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Responses of pepper to *Alfalfa mosaic virus* and manganese nutrition under greenhouse conditions: preliminary results

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ABSTRACT

Purpose: The present research was conducted to evaluate interactive effects of manganese (Mn) and *Alfalfa mosaic virus* (AMV) on some physiological characteristics of two pepper varieties including PS301 and California Wonder under greenhouse conditions. **Research Methods:** Treatments included two levels of virus infection (infected and non-infected), three levels of Mn concentrations (No manganese: 0ppm, 5ppm and 10ppm) and two varieties (California Wonder and PS301). After three weeks, total chlorophyll (Chl), carbohydrate, antioxidants, phenolic compounds, dry weight and proline were measured in aerial parts. **Findings:** The highest antioxidant activity observed in California Wonder treated with AMV and 5ppm Mn. The highest total Chl observed in PS301 when treated with 10 ppm Mn. Interaction between Mn and AMV showed the highest dry weight in virus infected plants without Mn supplying. Simple effects of AMV did not influence on total Chl, total carbohydrate and antioxidant, but significantly dcreased dry weight. Application of Mn significantly increased proline and carotenoid contents in infected plants. **Research limitations:** analysis of proteins that play important roles in resistance mechanism to plant viruses has not been measured because of restriction of accessibility of western blotting at the University of Birjand. **Originality/Value:** It is concluded that AMV infection could be ameliorated by manganese supplying because of improvement of antioxidant system and phenol content that lead to viral tolerance in pepper.

INTRODUCTION

Plant viruses are often discovered and studied as pathogenic parasites that cause severe diseases in many crops and are obligate intracellular symbionts. Viruses use host resources and change physiological status in plants to support their own reproduction and dissemination, so it is widely believed that virus infections are harmful to the host. *Alfalfa mosic virus* (AMV) is the type species of the *genus Alfamovirus*, family *Bromoviridae*.This virus can naturally infect many herbaceous and some woody plants (Xu & Nie, 2006). Solanaceae family is one of the major hosts for AMV and *Capsicum annum* shows huge trend for infection the infections of alfalfa, other cultivated plants, and weeds by the virus, and the ease of its transmission among them will contribute to the epidemiological significance of this disease (Abdollah et al., 2020). Since viral control is not possible by conventional methods, increasing plant tolerance in reducing its damage is very important.

Plant nutrition, although frequently unrecognized, has always been an important component of disease control and their tolerance by improving physiological characterizations of plants. Several elements such as selenium, zinc, copper, manganese, etc. have immunomodulatory functions and thus influence the susceptibility to the course and the outcome of a variety of viral infections. Some trace elements inhibit virus replication in the host cells, thus showing antiviral activity. Many elements act as antioxidants or help such functions that not only regulate immune responses of the host, but also may alter the genome of the viruses (Chaturvedi et al., 2004)

Manganese is one of the main micronutrients, which has an important role in physiological processes in plants such as a component of enzymes involved in photosynthesis. In addition, manganese is part of an important antioxidant (superoxide dismutase) structure that protects plant cells by deactivating free radicals which can destroy plant tissue. Manganese promotes the activity of various enzymes that helps in the photosynthetic light reactions, respiration and protein synthetic processes leading to better utilization of NPK to convert into functional seed carbohydrates. Crops yield increases with manganese foliar applications due to increasing photosynthesis efficiency and synthesis of carbohydrates such as starch (Millaleo et al., 2010). Uneven distribution of chlorophyll and accumulation of granules starch in chloroplasts is the effects of manganese toxicity. Moreover, amount of lignin in the plant will decline due to manganese deficiency, that this reduction is more severe in the roots, this matter is very important especially to reduction tolerance the roots of plants to fungal infection.

Manganese deficiency has very serious effects on non-structural carbohydrates, and roots carbohydrates especially. It is usually lower in tissues susceptible to viral pathogen and is required for multiple steps in the biosynthesis of secondary metabolites, such as lignin, flavonoids, cinnamic acid, and acyl lipids so it can play an important role in increasing tolerance in plants against viruses. Moreover, nutrition-mediated interactions within the host in particular Mn are likely to influence disease spread and pathogen density (Kendig et al., 2020) Up to now, there is no reports on interaction among AMV, manganese and pepper varieties. Therefore, in this research we aimed to investigate the interactive effects of virus and manganese nutrition on some physiological characteristics of different varieties of pepper plants, and also how Mn nutrition may ameliorates AMV infection effects on these varieties.

