J. Agr. Sci. Tech. (2017) Vol. 19: 145-155

Differential Expression of NADH Oxidase, Superoxide Dismutase, and Catalase in Wheat Seedling in Response to *Zataria multiflora* Essential Oil Incorporated into Polyvinyl Alcohol Dispersion

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ABSTRACT

Enhancement of antioxidant capacity of plants by natural antioxidants obtained from medicinal plants can be a simple way to produce stress tolerant plants. *Zataria multiflora* essential Oil (ZO) is one of the useful antioxidants with potent antioxidant activity. ZO is insoluble in water and can be degraded by light, oxygen, and high temperature. Incorporation of ZO into Poly Vinyl Alcohol (PVA) dispersion is a simple way to improve its stability. In this research, the antioxidant activity of PVA/ZO dispersion was measured by studying the effect of the Hydrogen peroxide (H_2O_2) and Hydroxyl radical ($HO^{\bullet}$) scavenging of the dispersion. The efficiency of the PVA/ZO dispersion on the production of Nicotinamide adenine dinucleotide phosphate Oxidase (NOX), SuperOxide Dismutase (SOD), and Catalase (CAT) mRNAs in the wheat seedlings in hydroponic condition was investigated too. PVA/ZO dispersion had a non-Newtonian shear-thinning liquid behavior with the negative zeta-potential (-12 mV) and nanoscale particle size (134 nm). PVA/ZO dispersion had effective H_2O_2 and $HO^{\bullet}$ scavenging in a dose dependent manner with IC_{50} value of 220 and 170 µg mL^{-1}, respectively. PVA/ZO dispersion up-regulated NOX (2 folds, at 30 µg mL^{-1}), SOD (10 folds, at 30 µg mL^{-1}) and CAT (8 folds, at 250 µg mL^{-1}) mRNAs production. Thus, there was a potent cooperation between NOX and SOD activity and low cooperation between SOD and CAT activity. The potent antioxidant activity of PVA/ZO dispersion implies that it can effectively be used as a promising natural antioxidant to reduce oxidative stress in the plants under stress.

Keywords: Essential oil, CAT, NOX, Oxidative stress, SOD.

INTRODUCTION

Superoxide anion and hydrogen peroxide are toxic by-products of aerobic metabolism (Bartosz, 1997). Plants actively produce these Reactive Oxygen Intermediates (ROI) as signaling molecules to control processes such as programmed cell death, abiotic stress responses, pathogen defense, and systemic signaling (Inze and Montagu, 1995). Superoxide anion can be produced in several ways including photosynthesis electron transport chain, respiration electron transport chain, excited chlorophyll, Nicotinamide adenine dinucleotide phosphate Oxidase (NOX) and xanthine oxidase (Foreman *et al*., 2003;). Superoxide anion is actively converted to hydrogen peroxide by superoxide dismutase (Alscher *et al.*, 2002). Hydrogen peroxide can also be produced by photorespiration (glycolate oxidase in peroxisome), fatty acid *β*-oxidation, amine oxidase and oxalate oxidase (Demidchik, 2015). Hydrogen peroxide eliminated by catalases and peroxidases such as ascorbate peroxidase and glutathione peroxidase (Mittler, 2011).

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[DOR: 20.1001.1.16807073.2017.19.1.8.3]

The balance between NOX, SuperOxide Dismutase (SOD) and Catalases (CAT) and/or peroxidases activities are crucial for determining steady-state level of superoxide anion and hydrogen peroxide (Sharma *et al.*, 2012). Plants have evolved non-enzymatic (ascorbic acid, glutathione, α-tocopherol, carotenoids, phenolic compounds) and enzymatic (superoxide dismutase, catalases and peroxidases) protection mechanisms that efficiently scavenge ROI (Gill and Tuteja, 2010). Tolerance to a wide variety of environmental stresses has been correlated with increased capacity of non-enzymatic and enzymatic antioxidant mechanisms. Enhancement of antioxidant capacity of plants by natural antioxidant obtained from medicinal plants is a simple and accessible way.

