Role of Bion in Reduce Replication of Zucchini Yellow Mosaic Virus in Cucurbita pepo L Plant

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Abstract

This study was conducted to evaluate the efficacy of foliar spray and irrigation by bion which induces systemic acquired resistance (SAR) in plant and determine the effect on Zucchini yellow mosaic virus in Cucurbita pepo L plant under field conditions. The study includes: Biological evaluation of virus by local lesion assay and serological evaluation by DAS-ELISA. Resultes of foliar spray and irrigation applications by Bion showed significant differences in Percentage of inhibition number of local lesions on chenopodium quinoa which were compared with control application (39.5). Serologically, DAS- ELISA values under 405 nm also showed significant differences compared with the control (1.41). foliar spray application by 2mM of bion was given the better results in inhibition of number local lesions (58.73%) and reduction of virus replication (68.08%) compared with all treatments. These finding suggested efficiency bion in enhancement systemic acquired resistance in squash plants against ZYMV under field conditions.

Key words - *Systemic acquired resistance, Zuccini yellow mosaic virus, bion, DAS-ELISA, Chenopodium quinoa*

I. INTRODUCTION

Squash crop (*Cucurbita pepo*L) it is cultivation in different parts of the world. Among those constrains was attacked by various diseases such as fungal and viral diseases, Recently viral diseases of Squash crop were considered the most important disease. There are more than 20 viruses infecting cucurbit crops [3]. *Zucchini yellow mosaic virus* (ZYMV) is one of the most important viruses

affect cucurbit production. It causes destructive diseases to a large variety of economically important plants including squash[11]. ZYMV was reported in many countries including Syria and It is one of the more common viruses in Syria [5].Morphological symptoms observed on leaves are vein clearing, yellowing, mosaic, and deformations of leaves [3]. ZYMV Causes yield losses and the loss ratio was 76.4% in Syria [10].

Viral diseases have become a menace to the farmers as well as to the scientist who are involved in the production of seeds and seeds quality [7]. Systemic acquired resistance (SAR) is a broad-spectrum defense system present in plants, which was realized as early as by Ray [12]. Several synthetic chemicals have been shown to be inducers of SAR response in variety of crop-pathogen systems [9,12]. In recent years, bion (benzothiadiazole) was used in inducing systemic acquired resistance against several pathogens includes fungi and due to the shortage of information in studies related to using bion in inducing resistance against the viruses and in order to extend our knowledge on the effect of this agent as a chemical inducer against ZYMV in squash plants. this study was carried out:

1- Evaluate the efficiency of foliar spray and irrigation for bion by detection of inactivation of virus replication by Bioassay.
2- 2- Evaluate the efficiency of foliar sprays and irrigation for bion by detection of inactivation of virus replication by Serological assay (DAS-ELISA)

II. MATERIALS AND METHODS

A. Sampling

Infected plants with suspected infected with *Zucchini yellow mosaic virus* were collected from one of the open fields in Syrian coastal region in the spring season.

B. Detection of Virus

Depending of artificial inoculation, symptoms occurred on indicator plants, and TBIA serological test (Tissue blot immune assay) [8], the virus was identified.

1. The Indicator Plants

In regard to virus detection used plants as show (table 1)

Table 1. The indicator plants used in detect of virus

English name	Scientific name	Symptoms
Squash	*Cucurbita pepo* L	Chlorotic local lesions, Systemic vein clearing, Yellowing, leaf deformation, dwarf plant (fig 3,4)
Cucumber	*Cucumis sativus* L	Yellowing, mosaic (fig 2)
Quinoa	*Chenopodium quinoa* Willd	Chlorotic local lesions; not systemic (fig1)
Globe amaranth	*Gomphrena globosa*	Local lesions, not systemic
Angled loofa	*Luffa. acutangula* L	Systemic mosaic