MATERIALS AND METHODS

Plant material and growth condition

Two important varieties of pepper (Capsicum annum) including California wonder and PS301 were planted and grown in 2L pots containing an air-dried loamy soil, sterilized with hot air under greenhouse condition. Irrigation was done with fresh water based on field capacity (FC) during the experimental period (Table 1). Then, in the three to five leaf stages, different concentrations of manganese sulfate (0, 5 ppm and 10 ppm) were applied to the soil via irrigation water. The experiment was done for a period of 2 months in green house condition (Table 1) located in faculty of agriculture, University of Birjand.

Table 1. Soil and water characteristics and greenhouse conditions used in this experiment

Soil characteristics			
Variable	Rate	Variable	Rate
ECe	0.93 dS m–1	Mn2+	1.96 mg kg–1
pH	7.12	Fe2+	2.65 mg kg–1
Total N	0.08%	Na+	4.00 meq l–1
P	8 mg kg–1	Mg2+	3.14 meq l–1
K+	210 mg kg–1	Ca2+	2.60 meq l–1
Zn2+	0.63 mg kg–1	Cl–	0.50 meq l–1
Cu2+	0.25 mg kg–1	HCO3–	0.30 meq l–1
Water characteristics			
Variable	Rate	Variable	Rate
ECe	1.05 dS m–1	Cl–	13.00meq l–1
pH	7.54	HCO3–	3.90meq l–1
Mg2+	5.87 meq l–1	Na+	8.50 meq l–1
Ca2+	2.20 meq l–1	K+	0.20 meq l–1

Greenhouse conditions				
Night Temp. (°C)	Day Temp. (°C)	RH (%)	CO2 level (ppm)	Light intensity (mmol m–2s–1)
16	24	50±5	280±55	18

Table 2. Analysis variance of interaction among manganese (Mn), variety and virus infection

		Mean Square (MS)						
Sources of variations	df	Total Chl	Carbohydrate	Antioxidants	Dry weight	Total phenol	Proline	Carotenoid
Manganese	2	112.574**	44.94^{ns}	13.81^{ns}	0.035**	42.10**	2.20**	10.32**
Variety	1	255.84**	678.39**	19.37^{ns}	0.0082^{ns}	0.23^{ns}	0.10^{ns}	0.03^{ns}
Virus	1	0.001^{ns}	9.85^{ns}	0.058^{ns}	0.057**	105.81**	2.64**	2.27*
Manganese× Variety	1	98.41**	43.79^{ns}	6.83^{ns}	0.0062^{ns}	0.10^{ns}	6.00**	3.25^{ns}
Manganese× Virus	1	209.72**	45.57^{ns}	7.24^{ns}	0.040**	38.86**	0.41^{ns}	0.23**
Variety × Virus	1	12.33^{ns}	0.37^{ns}	10.81^{ns}	0.024*	0.19^{ns}	0.13^{ns}	0.25^{ns}
Variety × Virus× Manganese	1	211.62**	394.87**	49.67**	0.001^{ns}	1.95**	2.65**	17.25**
Error	24	4.233	15.19	4.866	0.003144	0.3122	29.56	0.3372

Table 3. Simple effect of manganese, Alfalfa mosaic virus (AMV) and variety on total Chl, carbohydrate, antioxidant activity and dry weight of pepper plants

Treatment	Rate	Total chl (mg g^{-1} F.W.)	Total carbohydrate (mg g^{-1} D.W.)	Antioxidants (%)	Dry weight (g)	Total phenol (mg gallic acid·g^{-1} DM)	Proline (µmol proline g^{-1} FW)	Carotenoid (mg g^{-1} F.W.)
Manganese (ppm)	0	7.58b	2.42[b]	5.15[b]	7.58[b]	62.56c	2.51b	3.76a
	5	12.02a	3.87[a]	8.15[a]	13.45[a]	63.74b	3.07a	2.074b
	10	13.45a	4.28[a]	9.16[a]	12.02[a]	66.23a	3.35a	3.58a
AMV	+	11.02[a]	26.11[a]	31.51[a]	0.2322[b]	65.89a	2.70b	2.88b
	-	11.01[a]	25.06[a]	30.10[a]	0.3119[a]	62.46b	3.250a	3.39a
Variety	California Wonder	8.35b	29.93a	31.57a	0.2569a	64.26a	3.033a	3.108a
	PS301	13.68a	21.24b	30.10a	0.2872a	64.101a	2.92a	3.17a

AMV+: infected plants; AMV-: healthy plants.
Mean values in each treatment followed by the same letter are not significantly different by the LSD ($P < 0.05$ and $p<0.01$).