One of these useful medicinal plants with strong anti-oxidant activity is *Zataria multiflora. Z. multiflora,* which is a thyme-like plant belonging to the Lamiaceae family that is extensively used as a flavor ingredient in a wide variety of fields in its native region. Modern pharmacological studies have shown that Zataria possesses a wide range of biological properties including antimicrobial and anti-oxidative properties (Sajed *et al.*, 2013). In this context, the Zataria essential Oil (ZO) has played a crucial role in pharmaceutical as well as in food industries. Previous studies revealed that the main components of ZO were thymol, carvacrol, *p*-cymene and *γ*-terpinene (Sadeghi *et al.*, 2015). ZO is one of the ten useful essential oils with potent antioxidant activity, which at low concentrations (10-20 µg mL^{-1}, depending on chemical composition) introduced strong antioxidant activity (Kavoosi and Rabiei, 2015).

However, due to hydrophobicity and insolubility in water, *Zataria* Oil (ZO) is unstable and can be degraded by light, oxygen, and high temperature (Turek and Stintzing, 2013). Thus, useful protection system is required to improve the stability of ZO along processing, storage, and application. Dispersion of ZO into different types of edible polymers with mild operating conditions is a simple way to formulation of ZO. Due to low price and strong gel-forming properties, polymers such as Poly Vinyl Alcohol (PVA) have high potentials to be applied in formulation of plant essential oil (Alves *et al.*, 2011; Gonzalez *et al.*, 2012).

In the present study, PVA solution was prepared and ZO, as a potent anti-oxidant agent, was dispersed into it to produce PVA/ZO dispersion. The zeta-potential, particle size and viscosity of PVA/ZO dispersion were determined. The antioxidant activity of PVA/ZO dispersion against Hydrogen peroxide (H_2O_2) and Hydroxyl radical ($HO^{\bullet}$) was examined. The efficiency of PVA/ZO dispersion in the production of NOX, SOD and CAT in wheat seedling in hypotonic condition was investigated. In this research, we aimed to find out whether PVA/ZO exhibits antioxidant activity in wheat seedling, in part, due to down-regulation of NOX and up-regulation of *SOD* and *CAT* genes expression.

MATERIALS AND METHODS

PVA (72 kDa), glycerol, glutaraldehyde, hydrogen peroxide, $FeSO_4$, EthyleneDiamineTetraacetic Acid (EDTA) and sodium salicylate were purchased from Sigma-Aldrich Chemical Co. (Saito Louis, Mo, USA). ZO was extracted from the air-dried leaves through hydro-distillation employing an all-glass Clevenger-type apparatus (Kavoosi and Rabiei, 2015). The density of ZO measured by digital balance had an average of 992 mg/mL. Thus, mass of each µL of ZO was approximately equal to 1 mg. ZO was dissolved in one volume of tween 20, thus, the final concentration of dissolved ZO was taken as 500 mg/mL and was used for preparation of PVA/ZO dispersion.

Preparation of PVA/ZO Dispersion

One gram of PVA was dissolved in 80 mL of distilled water under continuous stirring at 70°C until a homogenous solution was

[DOR: 20.1001.1.16807073.2017.19.1.8.3]

obtained. The homogenous solution was sonicated using an ultrasonic (Bandlin, Germany) at 140W for 4 minutes at the temperature 30°C. For preparation of PVA/ZO dispersion, 10% (w/w) of ZO (100 mg g^{-1} based on the weight of the PVA powder, equal to 1,000 µg mL^{-1} of PVA solution) was added to PVA solution and mixed under mechanical stirring at 800 RPM for 12 hours at 40°C. As an emulsifier, glycerol (30% w/w based on PVA powder) was added to PVA/ZO dispersion and mixed under mechanical stirring at 800 RPM at 40°C. As a PVA cross-linker, glutaraldehyde (10% w/w based on PVA powder) was added to PVA/ZO dispersion and mixed under mechanical stirring at 800 RPM at 40°C. At the end, distilled water was added until the final volume reached 100 mL under mechanical stirring at 800 RPM at 40°C. The final dispersions were stored at 4°C until further experiments.

