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Research Article

Inhibition of *Tomato Yellow Leaf Curl Virus* by *Zingiber officinale* and *Mentha longifolia* Extracts and Silica Nanoparticles

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ABSTRACT

Tomato yellow leaf curl virus (TYLCV) causes huge economic losses in tomato production in Egypt. The control of TYLCV is extremely difficult because it is easily transmitted by whitefly (*Bemisia tabaci*). The only available method to control TYLCV is primarily based on the use of insecticides. Consequently, it is important to search for an alternative method to control TYLCV. The potentials of silica nanoparticles and the extracts of ginger (*Zingiber officinale*) and horsemint (*Mentha longifolia*) to enhance systemic resistance against TYLCV were investigated under pot and field conditions. Disease severity was significantly reduced in tomato plants treated with ginger and horsemint extracts and silica nanoparticles. Treated plants exhibited less and delayed virus symptoms compared with non-treated control plants. Coat protein gene of TYLCV was not founded in treated plants compared with control plants. Similarly, virus concentration was also reduced in plants treated with ginger and horsemint extracts and silica nanoparticles using ELISA. The activities of peroxidase, polyphenol oxidase were significantly increased in comparison with control plants. All treatments significantly increased growth of tomato plants and quality characters of tomato fruits. This is the first report of silica nanoparticles and ginger and horsemint extracts as elicitors to suppress TYLCV infection in tomato plants.

Keywords: *Tomato yellow leaf curl virus*; *Zingiber officinale; Mentha longifolia;* silica nanoparticles; induced systemic resistance

INTRODUCTION

Tomato is considered the most widely grown and consumed vegetable in Egypt. However, tomato is susceptible to different plant pathogens including fungal, bacterial and viral pathogens. Among viral pathogens, *Tomato yellow leaf curl virus* (TYLCV, genus: *Begomovirus*, family: *Geminiviridae*) is economically one of the most devastating pathogens in the tropical and subtropical regions [1]. The reduction of tomato yield attributed to TYLCV ranges between 28-100% depending on the age of the plants at the time of infection and the percentage of infected plants and disease severity [2,3]. Commercial cultivars of tomato still lack effective resistance against the virus and the introduction of resistant genes into commercial cultivars by conventional breeding requires years of selection to eliminate unfavorable characteristics, even when a natural source is available [4]. The main obstacle to develop an effective chemotherapy is the nature of virus multiplication in the host cells [5]. Additionally, some viruses persist in latent infection in the host [6,7]. The common and available method to control TYLCV was to spray plants with insecticides, like Cypermethrin (0.01%) or Dimethoate (0.1%), to control its vector *Bemisia tabaci* [8]. But the undesirable effects of insecticides on human health and the environment have encouraged researchers to find alternative methods to control TYLCV infection. In this respect, management of TYLCV by plant extracts is relatively new, ecologically sound and environmentally safe. Hence, this study was mainly concentrated on the development of effective strategies for the management of TYLCV in tomato using plant extracts and silica nanoparticles.

Recently, a class of molecules called elicitors has been used to induce systemic resistance against different plant pathogens including fungi, bacteria and viruses [9-16]. Several natural and synthetic agents have been used such as benzothiadiazole-7-carbothioic acid-*S*-methyl ester (BTH), 2, 6-dicholroisonicotinic acid (INA), chitin and Eugenol [17-21]. Induction of plant defenses by natural elicitors such as plant extracts was implemented in the management of plant virus disease [22,23]. Although, systemic resistance against plant viruses was enhanced by plant extracts, the underlying mechanism has not been elucidated. Therefore, the objectives of this study were to assess the antiviral activity of some medicinal plants (*Zingiber officinale* and *Mentha longifolia* extracts) and silica nanoparticles against TYLCV in tomato and to evaluate the effects of the medicinal plants and silica nanoparticles on oxidative enzymes (peroxidase and polyphenol oxidase) as well as morphological and physiological characteristics of tomato plants infected with TYLCV such as plant height, leaf area, number of branches, fresh and dry weight, chlorophyll contents and yield (number of flowers and fruits per tomato plant). To the best of our knowledge, this is the first report on using the aqueous extracts of ginger and horsemint and silica nanoparticles against TYLCV infection in tomato.