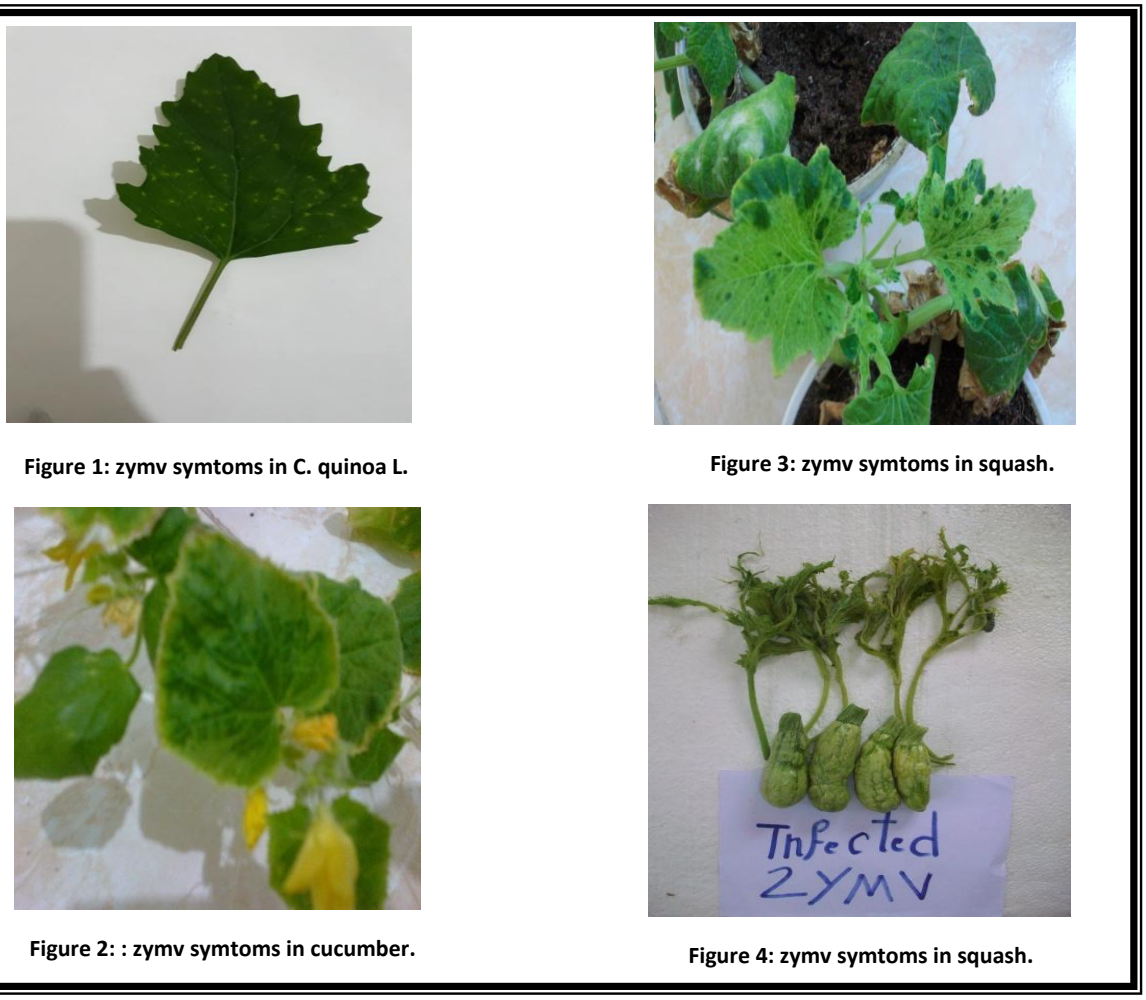

Figure 1: zymv symtoms in C. quinoa L.

Figure 3: zymv symtoms in squash.

Figure 2: : zymv symtoms in cucumber.

Figure 4: zymv symtoms in squash.

