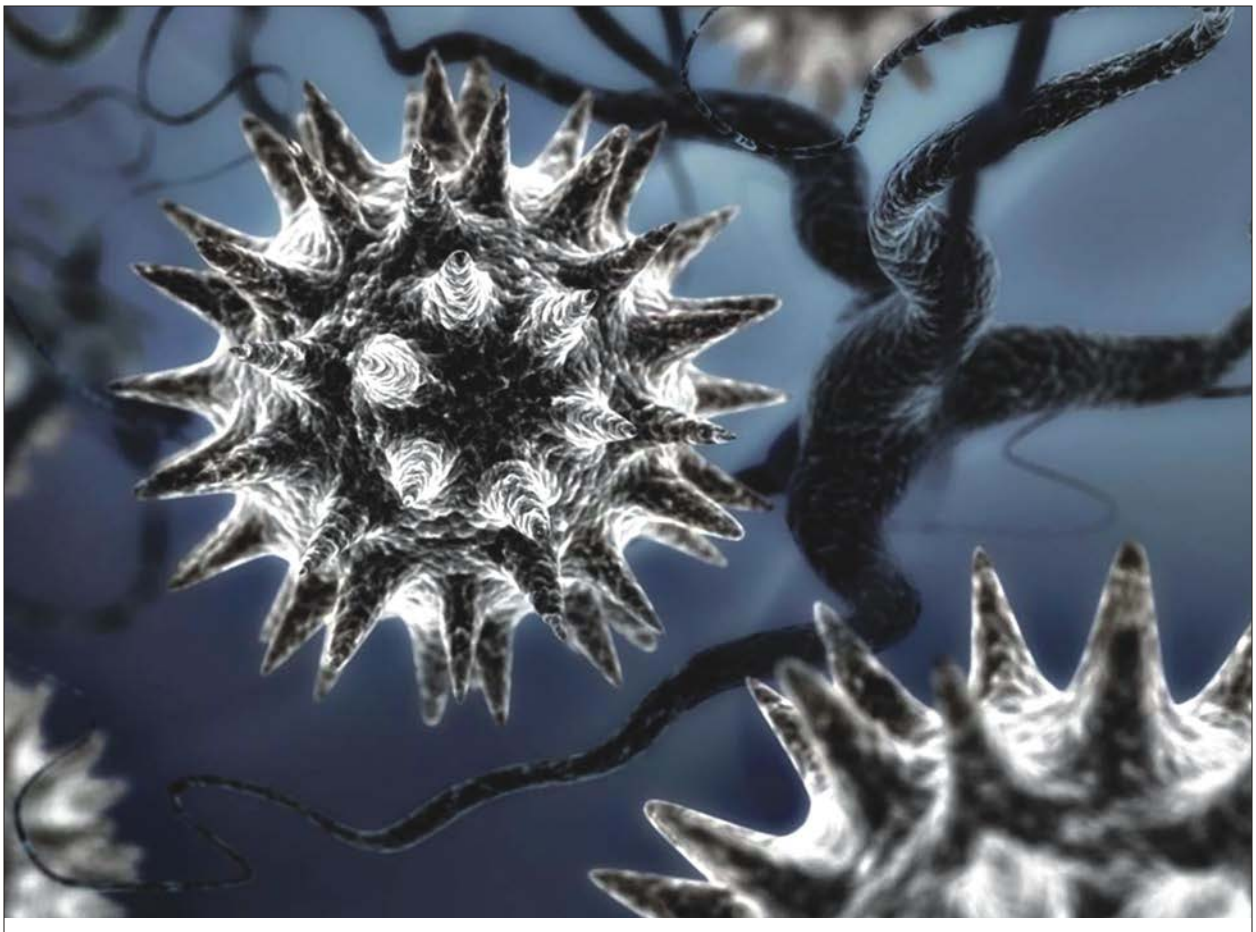

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Research Article

Effect of Jojoba Seed Extract and Riboflavin in Preventing the Transmission of Iris Yellow Spot Virus (IYSV): *Tospovirus* by *Thrips tabaci* L. to Onion Plants in Egypt

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Abstract

Background and Objective: Iris Yellow Spot Virus (IYSV) genus *Tospovirus*, family Bunyaviridae is one of the most important viruses affecting onion crops worldwide. In Egypt, IYSV was recorded for the first time from onion since, 2004. *Thrips tabaci* L. is the mainly vector of this virus. The present study was conducted to test the effect of jojoba seeds extract and riboflavin on decrease the transmission rate of IYSV by *Thrips*. **Materials and Methods:** The virus has been isolated from naturally infected onion plants grown in the experimental farm, Faculty of Agriculture, Cairo University, Giza. It was identified by mechanically and *T. tabaci* transmissibility, symptomatology, serology and transmission electron microscopy. Two pots experiments were conducted in 2013-2014 seasons to study the effect of spraying with jojoba seeds extract at 100, 500 and 1000 μg mL^{-1} and riboflavin at 0.5, 1.0 and 2.5 mM to inhibit infection with IYSV in onion plants (cv. Giza 20) exposed to *Thrips*. Data were subjected to analysis of variance and the means were compared using the Least Significant Difference (LSD) test at the 0.05 levels. **Results:** All treatments with jojoba seeds extract caused virus inhibition through reduction in the transmission rates of IYSV by *T. tabaci* and riboflavin through induced resistance against virus infection when plants were sprayed before the virus transmission. The highest concentration of jojoba seed extract (1000 μg mL^{-1}) and riboflavin at (1 mM) were the best treatments which resulted in the highest reduction of viral infection (58 and 36%), respectively. The different treatments resulted in significant increase in photosynthetic pigments, pungency compounds and activity of peroxidase (POD), polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL) and glutathione content compared with infected plants. Moreover, all treatments recorded decrements in activity of superoxide dismutase (SOD) compared with the untreated ones. **Conclusion:** It is suggested that reduction in the incidence and severity of IYSV infection in onion plant may be achieved by treatments with jojoba seeds extract and riboflavin when onion plants were sprayed before IYSV *Thrips* transmission.

Key words: Antioxidant enzymes, *Allium cepa* L., glutathione, Iris yellow spot virus (IYSV), jojoba, riboflavin, *Thrips tabaci* L., transmission, ultrathin section

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Onion (*Allium cepa* L.) is one of the most widely grown vegetable crops on a commercial scale. It is extensively cultivated all over the world[1]. Onion is the most economically significant member of family Alliaceae, it is ranked as the 3rd important vegetable crops after tomato and potato according to annual production (2024881 t) in Egypt[2]. Onion is used as a spice and food in most parts of the world however, it has been valued for millennia also for medicinal properties[3]. Onion plants are susceptible to several viral diseases such as Onion Yellow Dwarf Virus (OYDV), Iris Yellow Spot Virus (IYSV), Leek Yellow Stripe Virus (LYSV) and Garlic Common Latent Virus (GCLV)[4].

Iris Yellow Spot Virus (IYSV), genus *Tospovirus*, family Bunyaviridae has become the most studied onion plant virus. The IYSV is a serious yield-reducing pathogen of onion and other *Allium* crops worldwide. It is considered as an emerging virus whose world presence and distribution have recently dramatically increased[5]. The IYSV has been reported infecting onion in Egypt[6-9]. The virus causes irregularly shaped, tan to bleached white or straw colored and necrotic lesions on onion leaves[10]. Infected onion plants can be affected by tip dieback and also progressively by necrosis of older leaf area, resulting in reduced yield, bulb size and this infection is most damaging to onion seed crops where losses may reach upto 100%[11]. The IYSV has caused considerable onion losses in Brazil, Israel and the United States representing an immediate and serious threat to sustainable and productive onion cropping systems[12]. The IYSV is mechanically transmitted but not known to be seed transmissible[13] but potential inoculum sources include volunteer onion plants and onion cull piles as well as alternative hosts like winter annual, biennial, perennial weeds, other cultivated *Allium* species and several ornamental species[12]. The IYSV is vectored principally by onion *Thrips* only[14-18].

Thrips tabaci Lindeman; (Thysanoptera: Thripidae) is one of the major insect pests of onion[19,20] known to incur huge loses, 30-50% annually[21]. It is an important polyphagous species, sap-feeding pest worldwide with a broad variety of vegetable and ornamental host plants[22-26]. Adults and larvae feed on green leaf parts causes direct damage to the surface of leaves and different plant parts causing a silvery appearance[27-29]. This damage reduces the plant's photosynthetic ability and reduces onion bulb size[17,30].

Due to the distinct habit of *Thrips*, crawling into very small spaces and hiding in narrow crevices which is called thigmotactic behavior[17,31], chemical control is challenging. Also, its ability to develop resistances to many active ingredients[32-35] and the rising consciousness for the environmental and health impacts of synthetic pesticides[36-38] are driving forces for the search of new alternatives to synthetic pesticides such as jojoba seed extract and riboflavin (Vitamin B2).