Table 4. Interaction between AMV × Mn on total Chl, carbohydrate, antioxidant activity and dry weight of pepper plants

Virus	Mn	Total chl (mg g^{-1} F.W.)	Carbohydrate (mg g^{-1} D.W.)	Antioxidant (%)	Dry Weight (g)	Total phenol (mg gallic acid·g^{-1} DM)	Proline (µmol proline g^{-1} FW)	Carotenoid (mg g^{-1} F.W.)
AMV+	0	5.41[c]	27.61a	29.20[a]	0.23[c]	62.84c	2.25a	3.67a
	5	16.85[a]	24.70[a]	30.48[a]	0.44[a]	64.87b	2.61a	1.73c
	10	10.81[b]	26.01[a]	32.70[a]	0.26[b]	69.96a	3.26a	3.25a
AMV-	0	7.20[c]	23.15a	30.21[a]	0.22[c]	62.28c	2.76a	3.85a
	5	9.75[b]	22.82[a]	31.42[a]	0.23[c]	62.61c	3.53a	2.41b
	10	16.09[a]	29.21[a]	30.99[a]	0.23[c]	62.50c	3.44a	3.90a

AMV+: infected plants; AMV-: healthy plants.
Mean values in each column followed by the same letter are not significantly different by the LSD ($P < 0.05$ and $p<0.01$).

Table 5. Interaction between variety × Mn on total Chl, carbohydrate, antioxidant activity and dry weight of pepper plants

Variety	Mn	Total chl (mg g^{-1} F.W.)	Carbohydrate (mg g^{-1} D.W.)	Antioxidant (%)	Dry weight (g)	Total phenol (mg gallic acid·g^{-1} DM)	Proline (µmol proline g^{-1} FW)	Carotenoid (mg g^{-1} F.W.)
CW*	0	9.07c	31.85a	29.70a	0.41[a]	62.64a	2.80b	4.24a
	5	7.91c	27.52a	31.65a	0.17[a]	63.73a	2.33c	2.06a
	10	8.07c	30.40a	33.35a	0.22[a]	66.40a	3.96a	3.01a
PS301	0	7.24c	18.90a	29.71a	0.46[a]	62.48a	2.21c	3.28a
	5	14.97b	20.00a	30.25a	0.29[a]	63.76a	3.81a	2.08a
	10	18.82a	24.83a	30.34a	0.30[a]	66.062a	2.74b	4.14a

*= California Wonder.
Mean values in each column followed by the same letter are not significantly different by the LSD ($P < 0.05$ and $p<0.01$).

Inoculation of virus, magnesium treatment and disease symptom analysis

15 Pepper seedlings were used for inoculation with Alfalfa mosaic virus isolates and manganese treatment under greenhouse condition. Extracts of virus were prepared by grinding the inoculum in 1% (w/v) solution of K2HPO4 at pH 7.5 containing 0.01% Na2SO3, 2% polyvinylpyrrolidone (PVP) and 0.05% ethylene diamine tetra acetic acid (EDTA). In the five to six leaf stages, virus was carefully inoculated on all leaves using positive control (Certified by University of Kerman), phosphate buffered saline and endorum. After seeing the virus symptoms, it is transmitted to the laboratory for future tests.