MATERIALS AND METHODS

Isolation and identification of virus isolate

Tomato plants (*Lycopersicon esculentum* var. Alissa from Nunhems Seeds Ltd.) were grown in a greenhouse (25°C, with 12h dark, 12h light photoperiods). Six leaf stages were used for *Tomato yellow leaf curl virus* (TYLCV) infection.

An infectious clone of TYLCV in *Agrobacterium tumefaciens* was kindly provided by Agricultural Botany Department, Faculty of Agriculture, Kafrelsheikh University, Egypt. *Agrobacterium tumefaciens* strain was inoculated in YEP medium as described by Wang and Fan [24]. Puncture inoculation method was used for *Agrobacterium tumefaciens* inoculation [25].

Extract preparation

Rhizomes of *Zingiber officinale* and leaves of *Mentha longifolia* were purchased from the Research of Medicinal and Aromatic Plants, Institute of Horticultural Research, National Research Center (NRC), Giza, Egypt. About 500 g rhizomes of *Zingiber officinale* and leaves of *Mentha longifolia* were washed in tap water and then in sterile distilled water and were air dried at room temperature in dark conditions. The dried plants were milled to a fine powder in a mill and stored at room temperature in glass containers until used as described by Sultana et al. [26].

Silica nanoparticles

Nanosilica was obtained from Egypt Nanotech Company Limited, Cairo, Egypt. The size of the used nanosilica was 100 nm with a purity of 99.99%. Nanosilica used as spray solutions at concentration levels of 50, 100 and 200 μgmL^{-1}.

Pot experiment

The efficiency of plant extracts and silica nanoparticles in controlling TYLCV in tomato was assessed under greenhouse conditions. Tomato seeds were surface sterilized with 2% sodium hypochlorite. The seeds were dried under shade for 30 min and used for sowing. Tomato plants at six-leaf stage were selected for TYLCV infection. Spray treatments with extracts of *Zingiber officinale,*

Mentha longifolia and silica nanoparticles were applied at 50, 100 and 200 µg mL^{-1} to 'run-off' with a hand sprayer at 1 day before TYLCV inoculation. Foliar spray with distilled water was carried out as the control treatment. Fifteen plants were used per each treatment and the experiment was repeated in triplicate.

Field experiment

Induction treatments were evaluated also under field conditions. Three field trials were conducted in randomised complete block design with three replications during 2016-2017 in different regions of Kafr El-Sheikh, Egypt, which are endemic areas for TYLCV incidence. A standard plot size of 4 × 3.5 m^2 was maintained for all treatments. Foliar sprays were conducted as described previously.

Effect of plant extracts and silica nanoparticles on TYLCV disease

Disease severity was recorded one month after TYLCV inoculation. Disease severity was measured after the inoculation based on a scale of 0–4 as described earlier by Lapidot et al. [27]. A severity index was calculated, as described earlier by Raupach et al. [28] from the disease rating using the formula:

Disease severity index= [∑ (Rating no. × No. of plants in rating) × 100%] / (Total no. of plants × Highest rating)

The efficacy percentage (%) was determined by the following formula:

(Disease index of Ck1− Disease index of Pt1/Disease index of Ck1) × 100

where Ck1 is the control after spraying water; and Pt1 is the treatment.

DAS-ELISA

Double Antibody Sandwich Enzyme Linked Immunosorbent Assay (DAS-ELISA) was used for rapid detection as described by Elsharkawy et al. [11]. ELISA Kits were supplied by SANOEL, Santee Animal, Paris, France. In tomato, the second, third, fourth and sixth youngest leaf from the top of treated and untreated control of all the pot and field trials were collected randomly from six different plants for each replication and weighed to one gram and extracted using extraction buffer. The collected samples were placed individually into wells of a polystyrene microtiter plate, triturated with the blunt end of a glass rod with 250 ml of ELISA extraction buffer (0.01 M sodium phosphate buffer, pH 7.4 containing 0.02% sodium azide (w/v) 0.1% sodium chloride (w/v) 0.05% Tween 20 (v/v) and 2% polyvinyl pyrrolidone (w/v) added per well. Similarly, TYLCV infected tomato leaf tissue sap was diluted in ELISA extraction buffer and further steps were carried as normal DAS-ELISA. Before loading to the wells the protein concentration was adjusted to 250 mg/ ml in all the samples and a standard DAS-ELISA protocol was followed. 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 described. 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).