Jojoba *Simmondsia chinensis* (Link) schneider is a semiarid evergreen shrub. It grows wild in the desert South-Western United States and North-Western Mexico. However, the plant is cultivated in different parts of the world including Egypt due to its high economic value[39]. Jojoba seeds contain about 50-60% of a unique wax ester oil which is composed mainly of straight chain monoesters in the range of C40-C44. Jojoba oil has good markets in the cosmetics and lubricant industries and recently, it has been reported that the jojoba seeds possess anti-inflammatory activity[40]. After oil extraction of jojoba seeds, a protein rich residue remains known as defatted jojoba meal. The meal contains 20-32% of protein consisting mainly of albumins (79%) and globulins (21%). This meal also contains approximately 15% of a group of glucosides known as simmondsins[41]. Among these the methylated compounds simmondsin and simmondsin 2-ferulate exhibited food-intake inhibition in rodents and chickens and showed a strong insecticidal activity against the third instars larvae of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). Also, these compounds showed anti-feedant activity against *S. littoralis* in concentration-dependent manner. In addition these compounds showed moderate to high antifungal activity against four plant pathogenic fungi[42].

Riboflavin (vitamin B2) is a vitamin produced by plants and microbes that acts as a coenzyme in many physiological processes in animals, plants and microbes[43]. Riboflavin is involved in antioxidant and peroxidation both processes affect the production of reactive oxygen intermediates in oxidative burst and consequent Hypersensitive Response (HR). Foliar application of riboflavin effectively controls several diseases of tobacco and it reduces powdery mildew of strawberry plants in combination with methionine, metal ions and surfactant. Thus, riboflavin may function as a resistance elicitor or a mediator of resistance signal transduction or acts as a activator of a novel signaling pathway leading to systemic resistance[44,45].

In most target crops, use of synthetic pesticides is the most commonly used option for controlling *Thrips*. These treatments cause residue and insecticide resistance problems are costly and undesirable with regard to risks to operators, livestock and non target organisms[46]. Thus, certain plant

derived products have been promoted in recent years as alternatives to traditional chemical method. They possess good efficacy and are environmentally friendly against insect pests.

Thus, the objectives of this study were investigate the activities of jojoba seeds extract and riboflavin for controlling infection with Iris Yellow Spot Virus (IYSV) in onion plants under greenhouse conditions. Also, study the effects of different treatments on activities of antioxidant and transaminases enzymes in the young leaves of onion plants cultivar Giza 20 in order to select the effective treatments for inhibiting the virus infection. On the other hand, study the cytological differences between healthy plants and artificially infected one to confirm the infection with virus.

MATERIALS AND METHODS

Virus identification and propagation: Samples from naturally infected onion plants exhibiting characteristic IYSV were collected from the experimental farm of Faculty of Agriculture, Cairo University. The observed symptoms included chlorotic or necrotic, straw-colored to white, dry, elongate or spindle shaped lesions. Indirect ELISA technique described by Converse and Martin[47] was used to test the collected samples using an induced antiserum for IYSV[8]. The positive samples reacting with IYSV antiserum were used as a source of virus and mechanically inoculated to indicator host plants. The samples were inoculated to *Chenopodium quinoa*, *Chenopodium murale, Datura stramonium* and *Nicotiana benthamiana* plants though three consecutive passage for biological purification of the virus isolate[48], then mechanically transmitted to onion plant seedlings cv. Giza 20 which served as a source of *Thrips* transmission and for virus propagation.

Maintenance of culture virus free of *Thrips tabaci* L.: *Thrips* were collected from onion field at Giza region, adults were mounted on microscope slides according to the methodology proposed by Mound[49]. Identification of *Thrips* was conducted using a taxonomic key of Moritz *et al.*[50]. The stock culture of identified *T. tabaci* was maintained on healthy onion plants (*Allium cepa* L.) in the climate room. The onion plants were grown in small pots (10 cm diameter and 8 cm height) within a greenhouse. The fresh plants were added weekly and the old plants were removed when the *Thrips* had moved to new plants. The rearing of *T. tabaci* took place in a climatically controlled room at 25±1°C temperature, 60±10% RH and 16:8 h (L:D) photoperiod[51].

Acquisition and transmission of IYSV using *Thrips tabaci* L.: Newly hatched larvae, 0-4 h old were placed on onion infected IYSV as source of the virus with an Acquisition Access Period (AAP) of 4 h and then reared on healthy onion plants until adult emergence (Viruliferous adults). Another group of newly hatched larvae (0-4 h old) of *Thrips* caged on virus-free onion were used as controls (Virus free adults). All experiments were carried out at 25±0.5°C with a 16 h light photoperiod.

Preparation of jojoba seeds extract: Seeds of jojoba (*Simmondsia chinensis* L.) schneider were collected from the experimental station of Faculty of Agriculture, Cairo University, Egypt during September, 2013. Dried and powdered seeds of *S. chinensis* were extracted with petroleum ether (60-80°C) to removal oil and waxes substances then defatted jojoba meal was ground in a coffee mill to obtain a finely divided material which suitable for extraction studies. Batch extraction were performed at 25°C with 2 g samples of ground defatted jojoba meal for 30 min. Aqueous solution of 80% ethanol was used for extraction with 1:10 meal to solvent ratio at 25°C then the extract was filter and used as a source of simmondsin and simmondsin-2-ferulate according to Holser and Abbott[52].

Greenhouse experiment: A pot experiment was conducted in the greenhouse of Faculty of Agriculture, Cairo University, Department of Economic Entomology and Pesticides. Insect Virology Unit, Giza, Egypt during 2013-2014 winter seasons in order to evaluate the effect of viral infection and different treatments with antiviral compounds on the change of biochemical compounds in the young leaves of onion plants. Onion plants at the 6-8 leaf stage of healthy test plants were divided into four groups, 1st were sprayed with water (Negative control), 2nd group was infected with (IYSV) virus using *Thrips* transmission (Positive control), 3rd group was sprayed with jojoba seeds extract at (100, 500 and 1000 μg mL^{-1}) according to Abbassy *et al.*[42] and 4th group was sprayed with riboflavin at concentration (0.5, 1.0 and 2.5 mM) according to Dong and Beer[53]. Whole, plants were kept for 1 week then groups 3 and 4 were inoculated with virus using viroulifrous *Thrips* as follow:

***Thrips* transmission tests:** *Thrips* (adults) that reared on the IYSV source plant (Viruliferous) were used in the transmission tests for all treatments onion seedling plants, *Thrips* were transferred using a fin camel brush and were placed on onion seedling test plants, 10 viruliferous adults were used /each test plant, another group (10 virus-free *Thrips*)/each test plant

were used as (Negative control). After that, *Thrips* were killed by spraying with 0.01% malathion and plants were observed for developing virus symptoms for 3-4 weeks.

Indirect ELISA method: Indirect ELISA method was done as described by Converse and Martin[47] to detect the virus isolate and to study the reducing effect of treatments with jojoba seeds extract and riboflavin on infection with IYSV in onion plants.

Electron microscopy examination

Virus morphology: Electron microscopic examination of negatively stained leaf dip preparations by 2% phospotungestic acid was caried out according to Ahlawat and Varma[54] and examined under a JOEL-1400 Transmission Electron Microscope (TEM) at electron microscope unit. Faculty of Agriculture, Research Park, Cairo University (FARP).

Ultrathin section: Onion leaf tissues healthy and infected with IYSV through insect transmission (15 days post inoculation) were examined using thin sections by TEM. The healthy and infected leaves were cut into small pieces about 1-2 mm, fixed in 2% glutraldhyde in 0.1 M Na-cacodylate buffer pH 7.2 and subjected to a vacuum for 1-4 min every 15 min for 2 h on ice. Prior to vacuum treatment and floating samples were poked under the buffer surface with pointed metal pokers. Rinsing took place in 0.1 M Na-cacodylate buffer pH 7.2 for 45 min with buffer changes at 15 min with pointed metal pokers. Rinsing took place in 0.1 M pH 7.2 for 45 min with buffer changes at 15 and 30 min further fixation in 1% osmium tetraoxide in Na-cacodylate buffer pH 7.2, under intermittent vacuum and poking and took place for 1.5 h[55]. The samples were rinsed in the Na-cacodylate buffer then dehydrated through an ethanol series (35-50-70-80-95-100%) for 60 min and then infiltrate with the resin mixture through a graded serics in glass vials with polypropylene caps. Semi-thin sections were prepared on glass slides through cutting at 1 μ using the ultra-microtome. They were cut and stained with toludine blue for 5 min and examined by light microscopy model Olympus UC 30 BX53. Both semi and ultra-thin sections were cut using ultra-microtome (Leica model EM-UC6) at thickness 90 nm and mounted on copper grids (400 mish). Ultra-thin sections were stained with double stains (urinal acetate 2% for 10 min followed by lead citrate for 5 min and examined by transmission electron microscope (JOEL-JM- 100-C) at the candidate magnification at (AL-Azhar Univ.). Electron micrographs were captured by CCD camera model AMT and optronics camera with 1632×1632 pixel format as side mount configuration.

Chemical analysis of leaves

Photosynthetic pigments: Chlorophyll (a and b) and carotenoids were determined after extraction in 80% acetone according to the method reported by Holden[56].

Determination of flavonoids and total phenolic compounds: The ethanol extracts of onion leaves after 7 days from the virus infection were used to determine, total soluble phenols and total flavonoids. The total flavonoids content was determined according to the aluminum chloride colorimetric method described by Chang *et al.*[57]. Total phenolic contents were determined using the Folin-ciocalteu method Meda *et al.*[58].