Table 6. Interaction among AMV×variety×Mn on total Chl, carbohydrate, antioxidant activity and dry weight of pepper plants

Mn	Variety	Virus	Total chl (mg g^{-1}.W.)	Carbohydrate (mg g^{-1}.W.)	Antioxidant (%)	Dry weight (g)	Total phenol (mg gallic acid·g^{-1} DM)	Proline (µmol proline g^{-1} FW)	Carotenoid (mg g^{-1}.W.)
0	California Wonder	AMV+	9.82^{b}	38.07^{a}	29.00^{c}	0.41^{a}	62.86b	2.910b	2.73c
		AMV-	8.31c	25.63b	30.40b	0.25a	62.43d	2.700c	5.75a
	PS301	AMV+	7.47^{c}	31.26^{b}	30.17^{b}	0.17^{a}	62.82d	1.597d	4.62b
		AMV-	6.08c	20.66c	30.02b	0.20a	62.14d	2.837b	1.95c
5	California Wonder	AMV+	9.52^{b}	22.31^{c}	37.05^{a}	0.22^{a}	64.62c	2.170c	2.01c
		AMV-	8.34c	23.78c	33.13b	0.21a	62.84d	2.493c	1.953c
	PS301	AMV+	23.87^{a}	17.15^{d}	29.40^{c}	0.46^{a}	65.13c	3.050b	1.46d
		AMV-	11.15b	21.86c	29.71b	0.26a	62.38d	4.580a	2.71c
10	California Wonder	AMV+	3.34^{d}	18.13^{d}	30.78^{b}	0.29^{a}	70.66a	3.390b	3.58c
		AMV-	6.62c	38.49a	29.64b	0.26a	62.14d	4.537a	2.45c
	PS301	AMV+	12.10^{b}	29.72^{b}	30.34^{b}	0.30^{a}	69.26b	3.130b	2.93b
		AMV-	25.55a	19.94c	32.33b	0.20a	62.85d	2.353c	5.36a

AMV+: infected plants; AMV-: healthy plants.
Mean values (sampling) in each column followed by the same letter are not significantly different by the LSD ($P < 0.05$ and $p<0.01$).

Identification of systemic infection of *Alfalfa mosaic virus*

In order to detect and identify the virus, reverse transcription and polymerase chain reaction and specific primers for the RNA3 used. For this purpose, total RNA is extracted in pepper samples by CTAB method and then analyzed by RT-PCR for virus infection (Abdollah et al., 2012).

Extraction of total chlorophyll content

Acetone (80%) was used for assessment of Chl content (mg g–1 FM). Precisely, 0.25 g leaf disk was placed in 10mL acetone (80%) for extraction, and then centrifuged at 8000 g for 10 min and supernatant separated precisely for future experiment, and homogenization of leaf tissue with the buffer extraction was continued until colorless.

The collected supernatants were made to a final volume of 50 ml. Absorbance of the extract was read at 645 and 663 nm for chlorophyll and at 470 nm for carotenoid with a spectrophotometer (Shimadzo AA–670, Japan). Acetone 80% was used as blank. Then, total content was calculated based on method of Black et al. (1965). Carotenoid contents also evaluated using method of Lichtenthaler (1987).

Determination of total carbohydrate

The total leaf soluble carbohydrates were determined according to Irigoyen et al. (1992) and glucose (0–100 mg l–1, from MERCK) was used as a standard. Leaf samples of 0.5 g (FM) were homogenized in 5 ml ethanol (95%) and centrifuged at 4,500 × g for 15 min, the supernatant was removed from the sample and the residue was re-suspended in 5 ml of 70% ethanol. Then the supernatant was centrifuged again for final extraction.

Both supernatants were combined. Anthrone-sulfuric acid assay was used for determination. An aliquot of 100 µl was added to 3 ml of anthrone-sulfuric acid solution and the mixture was shaken, heated in a boiling water bath for 10 min and cooled at 4°C. The absorption at 625 nm was determined by spectrophotometer (Shimadzu AA-670, Japan). Equation used for standard curve preparation was as follow:

$Y = 545.04 x – 29.973$
$R^2 = 0.94$

Determination of antioxidants
For this purpose, 10 ml of 98% methanol added to 2 g of dry sample, and kept in room temperature for 24 hours. The solution will then be centrifuged at 6000 for ten minutes. Next, to the 0.1 ml of the above solution, 4 ml of 0.15 mM DPPH ethanolic solution was added and the resulting solution was stirred rapidly, and then kept in the dark chamber at room temperature for 30 minutes. The absorbance read at 517 nm by a spectrophotometer and antioxidant activity assessed by the method of Turkmen et al. (2005).

Dry weight measurement
For this purpose, the above ground parts of the plant dried by the oven in 70 °C for 24 h and then measured by the 0.0001 accuracy scale.

Total phenolic content
The total phenolic of tissues was determined by Folin-Ciocalteu method at a wavelength of 725 nm and expressed as a percentage of gallic acid (Chuah et al., 2008).