Electrophoretic Mobility and Zeta-potential

The dispersion was diluted to a final concentration of 50 µg mL^{-1} by deionized water. The electrophoretic mobility of particles in the dispersion was determined by Phase Analysis Light Scattering (PALS) technique using a Brookhaven instruments corporation 90 Plus zeta-sizer (New York, 11742, USA). The Bi-PALS zeta potential analyzer software provided an average of electrophoretic mobility and a measure of zeta potential using smolouchewsky model (Sanchez-Gonzalez *et al.,* 2011).

Effective Hydrodynamic Diameter of Particles

The equilibrated dispersion was diluted to a final concentration of 50 µg mL^{-1} by deionized water. The effective hydrodynamic diameter of particles were determined based on the principle of Dynamic Light Scattering (DLS) by using Brookhaven instrument corporation 90 Plus particle size analyzer (New York, 11742, USA). The Bi-9000 particle sizing software provided an average effective hydrodynamic diameter of particles (Sanchez-Gonzalez *et al.,* 2011).

Apparent Viscosity

The dispersion was decanted into a glass cylinder (80×70 mm) and left on an anti-vibration bench with flat level for 10 minutes for equilibrium. The apparent viscosity (mPa s) of the dispersion was measured using Brookfield viscometer (DVII-pro, USA) at different spindle (No. 02) rotational speed of 1, 2, 5 and 10 s^{-1}. The software of Brookfield instrument provided the apparent viscosity of the dispersions, directly (Sanchez-Gonzalez *et al.,* 2009).

H_2O_2 Scavenging Activity

Twenty microliters of PVA/ZO dispersion (0-1,000 µg mL^{-1}) were incubated with 1.0 mL of H_2O_2 (50 mM in 100 mM phosphate buffer pH 7.4) for 60 minutes at 37°C. After incubation, the Absorbance (A) was read at 230 nm employing spectrophotometer. H_2O_2 scavenging percentage was calculated via the following formula: $[(A_{H2O2}-A_{test})/A_{H2O2}\times 100]$ (Sies, 1981), where, A_{H2O2}= Absorbance of H_2O_2 solution and A_{test}= Absorbance of H_2O_2 solution in the presence of PVA/ZO. The concentrations that could provide 50% H_2O_2 scavenging (IC_{50}) was derived from the graph that plotted the H_2O_2 scavenging percentage against different PVA/ZO concentrations.

Hydroxyl Radical Scavenging Assay

Twenty microliters of PVA/ZO dispersion (0-1,000 µg mL^{-1}) were incubated with 3 mL of Fenton reaction solution and incubated at 37°C for 60 minutes. Fenton reaction solution was prepared as follow: 10 mM

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$FeSO_4$, 10 mM EDTA, 2 mM sodium salicylate, 200 µL of H_2O_2 (30%) in 100 mM sodium phosphate buffer pH 7.4. After incubation, the absorbance was read at 510 nm. The percentage of $HO^{\bullet}$ scavenging was calculated by using the following formula (Sanchez-Mareno, 2002): $[(A_{HO\bullet}-A_{test})/A_{HO\bullet}\times 100]$, where, $A_{HO\bullet}$= Absorbance of Fenton reaction solution and A_{test}= Absorbance of the Fenton reaction solution in the presence of PVA/ZO. The IC_{50} was derived from the graph that plotted the $HO^{\bullet}$ scavenging percentage against different concentrations of PVA/ZO.

Plant Growth Conditions

Seeds of *Triticum aestivum* L. cv. Shiraz were surface-sterilized with sodium hypochlorite (5%) and planted in 1 kg pot under hydroponic conditions. Wheat seedlings received tap water at greenhouse conditions with about 60% relative humidity for two weeks until the size of leaves reached about 10 cm. Diurnal cycles at each temperature (27/24°C) were set at 16 hours day and 8 hours night cycle. At this stage, the wheat seedling was treated with 50 mL of different concentrations (15-500 µg mL^{-1}) of PVA/ZO dispersion diluted. Equal concentrations (15-500 µg mL^{-1}) of PVA solution were used as the control. Treatment was continued for three days. Twenty four hours after treatment, young wheat leaves were harvested and freezed in liquid nitrogen and then stored at -70°C until use.