Detection of coat protein gene of TYLCV

TYLCV was detected using PCR according to Navot et al. [28]. The oligonucleotide geminivirus specific primers for all Eastern Hemisphere Whitefly-Transmitted Geminivirus (OWTGs) were C Forward: 5`-CAGTCCGTTGAGGAAACTTAC-3` C Reverse: 5`CCCACCAATAACTGTAGC-3`. Nucleic acid spot hybridization (NASH) was carried out for the detection of TYLCV concentration in the treated and untreated tomato plants according to Navot et al. [29].

Evaluation of defense-related enzymes activities

Peroxidase and polyphenol oxidase were measured on tomato plants treated with plant extracts and silica nanoparticles after 1 and 2 weeks after viral infection. The measurement process was as follows:

The upper tomato leaves (0.5 g) were harvested and immediately frozen in liquid nitrogen and stored at −80°C. The activity of Peroxidase (POD) was measured according to the method described by Sudhamoy et al. [30], while the activity of Polyphenol Oxidase (PPO) was assayed as described by Flurkey et al. [31]. The experiments were repeated at least thrice with three plants per each treatment.

Evaluation of plant growth and yield

Plant growth parameters, *i.e.* plant height, leaf area, fresh and dry weight, flowers number, fruits number, fruits weight and chlorophyll contents were measured. Moreover, quality characters of tomato fruits such as acidity, Total Soluble Solids (T.S.S) % and vitamin C mg/ 100 ml juice were recorded at the harvest time.

Statistical analysis

XLSTAT Pro statistical analysis software (Addinsoft, New York, NY, USA) was used for Analysis of Variance (ANOVA). Treatment means were separated using Fisher's Least Significant Difference (LSD) test [32]. All analyses were conducted at significance value of $P \leq 0.05$.

RESULTS

Effect of leaf extracts and silica nanoparticles on TYLCV disease

Plants were sprayed with plant extracts of ginger and horsemint and silica nanoparticles 1 day before TYLCV inoculation, to control TYLCVD in tomato plants (Table 1). The lowest disease severity was achieved using a concentration of 200 µgmL^{-1} in all treatments under pot and field experiments. Ginger extract was more effective than the extract of horsemint. The protective activity of plant extracts was more effective than the silica nanoparticles activity against TYLCV.

Serological detection using DAS-ELISA

The leaves from treated plants recorded lower virus concentrations compared to untreated control plants. Silica nanoparticles treated

Table 1: Efficacy of plant extracts and silica nanoparticles on controlling of TYLCV in tomato plants.

Treatments	Dosage (µgmL^{-1})	Pot experiment	Field experiment
Zingiber officinale	50 µgmL^{-1}	65.3b	48.6a
	100 µgmL^{-1}	58.7c	41.1b
	200 µgmL^{-1}	51.3d	35.2c
Mentha longifolia	50 µgmL^{-1}	71.2a	51.7a
	100 µgmL^{-1}	64.4b	43.3b
	200 µgmL^{-1}	56.8c	36.4c
Silica nanoparticles	50 µgmL^{-1}	57.3c	34.5c
	100 µgmL^{-1}	49.5d	29.7d
	200 µgmL^{-1}	40.1e	24.3e

plants showed higher virus concentrations compared to plant extracts (Table 2). TYLCV concentration in tomato leaves sprayed with ginger extracts was lower than horsemint-treated plants (Table 2).

Detection of coat protein gene of TYLCV

Coat protein gene was not founded in tomato plants treated with ginger and horsemint extracts and silica nanoparticles. In contrast, coat protein gene was founded in control plants (Figure 1).

Activity of defense-related enzymes

POD and PPO activities on tomato leaves were measured at 1 and 2 weeks post TYLCV inoculation. The activity of POD significantly increased 3-fold in leaves treated with plant extracts compared with control leaves. The activity of PPO significantly increased 2-fold in tomato plants treated with plant extracts compared with control plants (Table 3). The activities of POD and PPO in leaf extracts-treated tomato plants were also higher than silica nanoparticles-treated plants.

