italy), at 2.5 ml/L (containing 3% nitrogen, 6% potassium oxide, and 13% carbon), were used.

Thuja extract were prepared according to Al-Ani et al. (2010). Samples of *T. orientalis* (leaves, shoots, and fruits) were collected and dried in oven at 45°C for 7 days. The dried parts were ground by mortar and pestle. The powder obtained (100 g) was added to 250 ml of 80% ethanol in Erlenmeyer flask with agitation for 24 h at room temperature. The extract was filtered through filter paper (Whatman 2) in Buchner funnel with vacuum. The filtrate was concentrated to a consistent liquid in a waterbath at 40 to 45°C. Concentrations of 3, 4 and 6 g/L of the extract were prepared in water.

Inoculation and treatments

Eggplants at 4 leaves stage were mechanically inoculated by extracts from EBMV-infected leaves, and assigned into four groups. The inoculated plants of 3 groups were sprayed by each concentration of the three products after 48 h of inoculation (15 plants each). The plants of the fourth group were sprayed by distilled water as control. Healthy plants (15 plants) were left without treatment as control also. Young upper expanded leaves of the treated plants were collected at time course of 4, 6, 8, 10, 12, 14, 16, 18, 20 days of treatment. The leaves were homogenized with buffer (35 mM $NaHCO_3$, 15 mM Na_2CO_3 and 0.2% bovine serum Albumin (BSA), pH 9.6)(1 : 10 = g/ml) and centrifuged at 5000 rpm for 10 min. The supernatants were collected and used for virus detection by serological DAS-ELISA technique.

Virus purification

Pure isolate of virus was obtained by successive isolation of single lesion from *Z. elegans* grown in insect–proof cages in a greenhouse. The virus was then propagated in eggplants that serve as source for virus purification. The virus was purified according to the procedure described by Rowhani and Stace-Smith (1979) for potato leaf roll virus.

Serological assays

The virus multiplication in the treated plant was followed using double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (Clark and Adams 1977).

Anti-EBMV antibodies were prepared by 4 intramusclar injections of pure virus, (1 ml at 0.5 mg each) emulsified with an equal volume of Incomplete Freund's Adjuvant, in to a rabbit at interval of 10 days. The antiserum was collected from the rabbit through the ear marginal vein 15 days after the last administration. The antibodies were purified and conjugated to alkaline phosphatase according to deBokx and Vander Want (1987). Leaf extracts (200 µl) were transferred into wells of ELISA plates pre-coated with anti-virus antibodies. The plates were incubated at 37°C for 1 h and the wells washed three times with phosphate-buffered saline pH 7.5 containing 0.05% Tween-20 (PBST). The wells then were loaded with 200 µl of antirabbit immunoglobulin-alkaline phosphatase at 1:1000. The plates were incubated at 37°C for 1 h. The wells were washed three times as previously. The absorbance value of each sample was measured at 405 nm by ELISA-reader 1 h after the addition of the substrate (P-nitrophenyl phosphate at 1 mg/ml in 10% of diethanolamine pH 9.8).

Protection period determination

Groups of eggplants were sprayed at 4 leaves stage by; Thuja extract at 6 g/L, the chemical 2-nitromethyl phenol at 1 ml/L, and the nutrient Vit-org at 2.5 ml/L. The plant were then mechanically inoculated by an extract from EBMV-infected leaves 24 h after treatments. Samples of upper leaves of the plants were collected at interval of 2 days and homogenized with carbonate buffer, pH 9.6 as previously reported and used for virus detection by ELISA technique.

RESULTS

Results of ELISA-reactions with samples from treated plant indicated that the three products applied exhibited an inhibition effect on the multiplication of the EBMV in the plants. The more effective concentration were determined as 6 g/L for Thuja extract, 1 ml/L for 2-nitromethyl phenol, 2.5 ml/L for the nutrient Vit-org (Table 1). According to Table 1, the more effective product was found to be Vit-org with an absorbance value of ELISA reaction at 405 nm 0.073 after 10 days of application, compared with 0.092 and 0.091 for Thuja extract and 2-nitromethyl phenol, respectively after 14 days.

The absorbance values of ELISA reactions began to rise after reaching the minimum, more slowly with extracts from Vit-org treated plants than those treated with other products, but still exhibited an inhibition effect on the virus until 20 days of treatments. The absorbance values of ELISA readings were 0.411, 0.754, 1.420 and 1.840 for samples from plants treated with Vit-org, Thuja extract, 2-nitromethyl phenol, and EBMV-inoculated

Table 1. Effect of Thuja extract, Vit-org, and 2-nitromethyl phenol on multiplication of EBMV in eggplants.

