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INHIBITION OF TOMATO YELLOW LEAF CURL VIRUS (TYLCV) BY EXTRACT OF ALGAE *CLADOPHORA CRISPATE*

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Abstract

Tomato yellow leaf curl virus (TYLCV) causes enormous economic losses in tomato production in Iraq, so it is important to search for an alternative method to control TYLCV as compared with using of insecticides against whitefly (*Bemisia tabaci*). The present study aims to evaluate the effect of the alcoholic extract of algae (*Cladophora crispate*) with the different concentrations 0.5, 1.0, 1.5% against TYLCV disease on tomato. Results show that the spraying with concentration of 0.5% was significantly superior from other treatments in increase of plant length 130.00 cm and leaf area 28.00 cm as well as decrease of disease incidence and disease severity which reached 20% and 0.23% respectively, then followed by the concentration 1.0% in increase of plant length 99.00 cm and leaf area 23.66 cm and decrease of disease incidence and disease severity 40% and 0.56% respectively and finally the concentration 1.5% in increase of plant length 72.00 cm and leaf area 11.66 cm, also decrease of disease incidence and disease severity 40% and 0.43% respectively as compared with control treatment that sprayed with only distilled water, which recorded in traits previous 72.00 cm, 11.66 cm, 100% and 2.03% respectively.

***Key words*:** TYLCV, *Cladophora crispate*, *Bemisia tabaci*

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most significant vegetables in the world, ranking second in importance after potato in many countries (Salim and Jasim, 2016). Major constraints to production of tomatoes are diseases caused by fungi, bacteria and viruses (Salim *et al.*, 2017; Jones *et al.*, 2016; McGovern, 2015). Apart from a number of bacterial and fungal pathogens which cause severe infections on tomato, it is infected by a number of viruses. Among the viral pathogens, Tomato yellow leaf curl virus (TYLCV) is the most destructive disease of tomato that cause severe disease in economically important crops, including tomato, with yield losses of up to 100%, Tomato yellow leaf curl, genus Begomovirus is belong to Geminiviridae family, the genome of the virus contains a single-stranded circular DNA and the length of the DNA is in all cases about 2800 nucleotides (Glick *et al.*, 2009). TYLCV is transmitted by whitefly *Bemisia tabaci* in a persistent manner (EPPO/CABI, 1996). Acquisition and inoculation feeding periods range from 20 to 60 min and from 10 to 30 min depending on the isolates (Mansour & Al-Musa, 1992; Ioannou, 1985; Cohen & Nitzany, 1966). Symptoms of TYLCV are appearing on the tomato after 2-3 weeks, the new growth of plants with TYLCV has reduced inter nodes, giving the plant a stunted appearance. The new leaves are also greatly reduced in size and wrinkled, are yellowed between the veins, and have margins that curl upward, giving them a cup-like appearance. Flowers may appear, but usually will drop before fruit is set (Melzer *et al.*, 2009). It is difficult to control of TYLCV, but the common method is based on using of extensive insecticide to manage vector insects (Palumbo *et al.*, 2001). But sole dependence on insecticides leads to environmental pollution and it is not effective, therefore other alternative methods need to be investigated. Induced systemic resistance (ISR) can be stimulated in many plants by biotic or abiotic elicitors that increase the capacity of the plant to resist pathogens (Murphy *et al.*, 1999; Beckers and Conrath, 2007). There are several strategies for control of plant viral diseases, but not effective in reducing or avoiding of viral infections as compared with chemical