RNA Preparation

Total RNA was extracted from 100 mg of leaf matter using RNX-Plus buffer (Cinnagen, Tehran, Iran) according to the manufacturer's instructions. Briefly, leaf samples were grounded in liquid nitrogen with a mortar. Then, 1 mL of RNX-Plus extraction buffer was added to the ground powder in RNase-free microtube, mixed thoroughly and left for 5 minutes at room temperature and then 200 µL chloroform was added and mixed gently. This mixture was centrifuged at 10,000×g for 15 minutes at 4°C, and the supernatant precipitated with an equal volume of isopropanol for 15 min on ice in a new tube. The resulting pellet was washed using 75% ethanol, dried for 5 minutes at room temperature, and re-suspended in 50 µL of RNase-free water. Quantification of total RNA was performed with a Nano Drop ND 1,000 spectrophotometer at 260 nm (Thermo Fisher Scientific, Wilmington, DE, USA). The integrity RNA was checked by visual observation of *28S rRNA* and *18S rRNA* bands on an agarose gel electrophoresis before real-time PCR analysis.

DNase Treatment and cDNA Synthesis

DNase treatment was carried out using a Fermentas DNase Kit (Fermentas, Hanover, MD, Germany) using the manufacturer's protocol and again the integrity of total RNA was checked by electrophoresis in agarose gel. Five µg of DNase-treated RNA was converted to cDNA with a Revert Aid First Strand cDNA Synthesis Kit (Thermo Scientific, Fermentas) using the manufacturer's protocol in a 20 µL final volume.

Primer Design and Real-time Quantitative PCR Analysis

Primers were designed using Allele ID 7 software (Premier Biosoft Intl, Palo Alto, CA, USA) for NOX (AY561153), SOD (primer for SOD gene designed based on the aligned nucleotide file) and CAT (primer for CAT gene designed based on the aligned nucleotide file) target genes (Table 1). The wheat *18S rRNA* (AJ272181) gene was used as internal control for data normalization (Jain *et al*., 2006). Primers for the PCR reactions were designed to have a melting temperature of about 55 to 65°C and to give a PCR product between 100 and 200 bp.

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Table 1. Primers used for real-time PCR analysis.[a]

Genes name	Orientation	Sense 5′-3′ sequence	Product
18S rRNA	Forward	*CGCTCCTACCGATTGAATGG*	127
	Reverse	*CCTTGTTACGACTTCTGCTTCC*	
NOX	Forward	*AGTGTCGCCCTTTGAATG*	182
	Reverse	*TCTTAGTTGTCTCGTCTGC*	
SOD	Forward	*TCCGCCGTCGTCCACCTC*	106
	Reverse	*CACCACCACCCTCGCTGATG*	
CAT	Forward	*CTTCCCCGTCTTCTTCATCC*	193
	Reverse	*GTCCATGTGGCGGTAGTC*	

[a] Primer design (in form of exon junction) was carried out using Allele ID 7 software for the internal control *18s rRNA* and test genes Nicotinamide adenine dinucleotide phosphate Oxidase (NOX), SuperOxide Dismutase (SOD) and Catalase (CAT).

Real-time PCR was performed using a line Gene K Thermal cycler (Bioer Technology Co, Hangzhou, China). The cDNA samples were diluted 1:5 by using nuclease-free water, and 5 µL of cDNA was used for real-time PCR. The final volume for relative real-time PCR was 20 µL containing 4 pmol of each primers, 5 µL (diluted) of the first-strand cDNA and 1X SYBR Premix Ex Taq TM II (Takara, Japan). The initial denaturing time was 5 minutes, followed by 40 PCR cycles consisting of 94°C for 10 seconds, annealing temperatures of each primer 15 seconds, and 72°C for 30 seconds. A melting curve was run after the PCR cycles followed by heating from 50 to 95°C. Proper control reaction was carried out without the reverse transcriptase treatment. For each sample, the subsequent real-time PCR reactions were performed twice under identical conditions.