Determination of pungency in onion leaves: Pungency in onion leaves was determined as pyruvate content according to Schwimmer and Weston[59].

Preparation of cytosolic fraction: Leaves tissue of onion about 1 g was excised and homogenized using mortar and pestle in 4 mL of ice-cold grinding buffer containing 250 mM sucrose, 25 mM tris and pH was adjusted to 7.2. The homogenate was centrifuged at 12000 g for 15 min at 4°C. The resulting supernatant was used for analyses of enzyme activities, GSH, lipid peroxidation and protein content.

Enzymes assay: The activity of cytosolic superoxide dismutase (SOD) was assayed by measuring its ability to inhibit the photochemical reduction of NBT according to method of Ginnopolitis and Ries[60]. Spectrophotometrically method as described by Amako *et al.*[61] was used to assay the peroxidase (POD) activity, polyphenol oxidase (PPO) activity was assayed by using photochemical method as described by Coseteng and Lee[62] and phenylalanine ammonia-lyase (PAL) activity was done according to Dickerson *et al.*[63].

Determination of lipid peroxidation products: The lipid peroxidation products was estimated by the formation of thiobarbaturic acid reactive substances (TBARS) and quantified in term of malonaldheyde (MDA) as described by Haraguchi *et al.*[64]. The lipid peroxidation was expressed as μM of MDA. The extinction coefficient of TBARS was taken as 1.56×10^5 mM at wave length 532 nm.

Determination of glutathione (GSH): The GSH content was estimated by the acid-soluble sulfhydryl (SH) level in the tissue homogenates as described by La Vecchia *et al.*[65].

Determination of protein: Protein levels of cytosolic were determined spectrophotometrically at 595 nm using comassie blue G 250 as a protein binding[66]. Bovine serum albumin (BSA) was used as a protein standard.

Statistical analysis: The obtained data were subjected to an analysis of variance and the means were compared using the Least Significant Difference (LSD) test at the 0.05 levels according to Snedecor and Cochran[67].

RESULTS

Virus identification and propagation: Symptoms of the virus in naturally infected onion plant showed chlorotic or necrotic, straw-colored to white, dry, elongate or spindle-shaped lesions on onion leaf and spindle to diamond shaped lesions on the flower scapes and tip dieback (Fig. 1a-d). To confirm symptom development and Koch's postulates, virus-free onion seedlings cv. Giza 20 in the greenhouse were inoculated mechanically with plant sap from naturally infected onion plants developed symptoms identical to those observed on naturally infected plants. Symptoms appeared 7-10 days post inoculation. On the other hand, similar symptoms of IYSV *Thrips* transmitted to onion plant showed chlorotic or necrotic, straw colored to white, elongate or spindle lesions on onion leaf after 3-4 weeks on onion test plants and spindle to diamond shaped lesions on the flower scapes and tip dieback 7-8 weeks after IYSV transmission (Fig. 1e). The virus infectivity of isolate was confirmed biologically that gave chlorotic local lesions developed on *Chenopdium quinoa* and *Chenopodium murale*, necrotic local lesions on *Datura stramonium* and *Nicotiana benthamiana* and necrotic spot on *Petunia hybrid* after 1 week as show (Fig. 2a-e). Virus was detected by ELISA in all symptoms inoculated plants which gave positive result with IYSY polyclonal antibodies by indirect ELISA.

Electron microscopy examination: Leaf samples of onion were analyzed by TEM in leaf dip preparations. Virus particles typical of a IYSV were observed only in samples taken from symptomatic leaves stained with 2% phospotungestic acid. Spherical particles with approximately 90 nm of diameter were observed (Fig. 3a). On the other hand, electron microscope examination of ultrathin section of healthy and infected onion

Fig. 1(a-e): Symptoms of an infected onion plants with IYSV in a commercial onion field and greenhouse, (a) The IYSV Symptoms development in the field showing chlorotic, elongate ringed lesions on leaves and tip dieback, (b) Straw-colored, lenticular or spindle-shaped lesions, (c,d) Chlorotic, spindle-shaped lesions on onion leaf and spindle to diamond shaped lesions on the flower scapes and (e) Symptoms of IYSV in the greenhouse transmitted with virulifierous *Thrips tabaci* showing the typical symptoms of infected plants

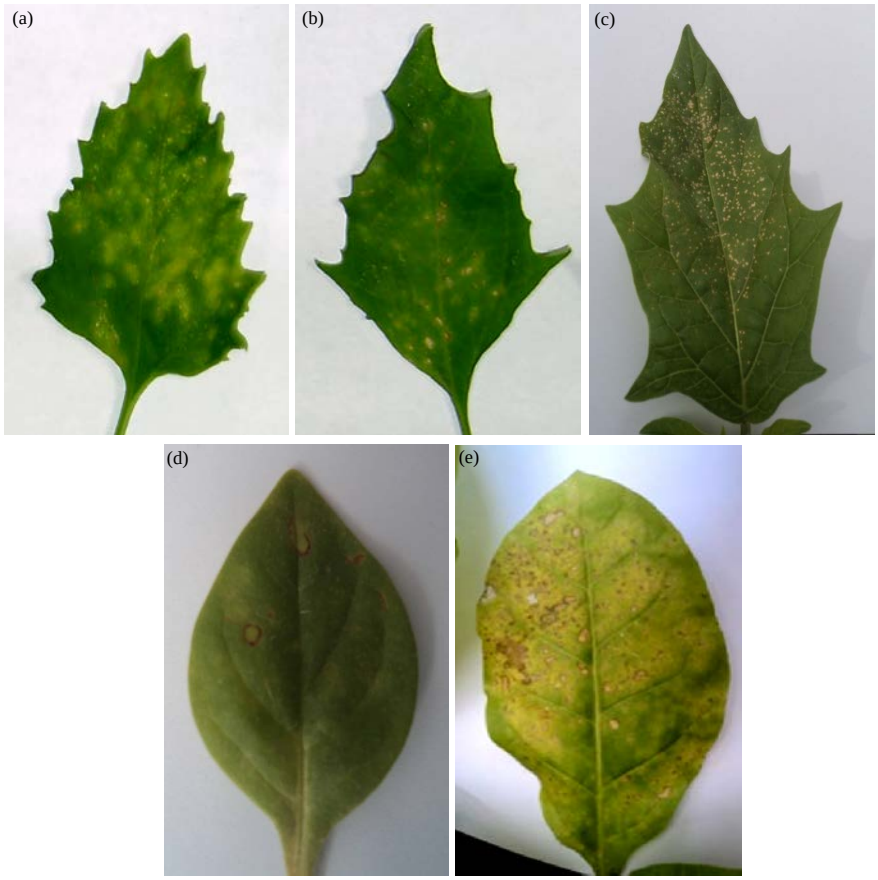

Fig. 2(a-e): Showing symptoms on different diagnostic hosts, (a) *Chenopodium quinoa* showing chlorotic local lesion, (b) *Chenopodium murale* showing chlorotic local lesions, (c) *Datura stramonium* showing necrotic local lesions, (d) *Petunia hybrid* showing necrotic spot and (e) *Nicotiana benthamiana* showing necrotic local lesions

plants with IYSV was done. Ultra-thin section of healthy samples showed normal cell wall, nucleus, mitochondria and chloroplast (Fig. 3b). However, extensive changes have been observed in infected mesophyll cell including severe degeneration of nucleus structure appeared in the large size of the nucleus, dense aggregation and segmented chromatin (Fig. 3c) lyses of rough endoplasmic reticulum and nuclear membrane producing many small vacuoles (Fig. 3d) in addition to the nucleus takes elongated shape and lyses of mitochondria and chloroplast in most cells (Fig. 3e). On the other hand, infected sample showed numerous spherical virus like particles in cytoplasm adjacent to plasma membrane (Fig. 3f).

Effect of treatment with jojoba seeds extract and riboflavin on the transmission rates of IYSV by *Thrips* and infection in onion plants: Data demonstrated in Table 1 according to indirect ELISA test showed that all concentrations of jojoba seeds extract (100, 500 and 1000 μg mL^{-1}) and riboflavin (0.5, 1.0 and 2.5 mM) significantly reduced transmission rates of IYSV by *Thrips* and the viral infection. The highest jojoba seeds extract concentration (1000 μg mL^{-1}) revealed the highest reduction (58%) while, the highest effective of riboflavin treatment against viral infection which realized the highest reduction compared with other treatments was (1 mM) and realized (36%).