Determination of proline
Proline content was determined using the toluene reagent described by Bates et al. (1973) at a wavelength of 520 nm and data expressed as micromole proline per g FW.

Experimental design
This research was done in a factorial based on completely randomized design. Factors including virus infection (infected or not infected), manganese nutrition (0, 5 and 10 ppm) and different varieties (California wonder and SP301). Each treatment consisted of five replicates. Obtained data were analyzed by the Genstat program (Discovery Edition, Version 9.2, 2007, VSN International Ltd., UK) and mean values were compared at the level of 1 and 5% probability according to LSD test.

RESULTS AND DISCUSSION

Analysis of variance
The ANOVA table (Table 2) show significant simple effects of manganese concentrations (on total chlorophyll and dry weight), variety (on total chlorophyll and carbohydrate), and virus infection (on dry weight). Moreover, results showed that interaction among variety× virus× manganese was significant on all characters with the exception of dry weight .

Virus infection
Symptoms including necrosis and yellow mosaics were observed after 14 days of *Alfalfa mosaic virus* inoculation in both of pepper varieties. Then total RNA of inoculated leaves were extracted by RNA extraction kit (Dena Zist). Using specific primers in RT-PCR specific fragments were amplified in all samples that were inoculated by AMV.

Total chlorophyll (Chl) contents
Simple effects of manganese (Mn) supply and also variety were significantly different on this variable. The highest Chl content observed in plants treated with 5 and 10ppm. There was a significant difference between varieties on Chl content, however, AMV infection did not have an effect on this variable (Table 3) that was disagreement with findings of other research

(Christov et al., 2005; Radwan et al., 2007; Palanisamy et al., 2009; Kyseláková & Prokopová, 2011). The mentioned differences might be resulted from genetic basis of each variety and also about how they cope with the stresses under different environmental statuses (Rao et al., 2019). It means that some changes might be occurred related to chlorophyllase activity (Roca and Minguez-Mosquera, 2003), the Chl synthesis (Lucas, 1998) or replacement of Mg^{2+} by Mn^{2+} (Chatterjee et al., 1994). In presence of AMV (Table 4), increment of Mn led to lower level of Chl that was in accordance with results on TMV infected plants (Edreva et al., 1989), PMMoV (Petrova et al., 2012) and PaMMoV1. They found that chlorophyll in tobacco leaves had lost after virus-infection. Interaction between variety×Mn led to the lowest Chl content in California Wonder, and the highest Chl observed in PS301 under 10ppm Mn (Table 5). Supplying 5ppm Mn for PS301 variety led to Chl increase in infected plants (Table 6). It could be a prominent characteristic for this variety, even under AMV infection Chl increased via Mn nutrition that might be related to Mn-containing superoxide dismutase (MnSOD) as an antioxidant. This antioxidant playing an important role in the survival of cells in the presence of oxygen (Fridovich, 1983). In green leaves more than 90% of the SOD is located in the chloroplasts and only 4–5% in the mitochondria (Jackson et al., 1978), which may have useful protection for chlorophyll molecules.

Carotenoid

A considerable decrease in carotenoid contents was observed in the Virus-infected plants, compared with control (Table 3). Data showed that increase in Mn from 5 to 10 ppm significantly increased carotenoid content (Table 4). Interaction of Mn and variety had no significant effects on carotenoid contents (table 5). Interaction among AMV×variety×Mn showed that the highest carotenoid content is in 10 for PS 301 and 0 for California Wonder variety (Table 6). There is a significant difference between varieties in this study and their reply to Mn supply under AMV infection. Genetic background or photosynthetic capacity might be important in this reply (Ullah et al., 2020).

Recent studies have shown that carotenoids serve a protective function against biotic stress. There are some contradictory results about effects of different viruses on carotenoid content. Ehinmore and Kareem (2010) stated that TMV unaffected this variable, but Palanisamy et al. (2009) on *Abelmoschus esculentus and* Hosseini et al. (2018) on tomato found that TMV significantly influenced this variable.

Total carbohydrate

Supplying Mn nutrition to plants increased total carbohydrate compared with control, and the highest rate of this variable observed in Mn10ppm. Manganese deficiency has the most severe effect on the concentration of non-structural carbohydrates.