Figure: symptoms of ZYMV in indicator plants

2. Serological test (TBIA)

was performed by using viral antiserum for ZYMV (provided from Bioreba, Switzerland) according to Makkouk and Kumari[8].

3. ***Mechanical Inoculation***

The standard virus inoculum was prepared by using the squash leaves showing mosaic symptoms. The leaves (1g) were homogenized in 5ml of the phosphate buffer pH 7.0 in a prechilled pestle and mortar. The sap extract was passed through muslin cloth then, the filtrate was used as a source of inoculum. The cotton swab was dipped in the virus inoculum and rubbed over previously dusted with an abrasive powder (carborundum 600 meshes).. After inoculation the leaves were washed with water. phosphate buffer solution consist of (1.362 gm KH2PO4 in 1000 ml distilled water ,1.781g No2Hpho4. 2 H2O2 in 1000 ml water, 51 ml of the Na2pho4 solution mixed with 49 ml KH2Po4 solution gives solution pH 7.0[1]. The inoculated plants were kept at the greenhouse isolated from insects and watched daily to record any symptom which possibly appeared on the plants.

4. Propagative Plants

For propagating the virus, squash and cucumber plants were used as a propagate host for virus.

5. Preparation of Pure Isolate of ZYMV

The *Chenopodium quinoa* plants were inoculated with a diluted crud sap of infected squash leave, after 2day the local lesions occurred on the leave surface. Sap of single local lesion was extracted and used as inoculum for mechanical inoculation on Chenopodium quinoa. A serious of inoculation on a local lesion *Chenopodium quinoa* was done and pure isolate from the virus was obtained[1].

C. Preparation of bion solutions

bion was supplied by Novartis Company (USA) at 50% active ingredient in a wet- able powder formulation (benzothiadiazole, MW 210) was used. Each one of the 0.84g, 0.42g, 0.21g of bion were dissolved separately in 1 L of distilled water to obtain 2mM, 1mM, 0.5mM of bion solution respectively[6]. Foliar spray and irrigation applications were applied at age real five leafs.

D. field Experiment

1. Agricultural Processes

One house coverd with Muslin (to prevent the entry of insects) at dimensions (40x8) m was Prepared then; the agricultural processes were performed including tillage, leveling, installing drip irrigation system, fertilizating with organic manure and Mineral fertilizer and Soil sterilization by metam sodium before planting. The Experiment was divided into 6 lines and between one plant and another 50 cm, leaving space between the treatment and another 1m.

2. Distribution of the Treatments and Experimental Design

The Experiment was divided into 7 treatments, each treatment included 3 replicates, each replicate included five plants then, treatments were arranged randomly according to RCBD. The means were tested according to LSD (P=0.05). Data was analyzed by GenStat discovery 12th Edition with factorial design two way ANOVA with blocking and Duncan test [4].

4-3. ***Methods of applications:*** applications were applied at the stage of five real plant leaves 3 days before the mechanical inoculation, at a rate of 10 ml per plant for foliar spray applications and 30ml for irrigation applications. The experiment included 7 treatments as follows :

1- Control:Foliar spray with distilled water with mechanical inoculation by ZYMV.
2- Foliar spray with bion at concentration 0.5 mM/L with mechanical inoculation by ZYMV.
3- Foliar spray with bion at concentration 1 mM/L with mechanical inoculation by ZYMV.
4- Foliar spray with bion at concentration 2 mM/L with mechanical inoculation by ZYMV.
5- Plant irrigation with bion at concentration 0.5 mM/L with mechanical inoculation by ZYMV.
6- Plant irrigation with bion at concentration 1 mM/L with mechanical inoculation by ZYMV.
7- Plant irrigation with bion at concentration 2 mM/L with mechanical inoculation by ZYMV.