Effect of jojoba seeds extract and riboflavin on photosynthetic pigments of onion leaves: The effect of jojoba seeds extract and riboflavin with different concentrations on the photosynthetic pigments of onion leaves after foliar application and inoculation with virus are shown in Table 2. The obtained results proved that the healthy plants (negative control) recorded the highest content of chlorophyll a, b and carotenoids (91.32, 82.68 and 18.56 mg g^{-1} fresh weight, respectively)

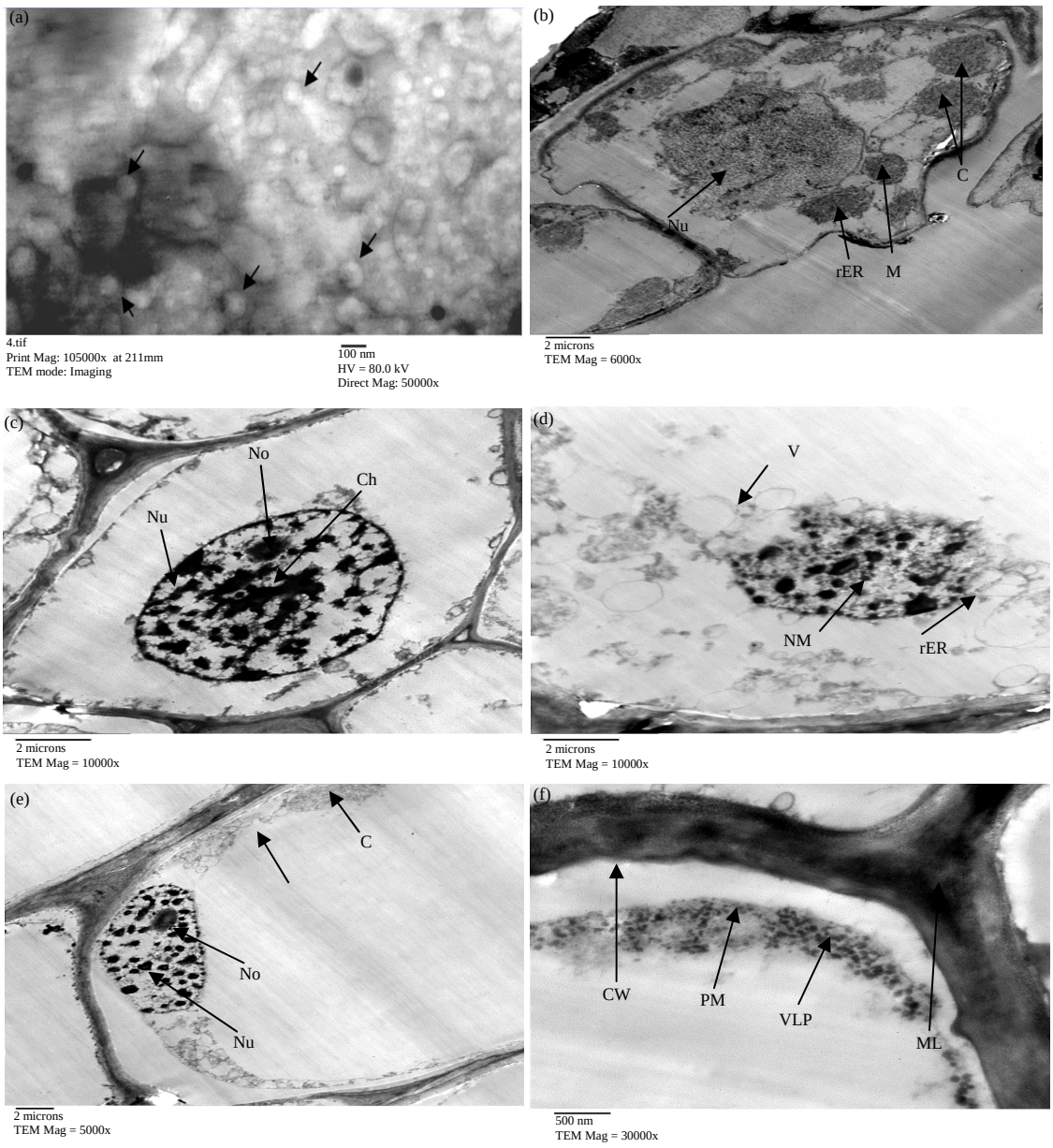

Fig. 3(a-f): Transmission Electron Microscopy (TEM) in leaf dip preparations and thin sections of onion leaf tissues, (a) Transmission electron microscope image of a leaf dip extract stained with 2% phosphotungestic acid showing enveloped viral particles in systemically infected onion leaves, electron micrographs representing the effect of IYSV-infection on onion cell organelles compared with those of healthy cells, (b) Mesophyll cell of healthy onion leaf with normal shape nucleus (Nu), Nucleolus (NO) and No. of chloroplasts (arrow), (c) Cell of IYSV-infected leaf showing large size of the nucleus (Nu), dense aggregation and segmented chromatin (Ch), (d) Lyses of rough endoplasmic reticulum (rER) and nuclear membrane (NM) producing many small vacuoles (V), (e) Elongated nucleus and lyses of mitochondria (M) and chloroplast (C) of infected cell and (f) Showing virus like particles (VLP) in cytoplasm adjacent plasma membrane (PM)

while, the lowest values were found in plants infected with virus (Positive control) (46.57, 34.28 and 10.95 mg g^{-1} fresh weight, respectively). In addition, a significant increase was noticed in chlorophyll and carotenoids content in different treatments of jojoba seeds extract and riboflavin, the highest effect were obtained with jojoba seeds extract at 1000 µg mL^{-1} and riboflavin at 1 mM.

Effect of treatments with jojoba seeds extract and riboflavin on pungency content in onion leaves: Data demonstrated in Table 3 reveal that healthy plants recorded the highest content of pyruvic acid (4.91 μmol g^{-1}) while, the lowest value were found in plants infected with virus (3.66 μmol g^{-1}) and different treatments with antiviral compounds improvement pungency content. In addition, jojoba seeds extract at (1000 μg mL^{-1}) and riboflavin at (1 mM) give the highest content from pyruvic acid (4.81 and 4.21 μmol g^{-1}, respectively) compared with infected plants (3.66 μmol g^{-1}).

Influence of treatments with jojoba seeds extract and riboflavin on total phenols and flavonoids in onion leaves: Regarding to the results in Table 4, it could be noticedthat viral infection increased the phenols and flavonoids contents as a natural plants defense compounds.

Jojoba seeds extract and riboflavin administration positively increased the phenols and flavonoids accumulation until the highest jojoba seeds extract (1000 μg mL^{-1}) which reached to (43.70 and 54.52 mg/100 g fresh weight) while, the riboflavin at (1 mM) phenols and flavonoids contents reached to (41.12 and 49.13 mg/100 g fresh weight) compared with infected plants (25.71 and 30.82 mg/100 g fresh weight, respectively).

Lipid peroxidation, resistance and antioxidant enzymatic activities: Results in Fig. 4 indicated that the lipid peroxidation product (as MDA) was lower in healthy plants than infected plants which their values were (13.14 and 44.39 μmol, respectively). Their values decreased in different treatments with antiviral compounds (Fig. 4). The decreasing values of MDA in treatment with jojoba seeds extract was lower than treatment with riboflavin, the decreasing values of TBARS at 100, 500 and 1000 μg mL^{-1} jojoba seeds extract were 30.22, 24.44 and 18.19 μmol and in riboflavin treatment at 0.5,1 and 2.5 mM the decreasing values were 31.53, 27.79 and 35.11 μmol, respectively compared to infected plants (Positive control). At the same time under viral infection glutathione content was increased compared with healthy plants which their values were (3.64 and 2.41 μmol, respectively). These values increased in different treatments with jojoba seeds extract and riboflavin which recorded (6.29 and 5.83 μmol) in jojoba seeds extract at 1000 μg mL^{-1} and riboflavin at 1 mM, respectively. To counteract the toxicity of ROS in response to the variety of stress antioxidant defiance system will be activated in plants. Thus, the antioxidant and resistance enzymes peroxidase (POD) superoxide dismutase (SOD) polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL) were extracted from onion leaves assayed as specific activities. Data in Fig. 4 indicated that infection with IYSV increase activities of these enzymes by three folds compared with healthy plants while, treatments with jojoba seeds extract and riboflavin increased the activities of these enzymes more than virus infection on plants.

DISCUSSION

In this investigation, IYSV was isolated from naturally infected onion plants showed symptoms including chlorotic or necrotic, straw-colored to white, dry, elongate or spindle-shaped lesions on onion leaf and spindle to diamond

Table 1: Effect of jojoba seeds extract and riboflavin on the transmission rates of IYSV by *Thrips* and infection with IYSV in onion plants cv. Giza 20

Treatments (μg mL^{-1})	IYSV transmission by *Thrips tabaci* (%)	Reduction of IYSV (%)
Jojoba seeds extract (100 μg mL^{-1})	69	31
Jojoba seeds extract (500 μg mL^{-1})	55	45
Jojoba seeds extract (1000 μg mL^{-1})	42	58
Riboflavin (0.5 mM)	71	29
Riboflavin (1 mM)	64	36
Riboflavin (2.5 mM)	77	23
Infected (Positive control)	100	0

Table 2: Chlorophyll and carotenoid content (mg g^{-1}) in leaves of onion c.v Giza 20 after treatment with antiviral compounds (mg g^{-1} fresh weight)

