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Full Length Article

Improving Wheat Performance by Fish Flour and Vermicompost Priming against Salt Stress

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Abstract

Fish flour (FF) and vermicompost (V) priming were used as exogenous growth enhancers to stimulate wheat (*Triticum durum* Desf. cv. Yelken) phenolic biosynthesis against high salt stress. The main aim was to address whether priming of wheat with fish flour and vermicompost-combined treatment could bring about supplementary benefits particularly against salt stress. Exogenous application of fish flour and vermicompost-combined treatment improved plant behavior in the presence of salt stress. However, the best results and synergy in terms of growth, seed vigor and total phenolic – flavonoids, chlorophyll – carotenoids contents, phenylalanine ammonia-lyase (PAL), peroxidase (POD) activities and lipid peroxidation content (LPO) were obtained in response to fish flour: vermicompost (1:1) -combined treatment.

Keywords: Fish flour; Individual phenolic biosynthesis; Salt stress; Vermicompost; Wheat

Introduction

Chemical fertilizers have been used extensively by farmers. However, the chemical fertilizers lead to lots of serious problems for health and environment in developing countries (Ongley *et al.*, 2010). Therefore, useful manure composting provide an ecologically procedure to promote agricultural production with less dependence on chemical fertilizers and the recycling of solid wastes (like vermicompost and fish flour). The transform of organic waste into the biodegraded products via combined action of earthworms and microorganisms is named vermicomposting and is a low-cost technique (Gomez-Brandon and Dominguez, 2013). The combined action of earthworms and microorganisms under non-thermophilic phase start the humification and mineralization of the waste (Pathma and Sakthivel, 2012). The last product "Vermicompost" contains a large quantities of trace elements, mineral and plant growth regulators like hormones and humic acids. Vermicompost alone is insufficient for all the demands of crop yield. In this regard, combination of fish flour and vermicompost was used to understand the synergistic interactions of both on plant performance against stress conditions.

Anchovy *(Engraulis encrasicholus*) is the most abundant fish species in Black Sea of Turkey (Anonymus, 2000). The fish waste, including the head and other residues, provides over 60% of its production. Therefore, using edible fish waste becomes highly important for economy and environmental pollution (Boscolo, 2001). Mechanically separated fish waste may be used to produce fish flour after drying. The amino acids of Turkish anchovy (72–74% protein) contain a higher quantity of glutamic acid (a proline precursor), proline, aspartic acids and arginine with respect to the species of other countries (Dincer *et al.*, 2010). Fish flour also contains high levels of proline and glutamic acid. In the plants these amino acids may regulate phenylpropanoid pathway that synthesize phenolic compounds (Shetty, 1997).

The goals of this research were determination of exogenous vermicompost and fish flour, *alone or in combination* to wheat seed may result in an increase in salt tolerance via increasing seed vigor or/and phenolic biosynthesis. To determine exogenous elicitor may result in an increase in both biochemical (individual phenolic compounds, PAL and POD enzyme activities, LPO levels and proline contents) and traditional agronomic indicators (germination percentage, shoot and root length) of seed vigor. High performance liquid chromatography (HPLC) analysis was done for separation and identification of major phenolic compounds of wheat.

Materials and Methods

Plant Material and Seed Treatment

Ten g of sterilized wheat cultivar (*Triticum durum* Desf. cv. Yelken) seeds were soaked in each treatment vermicompost (V, *Engraulis encrasicholus*), fish flour (FF) and FF+V-combined pretreatments) with shaking at 150 rpm overnight.

The vermicompost was prepared by vermicomposting of cow fertilizer with *Eisenia fetida* earthworms for 3 months (Green-PIK Waste Treatment Co., Ltd., Turkey). The

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vermicompost basic properties were moisture content 41.2%, pH 7.5, total organic carbon 55–65%, total N 1.0–2.0%, total P 1.5–3%, total K 1–2%, total Ca 4–6%, total Mg 0.6–2.3%, total Na 1–2%, total Fe 0.6–2.5, humic substances 25–32% and indole acetic acid (IAA) 0.31 mg kg^{-1}.

Fish flour (FF) was purchased from a local anchovy fabric (Protein content 0.74 protein g FF^{-1} g). Anchovy fish flour includes 90.76% dry matter, 9.86% glutamic acid, 7.48% aspartic acid, 6.31% lysine, 5.78% leucine, 4.89% proline and 4.41% arginine. It was sieved to obtain a homogeneous size particle of 0.84 mm and ground to reach 0.5 mm particle size. The fish flour dilutions tested were 8, 10, 12, 13 mL and 15 mL/g fish flour.