There was no significant difference in total carbohydrate between infected and control plants. PS301 showed lower carbohydrate compared with another variety (Table 3). Interaction between AMV and Mn (Table 4) and also between Mn and variety showed no significant effects on this variable (Table 5). Interaction among AMV×variety×Mn showed the highest total carbohydrates in California Wonder, in both AMV+ and AMV- statuses and 0 and 10ppm Mn, respectively. In 0ppm Mn, AMV+ led to the highest total carbohydrates in both varieties, however, in 10ppm Mn, AMV+ decreased total carbohydrate levels (Table 6).

Influence of plant pathogenic viruses on the carbohydrate metabolism in the infected host is very important with regard to economic damage caused to host. Some viruses appear to have little effect on carbohydrates in the leaves, while others may alter both their rate of synthesis and translocation (Gaddam et al., 2012). Regarding viruses, carbohydrates appear to have a role in plant defense mechanism and it has been known since the 1930s that

accumulation of starch precedes the presence of virus symptoms In plants infected with *Zucchini yellow mosaic virus* (Radwan et al. 2007; Gaddam et al., 2012), *Sunflower mosaic virus* (Buss et al., 2011) and *Beet Yellow virus* increased sucrose content was observed. A link between carbohydrates and defenses system against oxidative stress has been observed in other studies (Loreti et al., 2005) and carbohydrates are essential in the production of many anti-oxidants (Couee et al., 2006).

Antioxidant activity

Application of Mn nutrition raised leaf antioxidant activity, compared with control. In general, abiotic and biotic stresses generate ROS such as O^{-}_{2} and H_2O_2 in plant cells, and their over production is a common consequence of stress factors. However, plants respond to stress by ameliorating the danger that results from the oxidative stress. Under these conditions, there should be a balance between the generation and the degradation of ROS, otherwise oxidative injuries are inevitable. Therefore, plants, in general, have evolved complex defense mechanisms for detoxifying O^{-}_{2} and H_2O_2 (Dikilitas et al., 2009). The primary components of antioxidant systems consist of antioxidant enzymes (superoxide dismutase, catalase, peroxidase, and glutathione reductase) and non-enzymatic low molecular weight antioxidants (glutathione, proline, carotenoids, ascorbate, and tocopherols) (Panda and Khan, 2009). One of the Mn-containing enzymes is superoxide dismutase (MnSOD) that plays an essential role in the survival of plant cells in the presence of oxygen.

Virus infection had no significant effect on this variable (Table 3) that might be related to physiological characteristics of varieties coping with infection. It might be influenced by proline and total phenols accumulated within plant leaves. There are some reports showed that TMV infection affects this variable. They found that changes in ascorbate and glutathione levels and activities of ascorbate peroxidase, catalase, dehydroascorbate reductase (DHAR), glutathione reductase (GR), glutathione S-transferase (CST), and superoxide dismutase (SOD) declined following a TMV inoculation of tobacco plants (Fodor et al., 2001). Radwan et al. (2010) findings showed that stress metabolites such as H_2O_2 and MDA (malondialdhyde) tend to increase following infection by *Bean yellow mosaic virus* (BYMV). The infected leaves of bean plants had protein content higher than the control, indicating the accumulation of pathogenesis-related proteins and concluded that changing antioxidant status and the accumulation of some antioxidant metabolites, as well as pronounced alterations in the protein composition, indicated a kind of plant response against pathogen invasion (Radwan et al., 2010). Similarly, Hernandez et al. (2004) reported that a long-term effect of *Plum pox virus* (PPV) infection produced an oxidative stress via lipid peroxidation and protein.

Comparison of Aantioxidant activity showed no significant difference between two varieties (Table 3). Interaction between AMV and Mn (Table 4) and Variety and Mn (Table 5) on this variable also showed no significant effects. Interaction among AMV, Mn and variety showed the highest antioxidant activity in California Wonder treated with 5ppm Mn and AMV (Table 6). Supplying Mn to California Wonder may increase the tolerance of that to AMV infection, compared with PS301 because of improving antioxidant activities according to Panda and Khan (2009) statements (Table 6).

Dry weight

Manganese nutrition significantly increased dry weight content of pepper plants, compared with control (Table 3). Photosynthesis in general and photosynthetic O_2 evolution in PS II in particular is the processes that are most strongly depressed by Mn deficiency (Shenker et al., 2004).