Treatment	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoids
Jojoba seeds extract (100 μg mL^{-1})	63.27±1.93^{cd}	94.19±2.70^{a}	157.46±4.63^{b}	12.70±0.64^{cd}
Jojoba seeds extract (500 μg mL^{-1})	73.58±2.62^{b}	56.59±3.04^{d}	130.17±0.69^{d}	13.25±0.81^{cd}
Jojoba seeds extract (1000 μg mL^{-1})	79.17±3.44^{b}	61.51±1.76^{c}	140.68±4.58^{c}	15.99±0.72^{b}
Riboflavin (0.5 mM)	61.01±2.12^{cd}	48.14±1.03^{e}	109.15±3.56^{e}	13.03±0.59^{cd}
Riboflavin (1 mM)	65.66±1.89^{c}	50.76±1.63^{de}	116.42±0.26^{e}	13.57±0.45^{c}
Riboflavin (2.5 mM)	56.35±1.48^{d}	39.05±0.74^{f}	95.40±1.50^{f}	12.15±0.25^{cd}
Healthy plants	91.32±4.03^{a}	82.68±2.36^{b}	174.00±3.51^{a}	18.56±1.09^{a}
Infected (Untreated)	46.57±1.72^{e}	34.28±2.33^{f}	80.85±2.09^{g}	10.95± 0.46^{d}
LSD (0.05)	6.21	5.32	7.46	1.63

Values are Means±SE. Mean values with different small letters within a column indicate significant differences ($p \leq 0.05$)

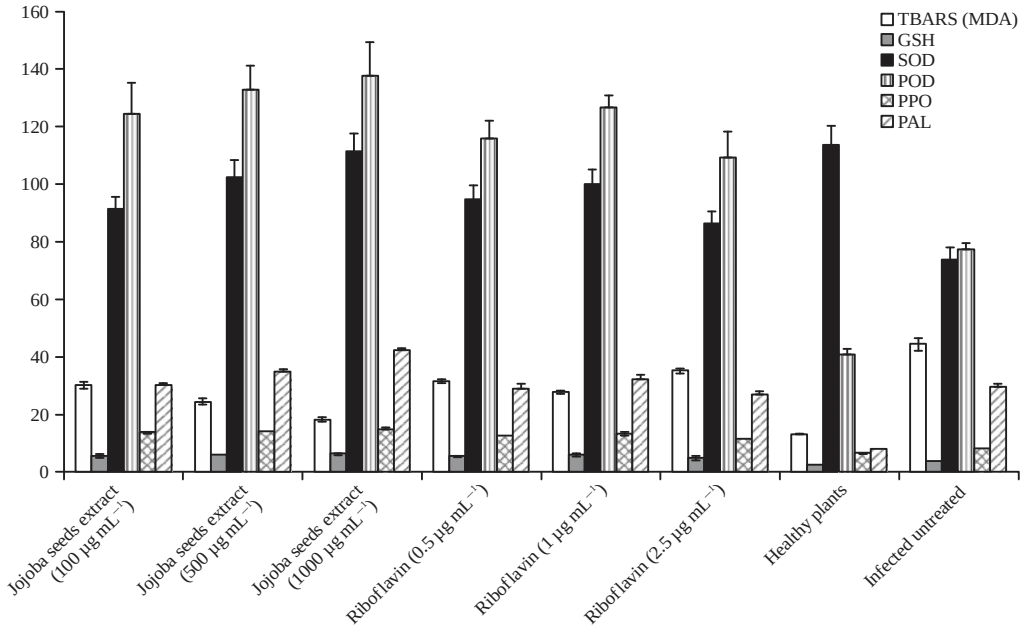

Fig. 4: Effect of treatment with antiviral compounds on lipid peroxidation (TBARS), glutathione(GSH), superoxidedismutase(SOD), peroxidase (POD), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) in onion leaves

Table 3: Effect of treatments with jojoba seeds extract and riboflavin on pungency content in onion leaves (µmol g^{-1} as pyruvic acid)

Treatment	Pungency content
Jojoba seeds extract (100 µg mL^{-1})	4.10 ± 0.19^{bc}
Jojoba seeds extract (500 µg mL^{-1})	4.35 ± 0.25^{bc}
Jojoba seeds extract (1000 µg mL^{-1})	4.81 ± 0.14^{a}
Riboflavin (0.5 mM)	4.04 ± 0.11^{bc}
Riboflavin (1 mM)	4.21 ± 0.14^{bc}
Riboflavin (2.5 mM)	3.96 ± 0.11^{bc}
Healthy plants	4.91 ± 0.12^{a}
Infected (Untreated)	3.66 ± 0.06^{c}
LSD (0.05)	0.36

Values are Means±SE. Mean values with different small letters within a column indicate significant differences ($p \leq 0.05$)

Table 4: Influence of jojoba seeds extract and riboflavin on total soluble phenol and flavonoids content (mg/100 g fresh weight) of onion leaves

Treatment	Total soluble phenol	Total flavonoids
Jojoba seeds extract (100 µg mL^{-1})	35.99 ± 1.43^{b}	39.88 ± 0.76^{c}
Jojoba seeds extract (500 µg mL^{-1})	39.85 ± 2.23^{a}	47.47 ± 1.58^{b}
Jojoba seeds extract (1000 µg mL^{-1})	43.70 ± 1.63^{a}	54.52 ± 2.21^{a}
Riboflavin (0.5 mM)	32.76±0.59b	32.22 ± 0.98^{d}
Riboflavin (1 mM)	41.12 ± 1.68^{a}	49.13 ± 1.32^{b}
Riboflavin (2.5 mM)	26.14 ± 1.38^{c}	24.46 ± 0.18^{e}
Healthy plants	$16.07\pm0.40_{d}$	17.91 ± 0.79^{e}
Infected (Untreated)	25.71 ± 0.99^{c}	30.82 ± 2.48^{d}
LSD (0.05)	3.44	3.6

Values are Means±SE. Mean values with different small letters within a column indicate significant differences ($p \leq 0.05$)

shaped lesions on the flower scapes and tip dieback. On the other hand, the virus under investigation was easily transmitted mechanically and by *Thrips tabaci* to onion seedling in the greenhouse. Symptoms resulting from mechanical and *Thrips* transmission were similar in all plants to those observed in naturally infected plants. Symptom descriptions on indicator plants for this virus are given in. Common symptoms were chlorotic or necrotic leaf symptoms were observed after 7 days post inoculation. Such collective symptoms have previously been described for IYSV infection[68-70]. In addition IYSV was serologically reactive to the induced antiserum using indirect ELISA which detected the presence of IYSV in naturally infected onion in the field and onion cv. Giza 20 in the greenhouse. Such results are confirmed by several researcher applying ELISA for virus identification[71,72,11].

Electron micrograph of a leaf dip extract showing spherical particles only in systemically infected onion leaves. On the other hand, analysis of ultra-structural changes reflected the external symptoms observed on infected onion leaves. The chlorotic and necrotic spots on the leaves were a result of effects on the chloroplasts which were completely or partially degenerated similar to that described by Garbaczewska *et al.*[73]. The nucleus in cells of infected plants was in some cases enlarged, swollen and contained segmented chromatine and in others elon.

Gated and lysed allowing virus RNA to release into the cytoplasm. Virus like particles observed in the cytoplasm and attached to plasma membrane. Such results were obtained by Garg *et al.*[74], Bag *et al.*[75] and Hafez *et al.*[9,76].

The present investigation showed that jojoba seed extracts have been shown to be repellent to the vector, *Thrips tabaci* this result goes online with another study, certain plant essential oils and their constituents have been shown to be repellent to *F. occidentalis* in laboratory assays[77] also, the essential oil from *Origanum majorana* repelled *Thrips tabaci* in a small scale field trial. The present results showed that the transmission rates of IYSV decreased by increasing the different concentration of jojoba seed extract, this agree with Abtew *et al.*[78] they reported that the repellency of the extracts increased with the concentration and effect on behavioral response of *M. sjostedti*.

The present results suspect that jojoba seed extracts interfered with *Thrips* feeding and transmission of IYSV. Also, Abtew *et al.*[78] reported that the repellent effect could be related to the presence of different active compounds or a blend of odors which induce an oriented movement away from the odor source. On the other hand, the reduction in infection by using jojoba seeds extract may be due to the extract contain two cyanoglucosides simmondsin and simmondsin 2-ferulate by (9.25% on dry weight basis) which have insecticidal, anti-feedant and antifungal activities which prevent *Thrips* from transmit IYSV to onion plants[52]. In this respect[42] showed that in topical application assay, simmondsin and simmondsin 2-ferulate showed strong insecticidal activity against the third instars larvae of *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) with LD50 values of 1.49 and 2.58 μg larva^{-1}, respectively. Both compounds showed anti-feedant activity against *S. littoralis* in a concentration-dependent manner. Also, these compounds give moderate to high antifungal activity against four plant pathogenic fungi. On the other hand, reduction in infection with different concentrations of riboflavin showed that concentrations of 0.5-1.0 mM were effective and sufficient for resistance, induction and higher concentration did not increase the inhibition effect. This result is in agree with previously studies by Dong and Beer[53].