Effect of ginger and horsemint extracts and silica nanoparticles on growth and yield characters of TYLCV infected tomato plants

Data presented in table 4 indicated that ginger and horsemint extracts as well as silica nanoparticles showed positive effects on growth parameters of tomato plants. Data showed that the treatment of ginger and horsemint extracts (200 µg mL^{-1}) exceeded the other treatments in plant growth characters. Silica nanoparticles gave an intermediate values, while the control treatment recorded the lowest values for all the studied parameters. Ginger extracts at 200 µg mL^{-1} exhibited the highest plants (91.2 cm), highest fresh and dry weight (447.9, 75.3, respectively) and the highest flowers and fruits number (115.3, 38.6, respectively).

Ginger and horsemint extracts and silica nanoparticles significantly increased number of flowers and fruits and fruits weight per plants as well as quality characters of tomato fruits such as acidity, total soluble contents and vitamin C compared with the control. Ginger and horsemint treatments achieved the best results in this respect followed by silica nanoparticles which was also significantly higher than the control. No significant difference was recorded between ginger and horsemint treatments (Table 5).

DISCUSSION

Induced systemic resistance to plant diseases has been well known for many years. The search for new inducing agents has intensified in recent years. The natural compounds as inducers of disease resistance could play a key role in this respect. Recently, there has been increasing interest in plant-induced resistance as new, environmentally friendly methods for disease control. In this study, we demonstrated that ginger and horsemint extracts and silica nanoparticles could greatly influence tomato resistance to TYLCV.

Table 2: Effect of Zingiber officinale and Mentha longifolia extracts and silica nanoparticles on multiplication of TYLCV.

Treatments	Dosage (µgmL^{-1})	Pot experiment	Field experiment
Zingiber officinale	50 µgmL^{-1}	0.81c	0.74b
	100 µgmL^{-1}	0.61d	0.63c
	200 µgmL^{-1}	0.48e	0.52d
Mentha longifolia	50 µgmL^{-1}	1.12b	0.79b
	100 µgmL^{-1}	0.82c	0.66c
	200 µgmL^{-1}	0.59d	0.55d
Silica nanoparticles	50 µgmL^{-1}	0.62d	0.57d
	100 µgmL^{-1}	0.45e	0.39e
	200 µgmL^{-1}	0.35f	0.25f
Control		1.750a	1.675a

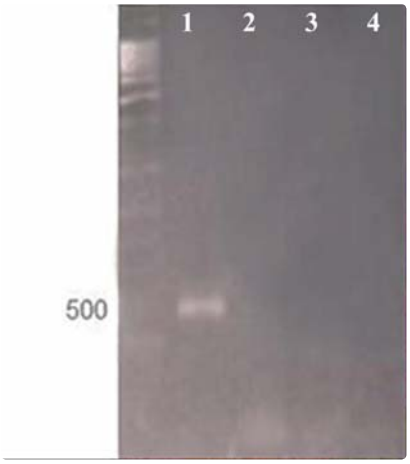

Figure 1: Detection of TYLCV in tomato plants by PCR. 1- Control, 2- Treated with *Zingiber officinale*, 3- Treated with *Mentha longifolia*, 4- Treated with silica nanoparticles

Table 3: Induction of the activities of Peroxidase (POD) and Polyphenol Oxidase (PPO) in tomato plants after ginger and horsemint extracts and silica nanoparticles treatments. Tomato leaves were sprayed with treatments at a concentration of 200 µg mL^{-1} and with water as control.

Treatments	Peroxidase		Polyphenol oxidase	
	1WPI	2WPI	1WPI	2WPI
Zingiber officinale	60.6a	43.6a	25.3a	22.7a
Mentha longifolia	57.3a	42.9a	23.9a	21.4a
Nanosilica	30.2b	28.5b	15.4b	13.5b
Control	20.3c	19.7c	10.5c	9.3c