Virus infection significantly decreased this variable (Table 3) that was in agreement with finding of Kollmann et al. (2007), however, there was no significant difference between varieties (Table 3).

Interaction between AMV and Mn showed the highest dry weight when Mn was supplied to infected plants. It is suggested that Mn supplying improves photosynthesis and carbon assimilation, and may effective on dry matter accumulation, even under AMV infection. Moreover, it was found that supplying 5ppm Mn in presence of AMV infection increased this variable compared with others (Table 4). However, interaction between variety and Mn supplying (Table 5) and also among AMV × Mn × variety showed no significant effect on this variable (Table 6).

Total phenolic

The simple effect of Mn was significant on this variable, so that 10ppm Mn application increased total phenolic content (Table 3). Also, analysis of data showed an increase trend in total phenolic content by AMV inoculation (Table 3) that was in agreement with Khalid et al. (2011), who reported that the phenolic compounds were increased in different plants after infection with pathogens. There was no significant difference between varieties on this trait (Table 3). Interaction between varieties and Mn supplying showed no significant effect on total phenolic contents (Table 5). Supplying of 10ppm Mn to California Wonder variety infected by AMV significantly increased total phenols (Table 6).

The phenolic compounds have play an important role in plant defense system (Ruelas et al., 2006). The defense system plays its role via pre-formed physical and chemical barriers (such as the strengthening of the cell wall) and activation of several biochemical pathways for the synthesis of different compounds (Ruelas et al., 2006). Moreover, manganese is a cofactor for some of enzymes involved in phenol and lignin production (Marschner, 1996).

Proline

Data showed that AMV inoculation led to the lowest proline content in leaves (Table 3). It should be mentioned that different viruses may activate different resistance reaction in plants, as Mandadi et al (2013) stated. They tried with identifying several resistance (*R*) genes in tomato (*Solanum lycopersicum*) and pepper (*Capsicum annuum*) to move them into different cultivars for protection of field and greenhouse-grown plants against viruses. They found that in some experiments proline production may not be activated by virus inoculation (Mandadi et al., 2013). Moreover, it is stated that proline might be consumed facilitating the virus resitance. This variable significantly increased under Manganese treatment compared with control (0 ppm) (Table 3). Proline contents were not significant by AVM × Mn treatments (Table 4). Interaction of variety and Mn supply showed the highest proline content in both ps 301 and california wonder supplied with 10 and 5ppm Mn, respectively (Table 5). Under non-infected conditions, the highest rate of this variable observed in 5ppm Mn for PS301 and 10ppm for California Wonder (Table 6).

The rapid induction of hypersensitive cell death led to the higher accumulation of proline in virus- inoculated leaves as hypersensitive response (HR). The induction of HR potentially limits the spread of disease from the infection points (Lee & Hwang, 2005). Similar results reported by Petrova et al. (2012). Radwan et al. (2007) who stated that the Zucchini yellow mosaic virus (ZYMV) infection increased proline content. Pazarlar et al. (2013) also reported similar results on pepper plants infected by Tobacco mosaic virus (TMV).

CONCLUSION

Plant viruses are globally responsible for the significant crop losses of economically important plants. All common approaches are not able to eradicate viral infection. Many non-conventional strategies are currently used to control viral infection, but unfortunately, they are not always effective. Therefore, it is necessary to search for efficient and eco-friendly measures to prevent viral diseases. It should be noted that some concentrations of nutrients such as Mn in plants may increase tolerance of plants against viruses (Beris, 2018). In the present study, we evaluated the interactive effect of AMV infection and manganese supplying on two pepper varieties California Wonder and PS301 for the first time. Data showed that Mn significantly improved some physiological aspects and increased total chlorophyll, total carbohydrate, antioxidant and dry weight. AMV infection decreased dry weight and had significant effect on total phenole and proline and did not have any influence on other characters. Interaction of AMV and Mn supplying raised total Chl, but decreased dry weight. Considering interaction among varieties, Mn supplying and AMV infection, it was found that California Wonder had the highest antioxidant activity when infected by AMV and treated with Mn 5 ppm. Finally, it is concluded that applying Mn nutrition improves total phenol and antioxidant activities under AMV infection that might be useful to promote tolerance in pepper varieties.

Conflict of interest

The authors declare there is no conflict of interest.

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