Riboflavin acts as elicitor of systemic resistance and an activator of novel signaling process in plants also, it induced expression of pathogenesis-related (PR) genes in the plants, suggesting its ability to trigger a signal transduction pathway that leads to systemic resistance[44]. In plants inoculated with TMV, riboflavin treatment significantly reduced the number of lesions and lesion diameter at the same time riboflavin is able to induce resistance and activate defense genes in the absence of (SA) accumulation and previous studies[45] indicate that riboflavin has potential for practical use. Resistance-inducing chemicals have been applied for disease control but success has been limited. For example, salicylic acid (SA) and 2,6-dichloroisonicotinic acid (INA) are not feasible for disease control because the concentration needed to induce resistance in nearly phytotoxic[79] but riboflavin is not phytotoxic at concentrations much higher than that needed for resistance induction. Riboflavin-induced resistance is non-specific and can provide a basis for protection to a broad range of plants against an array of pathogens therefore, riboflavin seems to prossess many important characteristics of a safe disease control agent and induces non-specific disease resistance by triggering a novel signal transduction pathway that appears distinct from that typical Systemic Acquired Resistance (SAR) and Induced Systemic Resistance (ISR). Thus, external application of riboflavin elicits resistance and initiates a signal transduction process[45]. On the other hand, the obtained data show a significant increase in chlorophylls and carotenoids content in different treatments of jojoba seeds extract and riboflavin. Changes in chlorophylls and carotenoids content may be due to virus infection frequently involves chlorotic or necrotic, straw-colored to white, dry, elongate or spindle-shaped lesions. As more lesions develop and increase in size, they coalesce often completely girding the scape[80]. Also, virus infection inhibits chlorophyll biosynthesis of dark grown barely seedlings[81] and is attributed to stimulation of cell enzymes like chlorophyllase that degrades chlorophyll or it may be the effect of virus on pigment synthesis and disturbed physiological processes like photosynthesis and utilization of plastid proteins or their precursors for the synthesis of virus protein[82] and different treatments with jojoba seeds extract and riboflavin delayed systemic symptoms development by IYSV and suppressed virus multiplication and thus, increased the chlorophylls and carotenoids contents.

The present results revealed that healthy plants recorded the highest content of pyruvic acid compared with infected plant, this may be due to virus infection inhibite alliinase enzyme addition to light and CO_2 levels which had positive effect on pungency of onion leaves and bulb also, the damage caused by IYSV reduced green leaf area of the plant and causes reduction in the ability of the plant to fill the onion bulb which leads to reduces the harvest yield and grade of onions[83,84].

The obtained results showed that total phenols and flavonoids increased according to antiviral compound concentration. These changes may be due to cinnamic acid is the product of phenylalanine ammonia-lyase (PAL) activity.

This enzyme is a key regulator of the phenyl propanoid pathway that yields a diversity of phenolics with structural and defense-related functions[85]. Also, upon infection by pathogens the host phenolic compounds may increase and contribute to enhance the mechanical strength of host cell walls by the synthesis of lignin and suberin that are involved in the formation of physical barriers that can block the spread of pathogens[86]. The increased quantity of phenolics in infected plant may be contributing to the resistance against the infection of viral pathogen. Increased levels of phenolics also suggest an acceleration of phenol synthesizing pathway following pathogen infection. In addition, flavonoids and organosulfur compounds are the two major classes of secondary metabolites found in onion believed to promote beneficial health effects. These compounds are formed when an onion is cut and cell walls are disrupted and when infection with microbes[82]. The data reflect the positively increase of total soluble phenols and total flavonoids under viral infection stress that is may be resulted under different gene expression synthesis of various flavonoids compounds increase which act against viral infection. Multifold increase of phenolic compounds in host local lesion by treatments with antiviral compounds after challenging with IYSV in the present study may be due to the activation of hexose-monophosphate pathway, acetate pathway and release of bound phenols by hydrolytic enzymes. At the same time plants can produce antimicrobial compounds to protect themselves from biotic attack hat could be essential for microbial infection resistance[87].

Changes in enzymes activity may infer that virus infection promotes ROS formation significantly and exerts oxidative stress to the plant. Also, infection by pathogen increased lipid peroxidation CAT, APX, GPX and GR activites were also increased in infected roots and seeds[88]. In addition, GSH may play a protective role in scavenging of single oxygen, peroxides and hydroxyl radicals and is involved in recycling reduced of ascorbic acid (ASC) in the ascorbate-glutathione pathway in chloroplasts. The increasing content of GSH may be driven by enhanced of H_2O_2 formation. The increasing of H_2O_2 level has been shown to enhance antioxidant content and antioxidative enzyme in many plants[89]. Also, GSH content and antioxidant enzyme activities were reporting to play a key role in virus infection. Consequently, the mechanisms that reduce ROS species and increase antioxidant enzyme system in plant are an important role in imparting tolerance in plant under environmental stress[89]. The POX activity was significantly higher in IYSV infected plants of onion. This result is in accordance with the results of tobacco mosaic virus infected tobacco and bean yellow mosaic virus infected bean[90]. In this respect, POX is known to be involved in the Active Oxygen Species (AOS) mechanism. The AOX accumulation causes oxidative damage through actions such as lipid peroxidation and membrane destruction. The AOX levels increase during senescence so, there is a correlation between the decrease in chlorophyll content and increase in POX activity in green leaves infected with virus. Therefore, higher activity of POX leads to lignifications process which is considered as a resistance mechanism against pathogen attack. By way of oxidation of indole-3-acetic acid, upregulated peroxidases might also be responsible for growth reductions and malformations in virus-infected plants[91]. Polyphenol oxidase (PPO) is important in the initial stage of plant defense where membrane damage causes release of phenols such as chlorgenic acid. The PPO catalyzes the oxidation of phenolics to free radicals that can react with biological molecules thus, creating an unfavorable environment for pathogen development. The PPO activity was found to increase in leaves of onion-infected plants. Total solube phenols together with PPO play a role in resistance to viral pathogens[92]. Superoxide dismutase is one of the most important scavenging enzymes and catalyzes the dismutation of superoxide radicals to active oxygen species hydrogen peroxide. The SOD activity was significantly higher in leaves of healthy plants. This is in agreement with results of Anuradha *et al.*[82] who has observed a significant decrease in SOD from Banana Bunchy Top Virus (BBTV) inoculated banana. Higher activity of SOD could be a strategy of the plant to restrict virus colonization because the excess ROS can be removed and vice versa when SOD was lower resulting in oxidative stress[82]. Also, the higher activity of PAL enzyme due to this enzyme is a key regulator of thephenyl propanoid pathway that yields a diversity of phenolics with structural and defense-related functions and PAL enzyme plays a role in the active defenses of barley and wheat in response to pathogen attack[93].

CONCLUSION

In conclusion, treatments with jojoba seeds extract and riboflavin induced reduction and inhibition of IYSV infection when plants were sprayed before IYSV *Thrips* transmission. Also, all tested treatments gave a significant increase in photosynthetic pigments and activities of POD, PPO and PAL compared with infected plants. Moreover, all treatments

recorded decrements in activity of SOD compared with untreated ones. On the other hand, electron microscope examination of ultrathin section showed extensive changes in infected mesophyll cell including severe degeneration of nucleus structure appeared in the large size of the nucleus, dense aggregation and segmented chromatin, lyses of rough endoplasmic reticulum and nuclear membrane producing many small vacuoles addition to taking the nucleus the elongated shape and lyses of mitochondria and chloroplast in most cells.