The induced disease resistance by plant extracts and silica nanoparticles and the mechanisms involved were investigated. Our results revealed that both plant extracts as well as silica nanoparticles acted as a potential elicitor in producing resistance. Application of induction treatments significantly reduced disease severity and TYLCV concentration in tomato. The results presented here demonstrate that the induction treatments significantly reduced the TYLCV incidence both in pot and field conditions. These results agree with previous studies of Maurhofer et al. [33], Murphy et al. [34], and Kandan et al. [35] in the management of *Tobacco necrosis virus* (TNV) in tobacco, *Tomato mottle virus* (ToMoV) and *Tomato spotted wilt virus* (TSWV) in tomato, respectively. Additionally, the infection of *Sunflower necrosis virus* (SFNV) was significantly reduced in cowpea and sunflower plants treated with the Antiviral Protein (AVP) from plant extracts [36]. Salicylic acid treatment was significantly induced systemic resistance against *Tobacco mosaic virus* [34]. The aqueous extracts of *Plectranthus tenuiflorus, Azadirachta indica, Clerodendrum inerme, Schinus terebinthifolius* induced systemic resistance against *Bean common mosaic virus* under field conditions [23].

In the present study, the DAS-ELISA test was performed for the detection of TYLCV in treated tomato plants and the ELISA values were found to be lower in treated leaf samples compared to the untreated control plants under pot and field conditions.

Table 4: Effect of *Zingiber officinale* and *Mentha longifolia* extracts and silica nanoparticles on tomato growth and yield.

Treatments	Morphological and physiological characters								
	Plant height (cm)	Leaf area (cm)	No. of branches	Fresh weight (gm)	Dry weight (gm)	Chlorophyll content	No. of flowers	No. of fruits	Weight of fruits per plant
Z. officinale	91.2a	276.4a	24.2a	447.9a	75.3a	47.3a	115.3a	38.6a	5.9a
M. longifolia	75.0b	225.2b	20.0ab	417.3a	66.5a	44.5a	102.2a	32.6a	4.5b
Nanosilica	62.7c	192.4c	16.5b	330.0b	52.8b	31.0b	86.3b	23.2b	3.8c
Control	31.6d	58.2d	8.7c	226.0c	32.2c	22.7c	9.6c	3.8c	0.57d

Table 5: Effect of *Zingiber officinale* and *Mentha longifolia* extracts and silica nanoparticles on quality of tomato fruits.

Treatments	Data Analysis		
	Acidity	Total soluble solids (T.S.S)%	Vitamin C mg/ 100ml juice
Zingiber officinale	0.58a	5.41a	9.14a
Mentha longifolia	0.56a	5.29a	8.89ab
Nanosilica	0.49b	4.89b	8.61b
Control	0.47b	4.62c	8.22c

Applications of plant extracts and silica nanoparticles for the control of TYLCV showed significant increase in morphological and physiological characters of tomato plants such as plant height, leaf area, number of branches, fresh and dry weight, chlorophyll contents, number of flowers and fruits number and weight per plant. Moreover, the quality characters of tomato fruits such as acidity, total soluble contents and vitamin C were significantly increased in ginger and horsemint treated plants compared with the control. The best results was recorded in plots that received spray treatment with ginger extracts followed by horsemint treatment which was also significantly higher than silica nanoparticles treated plants. Similarly, shoot length and fresh and dry weights were significantly increased due to ginger extract treatment [37]. Extracts of ginger (*Zingiber officinale*) showed significantly increased resistance against soil-borne pathogens as well as growth characters of chili plants [38]. The thickness and the roughness of Gerbera leaves were increased due to silica treatment, thus improving light reception and photosynthesis which enhances plant growth and yield [39-41]. Moreover, the production of new pigments was stimulated due to silicon treatment, thus the contents of chlorophyll a and b of *Polygonatum multiflorum* were also increased [42].

The activities of POD and PPO were increased during the induction treatments. Hence, a variety of disease resistance mechanisms were involved in induced resistance by ginger and horsemint extracts and silica nanoparticles against TYLCV in tomato plants. In our experiments, treatment of tomato with ginger and horsemint extracts and silica nanoparticles induced stronger activities of POD and PPO. Several reports have suggested that POD is related to lignin and suberin synthesis, which increase the hardness of tissues, and to the production of quinones and active oxygen, which possess antibiotic properties [43,44]. In this study, we found that POD and PPO activities were increased after induction treatments. Several reports have suggested that POD is a key regulatory enzyme in local and systemic disease resistance [45,46]. The phenolic compounds can be oxidized by PPO and produce antimicrobial phenolic substances, such as quinines, which are more toxic to pathogens. In conclusion, our results indicated that ginger and horsemint extracts and silica nanoparticles had induced activation of defense-related enzymes which resulted in enhanced resistance against TYLCV under pot and field conditions.