Data Normalization and Quantitative PCR Verification

For real-time data analysis, the relative expression of the target gene in each sample was compared with the control sample (corresponding to the control plants) and was determined with the delta-delta *Ct* method (Livak and Schmittgen, 2001) using the following equations:

$2^{-[\Delta^{Ct\ sample} - \Delta^{Ct\ control]}}$, $\Delta Ct = CT_{\text{target gene}} - CT_{\text{internal control}}$

The *Ct* for each sample was calculated using the Line-gene K software (fqdPCR ver. 4.2.00), which refers to the threshold cycle determined for each gene in the exponential phase of PCR amplification. In this method of analysis, the relative expression of the target gene in the control sample was equal to one (2^0) by definition (Larionov *et al.*, 2005).

Statistical Analysis

All tests were conducted at least by three independent experiments with completely randomized design and were expressed as the mean values±standard deviations. The significant differences among the means were analyzed through Duncan test at $P< 0.05$ employing SPSS (SPSS Inc, Chicago IL, USA) software version 16.

RESULTS AND DISCUSSION

Physical Properties of PVA/ZO Dispersion

PVA/ZO dispersion had a non-Newtonian shear-thinning liquid behavior (Figure 1)

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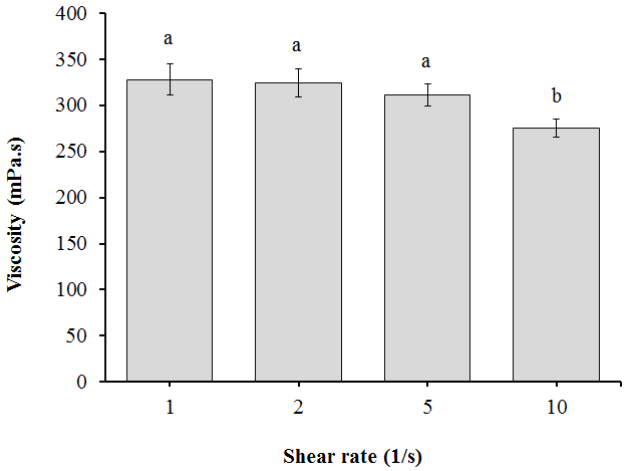

Figure 1. Viscosity as a function of shear rate of Poly Vinyl Alcohol (PVA)/Zataria Oil (ZO) dispersion. The apparent viscosity of the dispersion was measured using Brookfield viscometer at different spindle (No. 02) rotational speed of 1, 2, 5, and 10 s^{-1}. Increase in shear rate leads a decrease in the viscosity, thus, PVA/ZO dispersion had a non-Newtonian shear-thinning liquid behavior.

with the negative zeta-potential (-12 mV, Table 2) and with nanoscale particle size (134 nm, Figure S1 in supplementary file). Our experimental results are in accordance with the results of Sanchez-Gonzalez *et al.* (2009) on the HydroxyPropyl MethylCellulose (HPMC) emulsion incorporated with tea tree oil and the results of Sanchez-Gonzalez *et al.* (2011) on HPMC emulsion incorporated with bergamot oil. The physical properties of the dispersed particle, including the average particle size, zeta-potential and even viscosity greatly will influence the physical and functional properties of dispersions (Guzey and Mc Clements, 2007). When the particle size is decreased, the number of particles increases, so the number of particle-particle interactions increases, consequently, the viscosity of the sample usually increases (Saberi *et al.*, 2013). As the zeta-potential is increased, the particles are forced to stay away from each other due to the electrostatic repulsion of the particles. This effect principally prevents the particles from flowing freely; hence, the viscosity increases (Dickinson, 2009). The viscosity of PVA/ZO was differently modified as a function of shear rate. PVA/ZO showed non-Newtonian shear thinning flow behavior. For a shear thin fluid, the particle-particle interaction occurred with smaller forces, so this interaction could be broken down with an increase in the shear rate (Akhtar *et al.*, 2006). Accordingly, the authors suggested a lower interaction force between PVA/ZO particles at higher shear rate. The high zeta-potential, low particle size, and decrease in viscosity increased the repulsion forces in the dispersion and its stability (Huang *et al.*, 2001). Accordingly, the stability of the system and the mobility of the particles in the system increased. These events facilitated the flow of dispersion in the xylem and phloem between shoot and root.