Treatments foliage spray		ELISA readings at 405 nm according to days after inoculation								
		4	6	8	10	12	14	16	18	20
Thuja extract	3 g/L	0.375	0.410	0.490	0.592	0.791	0.981	1.121	1.121	1.521
	4 g/L	0.263	0.352	0.342	0.321	0.201	0.442	0.751	0.431	0.991
	6 g/L	0.273	0.340	0.241	0.111	0.092	0.121	0.194	0.225	0.554
2-nitromethyl phenol	0.5 ml/L	0.831	0.442	0.492	0.571	0.821	1.212	1.412	1.411	1.552
	1 ml/L	0.254	0.400	0.261	0.221	0.111	0.091	0.321	0.921	1.420
Vit-org	0.25 ml/L	0.253	0.172	0.078	0.073	0.077	0.091	0.121	0.172	0.411
Control/infected plant		0.425	0.725	0.972	1.231	1.372	1.552	1.891	1.891	1.840
Control/healthy plant		0.037	0.037	0.037	0.037	0.032	0.035	0.033	0.037	0.039

Table 2. Protection periods against EBMV infection by different treatments.

Treatments		2	4	6	8	10	12	14	16	18	20
Thuja extract	6 g/L	-	-	-	-	-	-	+	+	+	+
2-nitromethyl phenol	1 ml/L	-	-	-	-	+	+	+	+	+	+
Vit-org	2.5 ml/L	-	-	-	-	-	+	+	+	+	+
Control/infected plant		-	+	+	+	+	+	+	+	+	+
Control/healthy plant		-	-	-	-	-	-	-	-	-	-

(+) = Being infection, (-) = No infection. Each value in the table is a mean of 5 readings. The values in the table represent the absorbance at 405 nm.

non-treated plants, respectively after 20 days of application.

Protection period

The application of Vit-org nutrient at 2.5 ml/L, Thuja extract at 6 g/L, and 2-nitromethyl phenol at 1 ml/L has been found to confer a protection periods of 18, 12, and 10 days to the treated plants against EBMV-infection, respectively. No reactions were observed between anti-EBMV antiserum and extracts from treated plant during the periods mentioned above. Positive reactions began to appear at 20, 14, and 12 days of inoculation by the virus for the three compounds, respectively (Table 2), compared to 4 days with the samples from virus (EBMV)-inoculated, non-treated plants (control). The protection periods were accompanied by retardation of symptoms expression on the inoculated plants for 26 days with Vit-org nutrient, 20 days with Thuja extract, and 18 days with 2-nitromethyl phenol.

DISCUSSION

In the present study, we demonstrated the efficiency of pre and post applications of; Vit-org nutrient, 2-nitromethyl phenol, and Thuja extract on eggplants in reducing the multiplication of EBMV using DAS-ELISA protocol. The results showed that all products had reduced virus multiplication and retarding the development of disease symptoms in both pre and post applications.

Among the different products used against the viruses, it was found that the more effective one was the nutrient Vit-org which restricts virus multiplication up to 18 days followed by Thuja extract. The least effect on virus multiplication was 2-nitromethyl phenol as determined by ELISA-absorption at 405 nm. The restriction of virus multiplication was found accompanying with retardation of symptoms expression on the treated plants. The absorbance values of ELISA-reaction at 405 nm demonstrated protective and curative effects of these compounds.

Similar results were obtained in previous studies concerning the use of plant extracts to manage virus disease in both animals and plants (Al-Ani and Hassan 2002, Wannang et al., 2009; Yarmolinsky et al., 2009; Al-Ani et al., 2010).

The antiviral activity of the products used in this study is connected to their components which may acts directly by interaction with virus particles in early stage of infection and block the liberation of its nucleic acid that

lead finally to stop the virus multiplication. The curative action of these products may support this intention. These compounds may acts indirectly as a trigger of induce systemic resistance agents in the plants against the virus. The protective action of these compounds is in accordance with this phenomenon.

It was reported that plants can be induced to become more resistant to disease through treatment with various biotic (Pathogens, non-pathogens microorganisms), or non-biotic (Plant extracts, synthetic chemicals) agents (Hammerschmidt 1999; Walters et al., 2005). Shi et al. (2007) indicated that treated pumpkin plants with Osthol (a natural compound extracted from dried fruit of *Cnidii monnieri* Fructus.) could induce systemic resistance against powdery mildew. The resistance, induced or non-induced, is characterized by low concentration of virus accompanied with retardation of virus symptoms development.

Due to the infectivity of chemicals to control virus disease, the research in this field was focused on searching of products safe and innocuous and more effective to manage the virus disease. The results obtained in this study indicate that plant extracts may be promising in this direction.

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