REFERENCES

1. Fauquet CM, Briddon RW, Brown JK, Moriones E, Stanley J, Zerbini M et al. Geminivirus strain demarcation and nomenclature. Arch Virol. 2008; 153: 783-821. https://goo.gl/QR5Vzy
2. Aref NM, El-Dougdoug KhA. Effect of irrigation with waste water on some tomato viral diseases plants. Annals of Agric Science. 1996; 41: 151-172.
3. El-Sawy MM. Control measures of *Tomato yellow leaf curl virus* (TYLCV) under field conditions. M.Sc, Thesis, Faculty of Agricultural Kafr Elsheikh Tanta University. 2003.
4. Compton ME, Gray DJ. Somatic embryogenesis and plant regeneration from immature cotyledons of watermelon. Plant Cell Rep. 1993; 12: 61-65. https://goo.gl/yZ8Lf4
5. Yarmolinsky L, Zaccai M, Ben Shabat S, Mills D, Huleikel M. Antiviral activity of ethanol extracts of *Ficus binjamina* and *Lilium candidum* in vitro. N Biotechnol. 2009; 26: 307-313. https://goo.gl/2YBWYj
6. Horvath J. New artificial hosts and non-hosts of plant viruses and their role in the identification and separation of viruses: XVIII. Concluding remarks. Acta Phylopathol Acad Sci Hung. 1983; 18: 121-161. https://goo.gl/rfBn85
7. Hull R. Matthews' Plant Virology. 4th ed. Academic Press, London: UK; 2002; 1001. https://goo.gl/FPoLQZ
8. Abdelbacki AM, Taha SH, Sitohy MZ, Dawood AIA, Abd-El Hamid MM, Rezk AA. Inhibition of *Tomato yellow leaf curl virus* (TYLCV) using whey proteins. Virol J. 2010; 7: 26-31. https://goo.gl/pAh9eM
9. Chen YF, Zhan Y, Zhao XM, Guo P, An HL, Du YG et al. Functions of oligochitosan induced protein kinase in tomato mosaic virus resistance and pathogenesis related proteins in tomato. Plant Physiol Biochem. 2009; 47: 724-731. https://goo.gl/czjRVA
10. Wang FD, Feng GH, Chen KS. Burdock fructooligosaccharide induces resistance to tomato mosaic virus in tomato seedlings. Physiol Mol Plant Pathol. 2009; 74: 34-40. https://goo.gl/2DaGbD
11. Elsharkawy MM, Shimizu M, Takahashi H, Hyakumachi M. The plant growth-promoting fungus *Fusarium equiseti* and the arbuscular mycorrhizal fungus Glomus mosseae induce systemic resistance against Cucumber mosaic virus in cucumber plants. Plant Soil. 2012; 361: 397-409. https://goo.gl/q71k37
12. Elsharkawy MM, Hassan N, Ali M, Mondal SN Hyakumachi M. Effect of zoysiagrass rhizosphere fungal isolates on disease suppression and growth promotion of rice seedlings. Acta Agriculturae Scandinavica Section B Soil Plant Science. 2014; 64: 135-140. https://goo.gl/LN64nK
13. El-Kazzaz MK, Salem EA, Ghoneim KE, Elsharkawy MM, El-Kot GAN, Kalboush ZAE. Integrated control of rice kernel smut disease using plant extracts and salicylic acid. Archives of Phytopathology and Plant Protection. 2015a; 48: 664-675. https://goo.gl/q2V5Hc
14. El-Kazzaz MK, Salem EA, Ghoneim KE, Elsharkawy MM, El-kot GAN, Kalboush ZA. Biocontrol of Tilletia barclayana, the causal agent of kernel smut disease in rice. Egypt. J Biol Pest Con. 2015b; 25: 535-544.
15. El-Naggar MM, Elsharkawy MM, Almalla RA, El-Kot GAN, Alwakil AM, Badr MM. Control of *Ustilaginoidea virens*, the causal agent of rice false smut disease in Egypt. Egypt. J. Biol. Pest Con. 2015; 25: 555-564. https://goo.gl/vsQXBA
16. Hassan N, Elsharkawy MM, Villajuan-Abgona R, Hyakumachi M. A nonpathogenic species of binucleate Rhizoctonia inhibits the formation of infection structures caused by *Rhizoctonia solani* on cucumber. Acta Agriculturae Scandinavica Section B Soil Plant Science. 2015; 65: 208-214. https://goo.gl/t2Tc8Z