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control of fungi and bacteria (Hadidi *et al.*, 1998). The demand for antiviral substances to control of viral diseases has increased, but until now few of them are available (Song, 2010). Some plants contain a wide variety of natural stimulating compounds that have the potential to stimulate plant resistance against pathogens besides these plant materials are natural, effective, eco-friendly and can be used to resist plant viruses (Elsharkawy *et al.*, 2015). Plants are important sources of antimicrobial compounds (Das, *et al.*, 2010). Some antiviral compounds have also been isolated from plants, algae and Lichens (Abonyi, *et al.*, 2009). Over the last decades, research has shown that algal species have anti-microbial, anti-viral, anti-oxidant (Reichelt and Borowitzka, 1984; Pangestuti and Kim, 2011) Cladophora is a genus of green algae which contains many species that are very hard to classify, mainly because of the great variation in their appearances, which is affected by environmental conditions, habitat and age (Gestinari *et al.*, 2010). New anti-virus substances has been detected that include proteins, polyphenols, polysaccharides, flavonoids, alkaloids and oils from plants also polysaccharides and proteins from micro-and macro algae (Zhao *et al.*, 2017). In spite of no effective substances that can be used as effective viricides, so there is a continuous demand for the production of chemical substances for the purpose of stopping and treating viral infections in plants (Waziri, H., 2015). Therefore the present study was conducted to assess alcoholic extract of algae (*Chladophora crispate*) that spread in the sweet water and marshes against TYLCV disease on tomato.

Materials and methods

Collection of samples

The seedlings (*cv.* Huda) were obtained from Maysan nursery, while the virus isolate (Tylcv-Bsr) was obtained from Dr. abdulkareem kassim jabar, Faculty of Agriculture / University of Misan which Preserved on the Tomato plants under muslin protected cages, whereas the algae (*Cladophora crispata*) from Dr. Abdul Amir Obaid, College of Agriculture, University of Basra

Preparation of algae extract

5 gm of the algae powder was placed in 100 ml of ethanol 70% by using a magnetic stirrer for (24 hrs), the extract was filtered through filter paper (0.45 µ), the extract was placed in the petri dishes that exposed to the air under laboratory conditions in order to evaporate the alcohol, the remaining extract that represents the extract of algae was kept into a refrigerator (Obaed, 2015)

Pots experiment

This experiment was conducted at the Faculty of Agriculture / University of Misan, Iraq under the greenhouse conditions during October 2017- June 2018. Seedlings of the tomato at age 60 days were transplanted into plastic pots (20×20 cm) containing 2 kg of sterilized soil with Peat moss 3:1 under muslin protected cages. The experiment was laid out in a complete randomized design CRD with four treatments included three concentrations of algae extract 0.5, 1.0, 1.5%, the control plants were treated with distilled water with five replications and each of them contain 1 plant , the data was analyzed by SPSS Program. Adults of whiteflies (*Bemisia tabaci*) that virus-free were reared on cotton plants *Gossypium hirstum* under muslin protected cages (100 × 100 × 100 cm). Numbers of whiteflies were given an acquisition feeding period for 24 hrs on virus infected tomato plants Tylcv-Bsr, then transferred to healthy plants (10 insect / plant) at 2 leaf stage for a duration 24 hrs feeding period. After the appearance of virus symptoms during 2 weeks, the plants were sprayed with algae extract concentrations (0.5, 1.0, 1.5%) three times every 15 days, all the plants were maintained in insect proof cages in the greenhouse. The following measurements such as plant length, leaf area, disease incidence and disease severity were recorded 40 days post symptoms appearance. Data of disease incidence was recorded with the help of following formula

$$\text{Disease incidence} = \frac{No.of\ \text{inf}\ ected\ plants}{Total\ no.of\ plants} \times 100$$

The symptom severity scale described by (Friedmann *et al.* 1998) as follows:

0 = no visible symptoms, inoculated plants show same growth and development as non-inoculated plants.

1 = very slight yellowing of leaflet margins on apical leaf.

2 = some yellowing and minor curling of leaflet ends.

3 = a wide range of leaf yellowing, curling and cupping, with some reduction in size, yet plants continue to develop.

4 = very severe plant stunting and yellowing, pronounced leaf cupping and curling, and plant growth stops.

Disease severity index was calculated by the following formula (Raupach *et al.* 1996).