Table 2. Conductivity, pH, Zeta-potential, particle size and viscosity of Poly Vinyl Alcohol (PVA) dispersion incorporated *Zataria* Oil (ZO).

Properties	PVA/ZO [a]
Conductivity (µS cm^{-1}) (µS/cm)	250 ± 7
pH	5.66 ± 0.05
Zeta-potential (mV) (mV)(mV)	-12 ± 1.0
Particle size (nm) (nm)	134 ± 5
Viscosity (mPa s)	282 ± 10

[a] The values are expressed as means±standard deviation for three independent experiments.

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Hydrogen Peroxide and Hydroxyl Radical Scavenging Effect of PVA/ZO Dispersion

The antioxidant activities of PVA/ZO dispersion against H_2O_2 and hydroxyl radical were tested. Pure PVA did not have any inherent antioxidant activity, while PVA/ZO had potent anti-oxidant activity because of incorporation of ZO (Figure S2 in supplementary file). PVA/ZO dispersion had effective H_2O_2 and $HO^{\bullet}$ scavenging in a dose-dependent manner with IC_{50} values of 220 and 170 µg mL^{-1}, respectively (Table 3). Several studies have already reported the antioxidant activity of ZO *against* non-biological oxidants using DPPH and ABTS methods (Kavoosi and Rabie, 2015; Kavoosi *et al.*, 2012). However, there are scarce reports on the anti-oxidant activity of ZO against biological oxidants, for example, H_2O_2 and hydroxyl peroxide. H_2O_2 itself is less toxic but it is converted to the hydroxyl radical by the Fenton or Haber-Weiss reactions in the presence of transition metal. The hydroxyl radical damage to all major classes of macromolecules including protein, lipids, and specifically mitochondrial and nuclear DNA (Sharma *et al.*, 2012). Accordingly, H_2O_2 and hydroxyl radical scavenging activity of PVA/ZO imply its beneficial role in reducing oxidative damages to biological tissues as a protective material.

Expression of NOX, SOD and CAT in Response to PVA/ZO Dispersion

The stimulatory effects of PVA/ZO dispersion on the production/generation of NOX, SOD and CAT mRNAs are summarized in Figure 2. PVA/ZO at concentration of 30 µg mL^{-1} up-regulated NOX (2 folds) and SOD mRNAs (10 folds) production. PVA/ZO dispersion at concentration of 250 µg mL^{-1} up-regulated CAT mRNA (8 folds) production. Thus, there was a potent cooperation between NOX (superoxide anion producer) and SOD (uperoxide anion scavenger and H_2O_2 producer) activity and low cooperation between SOD and CAT (H_2O_2 scavenger) activity in response to PVA/ZO dispersion.

The activity of plant NOX has been reported to increase in response to biotic interactions and various environmental abiotic stresses such as heat, drought, cold, high-light intensity, salinity, or wounding (Suzuki *et al.*, 2011). The strong interplay between ROI-producing and ROI-

Table 3. Hydrogen peroxide and hydroxyl radical scavenging activity of Poly Vinyl Alcohol (PVA) dispersions incorporated *Zataria* Oil (ZO).[a]

	PVA/ZO [b]
IC_{50} (µg mL^{-1}) for H_2O_2	220 ± 15
Antioxidant index	5 ± 1
Galic acid equivalent (µg $µg^{-1}$)	15 ± 2
IC_{50} (µg mL^{-1}) for HO	150 ± 10
Antioxidant index	7 ± 2
Galic acid equivalent (µg $µg^{-1}$)	22 ± 4

[a] The concentrations that could provide 50% hydrogen peroxide or hydroxyl radical inhibition (IC_{50}) were calculated from the graph that plotted the hydrogen peroxide or hydroxyl radical inhibition percentage against different antioxidant concentrations. Antioxidant index was calculated by dividing 1,000 by IC_{50}. Galic acids equivalents were calculated by dividing IC_{50} of gallic acid for hydrogen peroxide inhibition by IC_{50} of tested antioxidant for inhibition of hydrogen peroxide or hydroxyl radical. PVA had not any antioxidant activity but ZO and PVA/ZO displayed antioxidant activity. [b]Data represent mean±standard deviation from at least three sets of independent experiments.