17. Elsharkawy MM, Shimizu M, Takahashi H, Hyakumachi M. Induction of systemic resistance against *Cucumber mosaic virus* by *Penicillium simplicissimum* GP17-2 in *Arabidopsis* and tobacco. Plant Pathology. 2012a; 61: 964-976. https://goo.gl/Rsozgv

18. Hassan N, Elsharkawy MM, Shimizu M, Hyakumachi M. Control of root rot and wilt diseases of roselle under field conditions. Mycobiology. 2014a; 42: 376-384. https://goo.gl/j8tBLr

19. Hassan N, Nakasuji S, Elsharkawy MM, Hushna AN, Kubota M, Ketta H, Shimizu M. Biocontrol potential of an endophytic *Streptomyces* sp. Strain MBCN152-1 against *Alternaria brassicicola* on cabbage plug seedlings. Microbes Environ. 2017; 32: 133-141. https://goo.gl/WrcYS4

20. Elsharkawy MM, Mousa KM. Induction of systemic resistance against *Papaya ring spot virus* (PRSV) and its vector *Myzus persicae* by *Penicillium simplicissimum* GP17-2 and silica (Sio2) nanopowder. Int J Pest Manage. 2015; 61: 353-358. https://goo.gl/oUGt8y

21. Elsharkawy MM, Shivanna MB, Meera MS, Hyakumachi M. Mechanism of induced systemic resistance against anthracnose disease in cucumber by plant growth-promoting fungi. Acta Agriculturae Scandinavica Section B Soil Plant Science. 2015; 65: 287-299. https://goo.gl/XnKoUq

22. Elsharkawy MM, El-Sawy MM. Control of *Bean common mosaic virus* by plant extracts in bean plants. Int J Pest Manage. 2015; 61: 54-59. https://goo.gl/depNxu

23. El-Sawy MM, Elsharkawy MM, Abass JM, Kasem MH. Antiviral activity of 2-nitromethyl phenol, zinc nanoparticles and seaweed extract against cucumber mosaic virus (CMV) in Eggplant. J Virol Antivir Res. 2017; 6: 2. https://goo.gl/9hxT7r

24. Wang C, Fan Y. Eugenol enhances the resistance of tomato against *Tomato yellow leaf curl virus*. J Sci Food Agric. 2014; 94: 677-682. https://goo.gl/PNzRJR

25. Abdallat AMA, Debei HSA, Asmar H, Misbeh S, Quraan A, Kvarnheden A. An efficient *in vitro*-inoculation method for tomato yellow leaf curl virus. Virol J. 2010; 7: 84-103. https://goo.gl/CEB6hk

26. Sultana B, Anwar F, Asharaf M. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules. 2009; 14: 2167-2180. https://goo.gl/373UGa

27. Lapidot M, Friedmann M, Pilowsky M, Ben-Joseph R, Cohen S. Effect of host plant resistance to *Tomato yellow leaf curl virus* (TYLCV) on virus acquisition and transmission by its whitefly vector. Phytopathology. 2001; 91: 1209-1213. https://goo.gl/r6nvXq

28. Raupach GS, Liu L, Murphy JF, Tuzun ST, Kloepper JW. Induced systemic resistance in cucumber and tomato against cucumber mosaic cucumovirus using plant growth-promoting rhizobacteria (PGPR). Plant Dis. 1996; 80:891-894. https://goo.gl/1BCdsj

29. Navot N, Zeidan M, Pichersky E, Zamier D, Czasneck H. Use of polymerase chain reaction to amplify *tomato yellow leaf curl virus* DNA from infected plants and viruliferous whiteflies. Phytopathology. 1992; 82: 1199-1202.