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REFERENCES

1. Nigussie, A., Y. Kuma, A. Adisu, T. Alemu and K. Desalegn, 2015. Onion production for income generation in small scale irrigation users agropastoral households of Ethiopia. J. Horticult., Vol. 2. 10.4172/2376-0354.1000145
2. FAOSTAT., 2014. FAO statistical databases. FAO., Rome, Italy. http://faostat3.fao.org/.
3. El Sagan, M.A.M. and A.A. El Baset, 2015. Impact of magnetic on metal uptake, quality and productivity in onion crop. J. Agric. Vet. Sci., 8: 43-50.
4. Sevik, M.A. and C. Akcura, 2013. Viruses occurring in onion crop in amasya province, the major onion producing region in Turkey. Indian J. Virol., 24: 78-81.
5. Pappu, H.R., R.A.C. Jones and A.K. Jain, 2009. Global status of tospovirus epidemics in diverse cropping systems: Successes achieved and challenges ahead. Virus Res., 141: 219-236.
6. Abd El-Wahab, A.S., 2004. Insects and insect-borne viruses associated with Alliaceae crops in Egypt. Ph.D. Thesis, Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, Egypt.
7. Elnagar, S., M.A.K. El-Sheikh and A.S. Abd El-Wahab, 2006. *Iris yellow spot virus* (IYSV-Eg.1): A newly isolated thrips-borne Tospovirus in Egypt. Proceedings of the 12th Congress of the Mediterranean Phytopathological Union, June 10-15, 2006, Rhodes Island, Greece.
8. El-Shazly, M.A., A.S. Abdel-Wahab and S.N. Zein, 2006. Comparative study on two thrips transmitted viruses Tomato Spotted Wilt Virus (TSWV) and Iris Yellow Spot Virus (IYSV) as a tospovirus. Egypt. J. Virol., 3: 49-70.
9. Hafez, E.E., A.A. Abdelkhalek, A.S.E.D. Abd El-Wahab and F.H. Galal, 2013. Altered gene expression: Induction/suppression in leek elicited by Iris Yellow Spot Virus infection (IYSV) Egyptian isolate. Biotechnol. Biotechnol. Equip., 27: 4061-4068.
10. Nischwitz, C., H.R. Pappu, S.W. Mullis, A.N. Sparks, D.R. Langston, A.S. Csinos and R.D. Gitaitis, 2007. Phylogenetic analysis of *Iris yellow spot virus* isolates from onion (*Allium cepa*) in Georgia (USA) and Peru. J. Phytopathol., 155: 531-535.
11. Munoz, R.M., M.L. Lerma, P. Lunello and H.F. Schwartz, 2014. *Iris yellow spot virus* in spain: Incidence, epidemiology and yield effect on onion crops. J. Plant Pathol., 96: 97-103.
12. Gent, D.H., H.F. Schwartz and R. Khosla, 2004. Distribution and incidence of Iris yellow spot virus in colorado and its relation to onion plant population and yield. Plant Dis., 88: 446-452.
13. Smith, E.A., A. Ditommaso, M. Fuchs, A.M. Shelton and B.A. Nault, 2011. Weed hosts for onion thrips (Thysanoptera: Thripidae) and their potential role in the epidemiology of *Iris yellow spot virus* in an onion ecosystem. Environ. Entomol., 40: 194-203.
14. Derks, A.F.L.M. and M.E.C. Lemmers, 1996. Detection of tospoviruses in bulbous crops and transmissibility by vegetative propagation. Acta Horticult., 432: 132-139.
15. Mound, L.A. and R. Marullo, 1996. The Thrips of Central and South America: An Introduction (Insecta:Thysanoptera). Associated Publishers, New York, USA., ISBN: 9781566650618, pp: 140-185.
16. Jones, D.R., 2005. Plant viruses transmitted by thrips. Eur. J. Plant Pathol., 113: 119-157.
17. Diaz-Montano, J., M. Fuchs, B.A. Nault and A.M. Shelton, 2010. Evaluation of onion cultivars for resistance to onion thrips (Thysanoptera: Thripidae) and *Iris yellow spot virus*. J. Econ. Entomol., 103: 925-937.
18. Gill, H.K., H. Garg, A.K. Gill, J.L. Gillett-Kaufman and B.A. Nault, 2015. Onion thrips (Thysanoptera: Thripidae) biology, ecology and management in onion production systems. J. Integrated Pest Manage., Vol. 6. 10.1093/jipm/pmv006
19. Afifi, F.M.L. and M.F. Haydar, 1990. Effect of different intercropping systems on onion and garlic infestation with the cotton and onion thrips, *Thrip tabaci* (Lind.). Bull. Fac. Agric. Cairo Univ., 41: 639-648.
20. Nault, B.A. and M.L. Hessney, 2010. Onion thrips control in onion, 2009. Arthropod Manage. Tests, 35: E13-E13.
21. Nault, B.A., C.L. Hsu and C.A. Hoepting, 2012. Consequences of co-applying insecticides and fungicides for managing *Thrips tabaci* (Thysanoptera: Thripidae) on onion. Pest Manage. Sci., 69: 841-849.
22. Hill, D.S., 1994. Agricultural Entomology. Timber Press, Portland, Oregon, Pages: 635.

23. Murai, T., 1990. Parthenogenetic reproduction in *Thrips tabaci* and *Frankliniella intonsa* Insecta Thysanoptera. Adv. Invertebrate Reprod., 5: 357-362.
24. Van Emden, H.F., 2013. Handbook of Agricultural Entomology. Wiley-Blackwell, New York, USA., ISBN: 9781118469347.
25. Elnagar, S., M.A.K. El-Sheikh and A.S. Abdel Wahab, 2005. *Iris yellow spot virus* (IYSV): A newly isolated thrips-borne tospovirus in Egypt. Proceedings of the 7th International Conference on Pests in Agriculture, October 26-27, 2005, Montpellier.
26. Fitiwy, I., A. Gebretsadkan and K.M. Ayimut, 2015. Evaluation of botanicals for onion thrips, *Thrips tabaci* lindeman, (Thysanoptera: Thripidae) control at Gum Selassa, South Tigray, Ethiopia. Momona Ethiop. J. Sci., 7: 32-45.
27. Kirk, W.D.J., 1997. Feeding. In: Thrips as Crop Pest, Lewis, T. (Ed.). CABI., Wallingford, UK., pp: 119-174.
28. Mound, L.A. and G. Kibby, 1998. Thysanoptera: An Identification Guide. CAB International, Wallingford, UK., Pages: 70.
29. Pedigo, L.P., 2002. Entomology and Pest Management. 4th Edn., Pearson Education, Upper Saddle River, NJ., ISBN-13: 978-0130195678, Pages: 742.
30. Shock, C.C., E.B.G. Feibert, K.J. Barlow, A.L. Rock, L.D. Saunders, E. Jemmett, and S. Reitz, 2013. Insecticide rotations for thrips control in onions, 2012. Oregon State University Agricultural Experiment Station, Malheur Experiment Station Annual Report 2012, Department of Crop and Soil Science, pp: 63-69.
31. Childers, C.C., 1997. Feeding and Oviposition Injuries to Plants. In: Thrips as Crop Pests, Lewis, T. (Ed.). CABI., Hertfordshire, UK., ISBN: 9780851991788.
32. Lewis, T., 1997. Thrips, their Biology, Ecology and Economic Importance. Academic Press, London, UK.
33. Martin, N.A., P.J. Workman and R.C. Butler, 2003. Insecticide resistance in onion thrips (*Thrips tabaci*) (Thysanoptera: Thripidae). New Zealand J. Crop Horticult. Sci., 31: 99-106.
34. Allen, W.R., B. Tehrani and R. Luft, 2005. Effect of horticultural oil, insecticidal soap and film-forming products on the western flower thrips and the Tomato Spotted Wilt Virus. Plant Dis., 77: 915-918.
35. Foster, S.P., K. Gorman and I. Denholm, 2010. English field samples of *Thrips tabaci* show strong and ubiquitous resistance to deltamethrin. Pest Manage. Sci., 66: 861-864.
36. Jeyaratnam, J., 1990. Acute pesticide poisoning: A major global health problem. World Health Stat. Q., 43: 139-144.
37. Alavanja, M.C.R., J.A. Hoppin and F. Kamel, 2004. Health effects of chronic pesticide exposure: Cancer and neurotoxicity. Ann. Rev. Public Health, 25: 155-197.
38. Pretty, J. and R. Hine, 2005. Pesticide use and the Environment. In: The Pesticide Detox: Towards a More Sustainable Agriculture, Pretty, J.N. (Ed.). Earthscan, London, UK., ISBN: 9781844071418.
39. Abu-Salem, F.M. and H.M. Ibrahim, 2014. Antimicrobial activity and phytochemicals screening of jojoba (*Simmondsia chinensis*) root extracts and latex. Int. J. Biol. Biomol. Agric. Food Biotechnol. Eng., 8: 517-522.
40. Habashy, R.R., A.B. Abdel-Naim, A.E. Khalifa and M.M. Al-Azizi, 2005. Anti-inflammatory effects of jojoba liquid wax in experimental models. Pharmacol. Res., 51: 95-105.
41. Van Boven, M., R. Holser, M. Cokelaere, G. Flo and E. Decuypere, 2000. Gas chromatographic analysis of simmondsins and simmondsin ferulates in jojoba meal. J. Agric. Food Chem., 48: 4083-4086.
42. Abbassy, M.A., S.A.M. Abdelgaleil, A.S.H. Belal and M.A.A. Abdel Rasoul, 2007. Insecticidal, antifeedant and antifungal activities of two glucosides isolated from the seeds of *Simmondsia chinensis*. Ind. Crops Prod., 26: 345-350.
43. Wolinsky, I. and J.A.D. Driskell, 1997. Sports Nutrition: Vitamins and Trace Elements. CRC Press, Boca Raton, FL.
44. Abdel-Monaim, M.F., 2011. Role of riboflavin and thiamine in induced resistance against charcoal rot disease of soybean. Afr. J. Biotechnol., 10: 10842-10855.
45. Zhao, Y.Y., D.F. Wang, T.Q. Wu, A. Guo, H.S. Dong and C.L. Zhang, 2014. Transgenic expression of a rice riboflavin synthase gene in tobacco enhances plant growth and resistance to Tobacco mosaic virus. Can. J. Plant Pathol., 36: 100-109.
46. Maniania, N.K., S. Sithanantham, S. Ekesi, K. Ampong-Nyarko, J. Baumgartner, B. Lohr and C.M. Matoka, 2003. A field trial of the entomogenous fungus *Metarhizium anisopliae* for control of onion thrips, *Thrips tabaci*. Crop Prot., 22: 553-559.
47. Converse, R.H. and R.R. Martin, 1990. ELISA Methods for Plant Viruses. In: Serological Methods for Detection and Identification of Viral and Bacterial Plant Pathogens: A Laboratory Manual, Hampton, R., E. Ball and S. DeBoer, (Eds.). The American Phytopathological Society, St. Paul, MN., USA. pp: 179-196.
48. Kuhn, C.W., 1964. Separation of Cowpea virus mixtures. Phytopathology, 54: 739-740.
49. Mound, L., 2007. Thysanoptera (Thrips) of the world-a checklist. http://www.ces.csiro.au/thysanoptera/worldthrips.html
50. Moritz, G., L. A. Mound, D.C. Morris and A. Goldarazena, 2004. Pest Thrips of the World: Visual and Molecular Identification of Pest Thrips. CBIT Publishing, Brisbane, Australia.
51. Li, X.W., J. Fail, P. Wang, J.N. Feng and A.M. Shelton, 2014. Performance of arrhenotokous and thelytokous *Thrips tabaci* (Thysanoptera: Thripidae) on onion and cabbage and its implications on evolution and pest management. J. Econ. Entomol., 107: 1526-1534.
52. Holser, R.A. and T.P. Abbott, 1999. Extraction of simmondsins from defatted jojoba meal using aqueous ethanol. Ind. Crops Prod., 10: 41-46.