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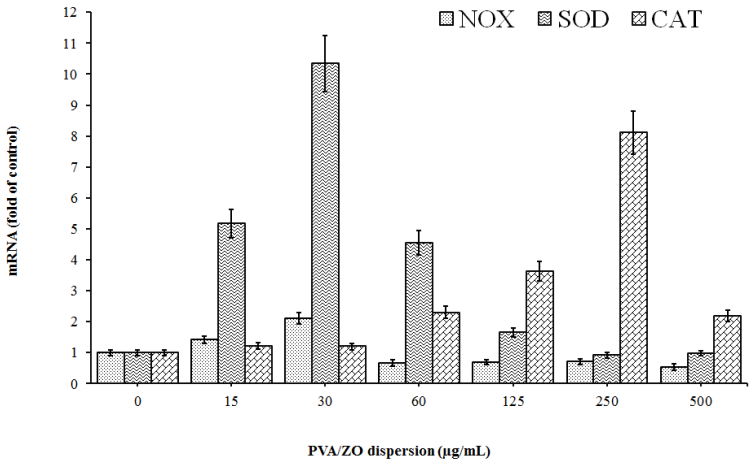

Figure 2. Differential expression of Nicotinamide adenine dinucleotide phosphate Oxidase (NOX), SuperOxide Dismutase (SOD) and Catalase (CAT) mRNAs in response to different concentration of Poly Vinyl Alcohol (PVA)/Zataria Oil (ZO) dispersion. The values of mRNA generation were examined using real-time PCR. PVA/ZO up-regulated NOX (2 folds, at 30 µg mL^{-1}), SOD (10 folds, at 30 µg mL^{-1}) and CAT (8 folds, at 250 µg mL^{-1}) mRNAs production. There was a potent cooperation between NOX and SOD activity and low co-operation between SOD and CAT activity.

scavenging enzymes enables cells to maintain a non-toxic steady-state level of ROI for signal transduction and to avoid plant cells to oxidative stress and oxidative damage (Baxter *et al*., 2014). The results of the present study indicated the modulatory effect of PVA/ZO on NOX mRNA induction/formation. By this effect, PVA/ZO may increase oxidative stress and related oxidative damage. Thus, the activity of superoxide anion-scavenging enzyme along NOX (e.g. SOD) will have a protective effect on the oxidative stress.

SOD present in most of the subcellular compartments (chloroplast, mitochondria, peroxisome and cytosol) generates activated oxygen and plays a central role in defense against oxidative stress (Gupta *et al.*, 1993). Thus, increment of SOD activity results in enhanced oxidative stress tolerance (Apel and Hirt, 2004). The up-regulation of SOD expression by PVA/ZO dispersion in this study suggested the potential of natural anti-oxidant from medicinal plants as an accessible agent for improvement of plant tolerance to stress.

Our results indicated that PVA/ZO dispersion as an antioxidant induced SOD and catalase in different concentrations. Thus, other hydrogen peroxide–degrading enzymes such as ascorbate peroxidase and glutathione peroxidase must be coupled to SOD (Racchi *et al.*, 2001). CAT is a heme-containing enzyme (in peroxisomes) that catalyzes dismutation of hydrogen peroxide into water and molecular oxygen without require to cellular reducing equivalent (Mhamdi *et al*., 2012). Moreover, CAT has a much lower affinity for hydrogen peroxide than peroxidase such as ascorbate peroxidase and/or glutathione peroxidase (Scandalios *et al.*, 1997). Thus, further investigations have to be managed to distinguish the coupling of enzyme systems for producing and degrading hydrogen peroxide.