30. Sudhamoy M, Adinpunya M. Reinforcement of cell wall in roots of *Lycopersicon esculentum* through induction of phenolic compounds and lignin by elicitors. Physiol Mol Plant Pathol. 2007; 71: 201-209. https://goo.gl/nKoajb

31. Flurkey WH. In vitro biosynthesis of *Vicia faba* polyphenoloxidase. Plant Physiol. 1985; 79: 564-567. https://goo.gl/HXP94Q

32. Hassan N, Elsharkawy MM, Shivanna MB, Meera MS, Hyakumachi M. Elevated expression of hydrolases, oxidase, and lyase in susceptible and resistant cucumber cultivars systemically induced with plant growth-promoting fungi against anthracnose. Acta Agriculturae Scandinavica Section B Soil Plant Science. 2014b; 64: 155-164. https://goo.gl/C28NDE

33. Maurhofer M, Hase C, Meuwly P, Metraux JP, Defago G. Induction of systemic resistance of tobacco to tobacco necrosis virus by the root colonizing *Pseudomonas fluorescens* strain CHAO: Influence of the gacA gene and of pyoverdine production. Phytopathology. 1994; 84: 139-146. https://goo.gl/1xFB4c

34. Murphy AM, Carr JP. Salicylic acid has cell-specific effects on Tobacco mosaic virus replication and cell-to-cell movement. Plant Physiol. 2002; 128: 552-563. https://goo.gl/f6APTM

35. Kandan A, Radjacommare R, Nandakumar R, Raguchander T, Ramiah M, Samiyappan R. Induction of phenylpropanoid metabolism by Pseudomonas fluorescens against tomato spotted wilt virus in tomato. Folia Microbiol. 2002; 47:121-129. https://goo.gl/BmJr3v

36. Lavanya N, Saravanakumar D, Rajendran L, Ramiah M, Raguchander T, Samiyappan R. Management of Sunflower necrosis virus through anti-viral substances. Arch Phytopathol Plant Prot. 2009; 42: 265-276. https://goo.gl/Ujj12K

37. Islam MT, Faruq AN. Effect of Some Medicinal Plant Extracts on Damping-off Disease of Winter Vegetable. World Appl Sci J. 2012; 17: 1498-1503. https://goo.gl/7b7NZq

38. Mahfuzul H. Control of major seed-borne fungi of chilli (*Capsioum annuum* L.). MS thesis, Bangladesh Agricultural University, Mymensingh, Bangladesh. 1997.

39. Belanger RR. Understanding the benefits of silicon feeding in plants through transcriptomic analyses. Proc. 4th International Conference «Silicon in Agriculture», 26-31 October, Wild Cost Sun, Transkei, South Africa. 2008.

40. Ma JF, Takahashi E. Soil, Fertilizer, and Plant Silicon Research in Japan. Elsevier, Amsterdam. 2002; 281. https://goo.gl/Wq49u2

41. Savvas D, Manos G, Kotsiras A, Souvaliotis S. Effects of silicon and nutrient-induced salinity on yield, flower quality and nutrient uptake of Gerbera grown in a closed hydroponic system. J Appl Bot. 2002; 76: 153-158. https://goo.gl/ztLQGH

42. Rubinowska K, Pogroszewska E, Laskowska H, Szot P, Zdybel A, Stasiak D et al. The subsequent effect of silicon on physiological and biochemical parameters of Polygonatum multiflorum (L.) All. 'Variegatum' cut shoots. Acta Sci Pol. Hortorum Cultus. 2014; 13: 167-178. https://goo.gl/G4XCmG

43. Bowles DJ. Defense-related proteins in higher plants. Annu Rev Biochem. 1990; 59: 873-907. https://goo.gl/QaD1EF

44. Stout MJ, Workman J, Duffey SS. Differential induction of tomato foliar proteins by arthropod herbivores. J Chem Ecol. 1994; 20: 2575-2594. https://goo.gl/LecT1y

45. Elsharkawy MM, Shimizu M, Takahashi H, Ozaki K, Hyakumachi M. Induction of systemic resistance against *Cucumber mosaic virus* in *Arabidopsis thaliana* by *Trichoderma asperellum* SKT-1. Plant Pathol J. 2013; 29: 193-200. https://goo.gl/1JyLjk

46. Karban R, Myers JH. Induced plant responses to herbivory. Annu Rev Ecol Syst. 1989; 20: 331-348. https://goo.gl/qRPo9q