Groups of vermicompost + fish flour-combined pre-treatments:

1. 0.1 g FF + 0.9 g V (1:9, FF:V)
2. 0.5 g FF + 1 g V (1:2, FF:V)
3. 0.9 g FF + 0.1 g V (9:1, FF:V)
4. 1 g FF + 0.5 g V (2:1, FF:V)
5. 0.5 g FF + 0.5 g V (1:1, FF:V)

Salinity Experiments

In order to examine the influences of salt stress on germination percentages. 15 wheat seeds were sown in glass containers with non-cellulosic paper. The seeds were constantly based-watered with Hoagland solution. Glass containers with germination seedlings were sealed to inhibit evaporation. They were germinated at 20ºC in a growth cabinet illuminated (25 µmol $m^{-2}s^{-1}$, 400–700 nm) on a 14 h day/10 h night regime. The salt concentration was 150 mM sodium chloride.

Germination Percentage Shoots Length and Roots Length

Seeds were considered to be germinated when the emergence of the radicula reached more than 2 mm in length. The number of germinated seeds for each cultivar and treatment was recorded every day. Germination percentages (GP) were calculated by the this formula: GP = n/N x 100%, where n is the number of germinated seeds and N is the total seeds number. The weight of whole seedlings including roots was measured.

Enzyme Determinations

After homogenization of one gram of the seedlings in 4 mL (20 mM phosphate buffer, pH 7.4) the mixtures were filtered and centrifuged (15.000 × *g* for 15 min). The phenylalanine ammonia-lyase (EC 4.3.1.5) activity was determined by Hodgins method (Hodgins, 1971). Assay concentrations contained 150 mM Tris-base buffer pH 8.5, 3 mM L-phenylalanine and enzyme (E = 19.7 mM^{-1} cm^{-1}).

The guaiacol-dependent peroxides (EC 1.11.1.7) activity was studied to Nakano and Asada method (Nakano and Asada, 1981). It was read in absorbance at 470 nm because of guaiacol oxidation (E = 26.6 mM^{-1} cm^{-1}). Assay concentration contained 0.05% guaiacol, 10 mM H_2O_2, 25 mM phosphate buffer (pH 7.0) and the enzyme.

Analytical Methods

Proline concentration was determined according to Bates and co-workers (Bates *et al.*, 1973). In order to precipitate protein, 50 mg of seedling was ground in 1.2 mL of 3% sulphosalicylic acid. After homogenization, the mixtures were centrifuged at 18.000 × g for 15 min. Then into the tubes with supernatants 1 mL glacial acetic acid and 1 mL ninhydrin reagent were added. Tubes heated in water bath at 90ºC for 1 h and then chilled in ice. The finally mixtures were mixed with 2 mL of toluene. Solvent absorbance recorded at 520 nm.

Total phenolic content in wheat samples were taken on first, third, fifth day of germination and was determined according to McCue method and gallic acid (25–200 µg/mL) was used as a phenolic standard curve (Mccue *et al.*, 2000). The flavonoid content was assayed according to Du and coworkers method with some modifications (Du *et al.*, 2009). Rutin standard (10-80 µg/mL) was prepared in 30% ethanol solution.

LPO was determined according to Buege and Aust method (Buege and Aust, 1978). The protein amount was determined according to Bradford method. Bovine serum albumin (BSA) was used as a standard (Bradford, 1976).

Quantitative Analysis of Individual Phenolic Compounds by HPLC

The phenolic acids and rutin standard solutions were prepared in methanol (1 mg/mL). Calibration curve ranged of seven concentrations (5.0, 2.5, 1.0, 0.5, 0.25, 0.125 and 0.0625 µg/mL). All solutions were filtered by a membrane filter (0.2 mm). Samples were extracted as described in Lee and Scagel (2009) with some modifications. 0.5 g wheat shoots were ground in a mortar with acidified methanol (0.1% formic acid, v/v) and were waited in the hot water bath (100ºC) for 5 min, then immediately kept in ice bath for 10 min. All the mixtures filtered with Whatman paper and the pellet was re-extracted. Analyses were carried out using Agilent 1100 HPLC system with a UV detector (Agilent Technologies, CA, USA). This system consisted of C18 Kinetex column (100 x 2.1 mm) with 2.6 µm particle size and the mobile phase of eluent A: 0.1% TFA in water and eluent B: acetonitirile. The absorbance of the eluent was scanned at 280 nm by the UV.

Statistical Analysis

Tukey test, one of the multiple comparisons, was used for

Table 1: Fresh weight and germination percentage of wheat for different treatments under salt stress 150 mM NaCl