E. Biological evaluation

The effect of bion was quantified by local lesions assay on *Chenopodium quinoa*. The six equal leaves of *Chenopodium quinoa* plants were inoculated with the infected squash crude sap extracted for each treatment separately in field experiment. Two leaves of each plant represent a replicate and were rubbed with the infected squash crud sap of each replicate in the treatment. Number of the local lesions were counted to each inoculated leaf after 14 days of incubation. The average number of local lesion per replicate was calculated.The effect of bion on ZYMV was quantified by number of local lesions which form on *Chenopodium quinoa*. The percentage inhibition of local lesions on the inoculated leaves was calculated by using the following formula [13]:

$$I = \frac{(C-T)}{C} * 100$$

Where, I = percent inhibition of local lesion formation over control; C = Number of local lesions in control; T= Number of local lesions in plants treated with bion.

F. Serological evaluation DAS-ELISA

The virus concentration in the inducer-treated as well as the control plants was quantified after 14days of inculation by using Serological assy DAS-ELISA . three plants from each replicate were used for detecting the virus concentration. The three upper leaves of each plant (1g) were harvested and crushed with 1ml of the tissue extraction buffer in a mortar and pestle then, passed through 2 layers of muslin clothe, the extract was subjected to DAS-ELISA. Whereas DAS- ELISA technique was applied as described by Clarke and adams [2] by using viral antiserum for ZYMV as ELISA kit (provided from Bioreba, Switzerland)

percentage reduction of virus replication**:** was calculated by using the following formula:

$$I = \frac{(C-T)}{C} * 100$$

Where, I = percent reduction of virus concentation over control; C = virus concentration in control; T=virus concentration in plants treated with bion.

III.RESULTS AND DISCUSSIONS

A. Biological assay

The results of bioassay experiment showed that the number of local lesions occurred by foliar spraying and irrigation of bion treated plants were reduced significantly compared with number of local lesions formed on control plants (untreated) (table2). The results showed that foliar spraying is better than irrigation in reducing the number of local lesions. foliar spraying with 2mM of bion gave the best results 58.73% in inhibition the number of local lesions compared to all treatments.

Table 2. Effect of bion in inhibition number of local lesions formation on *C. quinoa*.

Treatment	No. of S.L.L/Leaf	%inhibition of NO S.L.L
ZYMV control	39.5	0
Bion 0.5mM spray+ ZYMV	28.4	28.1
Bion 1mM spray+ ZYMV	18.7	52.65
Bion 2mMspray+ ZYMV	14.3	58.73
Bion 0.5mM irrigation+ ZYMV	34.6	12.4
Bion 1mM irrigation+ ZYMV	29.2	26.07
Bion 2mM irrigation+ ZYMV	24.7	37.46
lSD%=0.05	1.476	

***Each value represents the mean of three replicates.**

B. Serological Evaluation

The DAS-ELISA values for bion treated squash plants showed significant reduction in the viral concentration means compared with control plants (table 3). . foliar spraying with 2mM of bion gave the best results in reducing the viral concentration (68.08%) compared to all treatments. The results showed that foliar spraying applications is better than at irrigation in reducing the viral concentration of ZYMV in sequash plant.

Table 3. DAS-ELISA values for treatments with bion

Treatment	Virus concentration	% reduction of virus replication
ZYMV control	1.41	0
Bion 0.5mM spray+ ZYMV	0.97	31.20
Bion 1mM spray+ ZYMV	0.68	51.77
Bion 2mMspray+ ZYMV	0.45	68.08
Bion 0.5mM irrigation+ ZYMV	1.14	19.14
Bion 1mM irrigation+ ZYMV	0.92	34.75

Bion 2mM irrigation+ ZYMV	0.78	44.68
lsd5%	0.21	

***Each value represents the mean of three replicates. **Absorbance at 405 nm. Positive control are:1.5**

IV.CONCLUSIONS

These finding suggested efficiency bion in enhancement systemic acquired resistance in squash plants against ZYMV under field conditions and reducing of virus replication and minimizing the loss of squash yield due to ZYMV infection.

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