Disease severity index =

$$\frac{Disease\ grade \times number\ of\ plants\ in\ each\ grade}{Total\ number\ of\ plants \times highest\ disease\ grade} \times 100$$

Inoculated tomato plants were tested by ELISA to confirm TYLCV infection (Clark Adams, 1977).

Results and Discussion

The results in Table 1 showed that spraying of algal extract with the different concentrations 0.5, 1.0, 1.5% was the best in reducing the virus infection as compared with control, where the concentration of 0.5% was significantly superior from other treatments in increase of plant length and leaf area and decrease of disease incidence and disease severity which reached 130.00 cm, 28.00cm, 20% and 0.23% respectively followed by the concentration 1.0%, which recorded in traits previous (99.00 cm, 23.66 cm, 40% and 0.56%) and concentration 1.5% (83.33 cm, 17.66 cm, 40% and 0.43%) as compared with control (72.00 cm, 11.66 cm, 100% and 2.03%) respectively. Several plants have resistance mechanisms which induce by the treatment with extracted substances from other plants and leads to the formation of chemical substances that prevent the virus replication , thus stop the development of symptoms on plants and the existence of proteins in some plants indirectly prevents infection of virus, these proteins are not anti-virus, but induce the plant systems to produce new proteins in the treated plants, which are the actual anti-virus proteins (verma *et al.,* 1998). These proteins may be effective in giving of signaling to activate of defense mechanisms in sensitive plants, this is called systemic resistance inducers. Al-shakankerya, (2014) reported that the algae extract consists of a wide range of active compounds including (organic acids, amino acids, vitamins, hormones, enzymes), this supports our results in reducing the damage of TYLCV virus in addition to improve the health plant status, such as plant height, leaf area as well as corresponds with verma, *et al.,* (1998) they reported that bioactive compounds in plant products act as elicitors or induce for resistance in host plants. The effect of these substances may be direct or indirect on the virus through inducing systemic resistance in the plant against the virus, which may persist for long periods depending on both of plant cultivar and the virus strain (Mahdy *et al.,* 2007, AL-Jerisi, 1998, Kessman *et al.,* 1994). These results are in accordance with Salim *et al.,* 2016 where they reported that Newton cultivar was susceptible to infection by TYLCV virus, whereas other cultivars such as Magda, GS12 and Defnis were tolerant to infection in this disease. The plant extracts may play an essential role in integrated management of plant viruses in the future. Zaid *et al*., (2016), Mohamed *et al.,* (2012), El Gamal, (2010) and Pulz and Gross (2004) highlighted on the wide spectrum for marine macro algae and their antiviral effects. Separated polysaccharides from brown algae have efficient antiviral activities through blocking viral adsorption at the plant cell membrane (Sano, 1999; Pardee *et al*., 2004; Jiao *et al*., 2011), while Polysaccharides from brown algae led to inhibit potato virus X (PVX) at 95% with a concentration of 10 mg/ml (Pardee *et al*., 2004). Mechanisms of antiviral-algal effects are based on a combination between the polysaccharides with the target host cell to block the viral entry (Feldman *et al*., 1999), or through inhibition of the virus adsorption on host cells by competing with the virus binding (Duarte *et al*., 2004).

Table 1: Effect of different concentrations of alcoholic algae extracts on plant length, leaf area, disease incidence and disease severity of TYLCV virus on tomato.

Treat-ments	Plant length	Leaf area	Disease incidence %	Disease severity %
0.5	130.00 a	28.00 a	20 b	0.23 a
1.0	99.00 b	23.66 b	40 b	0.56 bc
1.5	83.33 c	17.66 c	40 b	0.43 dc
Control	72.00 d	11.66 d	100 a	2.03 e
LSD 0.5	1.598	0.912	59.96	0.818

Conclusion

Thus, it can be concluded that algae extract played an important role in reducing the severity of tomato yellow leaf curl virus infection in addition to improve some of characteristics, such as plant height, leaf area in tomato plants.

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