53. Dong, H. and S.V. Beer, 2000. Riboflavin induces disease resistance in plants by activating a novel signal transduction pathway. Phytopathology, 90: 801-811.
54. Ahlawat, V.S. and A. Varma, 1997. Serological detection of a mixed viral infection in onion seed crop and possible measure for its management. Indian Phytopathol., 50: 137-140.
55. Osmont, K. and M. Freeling, 2001. Characterization of extended auricle (eta) a developmental mutant that affects the blade/sheath boundary in maize. Proceedings of the 43rd Maize Genetics Conference, March 15-18, 2001, Lake Geneva, WI.
56. Holden, M., 1965. Chorophylls. In: Chemistry and Biochemistry of Plant Pigments, Goodwin, T.W. (Ed.). Academic Press, London, UK., pp: 462-488.
57. Chang, C.C., M.H. Yang, H.M. Wen and J.C. Chern, 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Anal., 10: 178-182.
58. Meda, A., C.E. Lamien, M. Romito, J. Millogo and O.G. Nacoulma, 2005. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem., 91: 571-577.
59. Schwimmer, S. and W.J. Weston, 1961. Enzymatic development of pyruvic acid in onion as a measure of pungency. Agric. Food Chem., 9: 301-304.
60. Giannopolitis, C.N. and S.K. Ries, 1977. Superoxide dismutases. I. Occurrence in higher plants. Plant Physiol., 59: 309-314.
61. Amako, A., G.X. Chen and K. Asada, 1994. Separate assays specific for ascorbate peroxidase and guaiacol peroxidase and for the chloroplastic and cytosolic isozymes of ascorbate peroxidase in plants. Plant Cell. Physiol., 35: 497-504.
62. Coseteng, M.Y. and C.Y. Lee, 1987. Changes in apple polyphenoloxidase and polyphenol concentrations in relation to degree of browning. J. Food Sci., 52: 985-989.
63. Dickerson, D.P., S.F. Pascholati, A.E. Hagerman, L.G. Butler and R.L. Nicholson, 1984. Phenylalanine ammonia-lyase and hydroxycinnamate: CoA ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *Helminthosporium carbonum*. Physiol. Plant Pathol., 25: 111-123.
64. Haraguchi, H., H. Ishikawa and I. Kubo, 1997. Antioxidative action of diterpenoids from *Podocarpus nagi*. Planta Med., 63: 213-215.
65. La Vecchia, C., E. Negri, S. Franceschi, B. D'Avanzo and P. Boyle, 1992. Tea consumption and cancer risk. Nutr. Cancer, 17: 27-31.
66. Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.
67. Snedecor, G.W. and W.G. Cochran, 1982. Statistical Methods. 7th Edn., Iowa State University Press, Ames, Iowa, USA.
68. Nagata, T., A.C.L. Almeida, R.D.O. Resende and A.C. de Avila, 1999. The identification of the vector species of *Iris yellow spot tospovirus* occurring on onion in Brazil. Plant Dis., 83: 399-399.
69. Kritzman, A., M. Lampel, B. Raccah and A. Gera, 2001. Distribution and transmission of *Iris yellow spot virus*. Plant Dis., 85: 838-842.
70. Srinivasan, R., S. Sundaraj, H.R. Pappu, S. Diffie, D.G. Riley and R.D. Gitaitis, 2012. Transmission of Iris yellow spot virus by *Frankliniella fusca* and *Thrips tabaci* (Thysanoptera: Thripidae). J. Econ. Entomol., 105: 40-47.
71. Hsu, C.L., C.A. Hoepting, M. Fuchs, E.A. Smith and B.A. Nault, 2010. Sources of *Iris yellow spot virus* in New York. Plant Dis., 95: 735-743.
72. Mandal, B., R.K. Jain, M. Krishnareddy, N.K.K. Kumar, K.S. Ravi and H.R. Pappu, 2012. Emerging problems of Tospoviruses (Bunyaviridae) and their management in the Indian Subcontinent. Plant Dis., 96: 468-479.
73. Garbaczewska, G., K. Otulak, M. Chouda and M. Chrzanowska, 2012. Ultrastructural studies of plasmodesmatal and vascular translocation of Tobacco Rattle Virus (TRV) in tobacco and potato. Acta Physiol. Plant., 34: 1229-1238.
74. Garg, I.D., V. Hegde, S.M.P. Khurana and R.A. Singh, 1999. Ultrastructural studies on different host-tospovirus combinations. Indian Phytopathol., 33: 215-222.
75. Bag, S., H.F. Schwartz, C.S. Cramer, M.J. Havey and H.R. Pappu, 2014. Iris yellow spot virus (Tospovirus: Bunyaviridae): From obscurity to research priority. Mol. Plant Pathol., 10: 224-237.
76. Hafez, E.E., A.A. El-Morsi, O.A. El-Shahaby and A.A. Abdelkhalek, 2014. Occurrence of iris yellow spot virus from onion crops in Egypt. VirusDisease, 25: 455-459.
77. Reitz, S.R., G. Maiorino, S. Olson, R. Sprenkel, A. Crescenzi and M.T. Momol, 2008. Integrating plant essential oils and kaolin for the sustainable management of thrips and Tomato Spotted Wilt on tomato. Plant Dis., 92: 878-886.
78. Abtew, A., S. Subramanian, X. Cheseto, S. Kreiter, G.T. Garzia and T. Martin, 2015. Repellency of plant extracts against the legume flower thrips *Megalurothrips sjostedti* (Thysanoptera: Thripidae). Insects, 6: 608-625.
79. Ryals, J.A., U.H. Neuenschwander, M.G. Willits, A. Molina, H.Y. Steiner and M.D. Hunt, 1996. Systemic acquired resistance. Plant Cell, 8: 1809-1819.
80. Boateng, C.O. and H.F. Schwartz, 2013. Temporal and localized distribution of Iris yellow spot virus within tissues of infected onion plants. Southwestern Entomol., 38: 183-200.
81. Harsanyi, A., M. Ryberg, M.X. Andersson, K. Boka and L. Laszlo *et al*., 2006. Alterations of NADPH: Protochlorophyllide oxidoreductase quantity and lipid composition in etiolated barley seedlings infected by *Barley stripe mosaic virus* (BSMV). Mol. Plant Pathol., 7: 533-541.

82. Anuradha, C., R. Selvarajan, S. Vasantha and G.S. Suresha, 2015. Biochemical characterization of compatible plant virus interaction: A case study with bunchy top virus-banana host-pathosystem. Plant Pathol. J., 14: 212-222.
83. Mishra, R.K., R.K. Jaiswal, D. Kumar, P.R. Saabale and A. Singh, 2014. Management of major diseases and insect pests of onion and garlic: A comprehensive review. J. Plant Breed. Crops Sci., 6: 160-170.
84. Alam, N., A.L. Abadi, M.L. Rayes and E. Zubaidah, 2015. The relationship of quality characteristics of fried red onions with the chemical compositions of red onion bulbs of Palu valley variety. J. Environ. Sci. Toxicol. Food Technol., 9: 43-49.
85. Enyedi, A.J., N. Yalpani, P. Silverman and I. Raskin, 1992. Signal molecules in systemic plant resistance to pathogens and pests. Cell, 70: 879-886.
86. Singh, H.P., S. Kaur, D.R. Batish and R.K. Kohli, 2014. Ferulic acid impairs rhizogenesis and root growth and alters associated biochemical changes in Mung bean (*Vigna radiata*) hypocotyls. J. Plant Interact., 9: 267-274.
87. Wojtaszek, P., 1997. Oxidative burst: An early plant response to pathogen infection. Biochem. J., 322: 681-692.
88. El-Baky, A., H. Hanaa, M.A. Amal and M.M. Hussein, 2003. Influence of salinity on lipid peroxidation, antioxidant enzymes and electrophoretic patterns of protein and isoenzymes in leaves of some onion cultivars. Asian J. Plant Sci., 2: 1220-1227.
89. Lechno, S., E. Zamski and E. Tel-Or, 1997. Salt stress-induced responses in cucumber plants. J. Plant Physiol., 150: 206-210.
90. Maksimov, I., N. Troshina, O. Surina and E. Cherepanova, 2014. Salicylic acid increases the defense reaction against bunt and smut pathogens in wheat calli. J. Plant Interact., 9: 306-314.
91. Riedle-Bauer, M., 2000. Role of reactive oxygen species and antioxidant enzymes in systemic virus infections of plants. J. Phytopathol., 148: 297-302.
92. Mohamed, H., A.A. El-Hady, M. Mansour and A.E.R. El-Samawaty, 2012. Association of oxidative stress components with resistance to flax powdery mildew. Trop. Plant Pathol., 37: 386-392.
93. Karabal, E., M. Yucel and H.A. Oktem, 2003. Antioxidant responses of tolerant and sensitive barley cultivars to boron toxicity. Plant Sci., 164: 925-933.