CONCLUSIONS

In summary, the results revealed that PVA/ZO dispersion showed a non-Newtonian and shear thinning fluid with nano-scale particle size and negative charge. PVA/ZO dispersion had effective hydrogen peroxide and hydroxyl radical scavenging in a dose-dependent manner with IC_{50} of 220 and 170 µg mL^{-1}, respectively. Thus,

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PVA/ZO is a potent hydroxyl radical (HO•) scavenger. PVA/ZO also up-regulated NOX SOD and CAT mRNA. The maximum production of SOD and CAT mRNAs was observed at 30 and 250 µg mL^{-1}, respectively. Thus, PVA/ZO is a potent SOD and CAT inducer, but at various concentrations. The potent antioxidant activity of PVA/ZO dispersion implies that such products can effectively be used as promising antioxidant to reduce oxidative stress and oxidative damages in the plants. However, more specialized study is required to investigate other enzymes in the ROI producing and scavenging networks for determination of hydrogen peroxide producing and degrading coupled systems. Since this study was focused on wheat seedling in the unstressed conditions, further studies are required for other plants under stressed and unstressed conditions. It will be very useful if PVA/ZO dispersion could diminish oxidative stress of plants in response to abiotic and biotic stresses.

ACKNOWLEDGEMENTS

This work was supported by the financial support of Shiraz University (grant No. 88-GRAGRST-108). The authors declare no conflict of interest and are alone responsible for the content of this manuscript.

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[DOR: 20.1001.1.16807073.2017.19.1.8.3]

بیان متفاوت نیکوتین آمید ادنین دی نوکلئوتید اکسیداز، سوپراکسید دیسموتاز و کاتالاز در نهال گندم در پاسخ به مخلوط پلی‌وینیل الکل و اسانس آویشن شیرازی

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چکیده

افزایش توان آنتی اکسیدنی گیاهان از طریق آنتی اکسیدان های طبیعی گیاهان دارویی یک راه ساده برای تولید گیاهان متحمل به تنش است. یکی از این آنتی اکسیدان های طبیعی اسانس گیاه آویشن شیرازی است، که دارای خواص آنتی اکسیدانی بسیار قوی است. اما اسانس نامحلول در آب و در دما و نور ناپایدار است. قرار دادن اسانس در پلی وینیل الکل یک را ساده برای تقویت پایداری ان است. بر همین اساس در مطالعه حاضر، فعالیت آنتی‌اکسیدانی مخلوط پلی‌وینیل‌الکل-اسانس از طریق مطالعه اثر مهار پراکسید هیدروژن و رادیکال هیدروکسیل اندازه گیری شد. همچنین اثر مخلوط پلی‌وینیل‌الکل-اسانس ببان ژن های نیکوتین آمید ادنین دی نوکلئوتید اکسیداز، سوپراکسید دیسموتاز و کاتالاز در گیاهچه‌های گندم در شرایط هیدروپونیک بررسی شد. نتایج نشان داد که مخلوط پلی‌وینیل‌الکل-اسانس از رفتار سیال غیر نیوتنی رقیق شونده پیروی می‌کند و دارای بار منفی و اندازه ذرات ۱۳۴ نانومتر است. مخلوط پلی‌وینیل‌الکل-اسانس اثر مهاری موثری بر پراکسید هیدروژن (۲۲۰ میکروگرم بر میلی لیتر) و رادیکال هیدروکسیل (۱۷۰ میکروگرم بر میلی لیتر) داشت. به علاوه مخلوط پلی‌وینیل‌الکل-اسانس بیان ژن های نیکوتین آمید ادنین دی نوکلئوتید اکسیداز (دو برابر در غلظت ۳۰ میکروگرم بر میلی لیتر)، سوپراکسید دیسموتاز (ده برابر در غلظت ۳۰ میکروگرم بر میلی لیتر) و کاتالاز (هشت برابر در غلظت ۲۵۰ میکروگرم بر میلی لیتر را افزایش داد. بنابراین، یک همکاری قوی بین ف ژن های نیکوتین آمید ادنین دی نوکلئوتید اکسیداز، سوپراکسید دیسموتاز و همکاری پایینی بین فعالیت سوپراکسید دیسموتاز و کاتالاز وجود داشت. فعالیت آنتی اکسیدانی قوی مخلوط پلی‌وینیل‌الکل-اسانس آویشن نشان می‌دهد که این نوع محصول می‌توانند به عنوان یک آنتی اکسیدان امیدوارکننده‌ای برای کاهش اسیب های اکسیداتیو در گیاهان به طور موثری استفاده شوند.

[DOR: 20.1001.1.16807073.2017.19.1.8.3]