Growth parameters	Plant growth (days)				
	Salinity	Treatments	1	6	11
	%		(% +g/ 10 mL)		
Fresh weight (mg)	0	Control	40±2	120±12	137±3
		0.1 g FF+.9 gV	53±2	100±12$^{\delta}$	168±8$^{\varepsilon}$
		0.5 g FF+1 g V	55±2	90±11$^{\delta}$	177±9$^{\varepsilon}$
	100	Control	40±2	60±10	90±6
		0.1 g FF+0.9 g V	51±2	100±08$^{\varepsilon}$	108±5$^{\varepsilon}$
		0.5 g FF+1 g V	50±2	110±09$^{\varepsilon}$	152±4$^{\varepsilon}$
		0.9 g FF+0.1 g V	50±2	90±11$^{\delta}$	129±11$^{\varepsilon}$
	0	Control	2	80	87
		0.1 g FF+0.9 gV	3	87	85
		0.5 g FF+1 g V	3	90	95
		0.9 g FF+0.1 g V	2	80	85
% Germination		Control	2	60	70
	100	0.1 g FF+0.9 g V	2	70	70
		0.5 g FF+1 g V	2	80	90
		0.9 g FF+0.1 g V	2	70	85
		1 g FF+0.5 g V	2	60	80
		0.5 g FF+0.5 g V	3	70	95

Data are the mean ± SD of three independent experiments
$^{\delta}p<0.05$ (probably significant); $^{\varepsilon}p<0.01$ (definitely significant)

Table 2: Shoot and root length of wheat for different treatments under salt stress 150 mM NaCl

Growth parameters	Plant growth (days)				
	Salinity	Treatments	1	6	11
	%		(mM +g/ 10 mL)		
Root length (cm)	0	Control	0.2	3.7±1.1	4.9±0.8
		0.1 g FF+0.9 gV	-	4.8±0.7	6.9±0.5
		0.5 g FF+1 g V	0.1	4.7±0.1$^{\delta}$	9.0±0.2$^{\delta}$
		0.9 g FF+0.1 g V	-	4.5±0.2$^{\delta}$	7.0±0.1$^{\delta}$
		1 g FF+0.5 g V	0.3	6.7±1.1	7.8±0.2
		0.5 g FF+0.5 gV	0.5	7.7±1.1	11.7±2.0
	100	Control	0.2	1.2±0.3	3.6±0.6
		0.1 g FF+0.9 gV	-	1.6±0.2$^{\varepsilon}$	2.4±0.5$^{\varepsilon}$
		0.5 g FF+1 g V	-	3.9±0.1$^{\varepsilon}$	5.4±0.4$^{\varepsilon}$
		0.9 g FF+0.1 g V	-	2.8±0.2$^{\varepsilon}$	4.3±0.1$^{\varepsilon}$
		1 g FF+0.5 g V	-	2.7±0.2$^{\varepsilon}$	5.0±1.2$^{\varepsilon}$
		0.5 g FF+0.5 gV	0.2	4.4±0.3$^{\varepsilon}$	5.8±0.6$^{\varepsilon}$
Shoot length (cm)	0	Control	0.2	2.2±1.1	6.8±0.8
		0.1 g FF+0.9 gV	-	4.7±0.7$^{\varepsilon}$	9.3±0.5
		0.5 g FF+1 g V	0.1	6.3±0.1$^{\varepsilon}$	10.0±0.2$^{\varepsilon}$
		0.9 g FF+0.1 g V	-	2.8±0.2$^{\varepsilon}$	7.9±0.1$^{\varepsilon}$
		1 g FF+0.5 g V		2.8±0.2$^{\varepsilon}$	7.4±0.1$^{\varepsilon}$
		0.5 g FF+0.5 gV		4.8±0.2$^{\varepsilon}$	9.1±0.1$^{\varepsilon}$
		Control	0.2	1.2±0.3	4.7±0.6
		0.1 g FF+0.9 gV	-	1.6±0.2$^{\varepsilon}$	3.3±0.5$^{\varepsilon}$
		0.5 g FF+1 g V	-	2.1±0.1$^{\delta}$	6.7±0.4$^{\varepsilon}$
		0.9 g FF+0.1 g V	-	2.9±0.2	6.4±0.1$^{\varepsilon}$
		1 g FF+0.5 g V	-	2.3±0.2$^{\delta}$	7.1±1.2$^{\varepsilon}$
		0.5 g FF+0.5 gV		3.3±0.2$^{\delta}$	8.1±1.2$^{\varepsilon}$

Data are the mean ± SD of three independent experiments.
$^{\delta}p<0.05$ (probably significant); $^{\varepsilon}p<0.01$ (definitely significant)

statistical significance analysis. The data reports are the mean ± SD of 3 independent experiments, or 3–4 replicates of biochemical tests. Also comparison was made with Pearson correlation for each substrate and/or enzyme.

Results

Fish flour-alone, vermicompost-alone and FF+V-combined treatment were used as growth enhancers to stimulate wheat phenolic biosynthesis against salt stress. The average plant height and weight resulted in 1.1-fold increase (7.2 cm) and 1.2-fold increase (169 mg) under fish flour-alone treatment when compared to control, respectively (data not shown). The similar results were also obtained for vermicompost-alone treatment (data not shown). Without vermicompost, fish flour had only slight effects on wheat phenolic biosynthesis and vice versa (data not shown).

Fresh weights (FW) and germination percentages of wheat seedlings increased gradually from day 1 to 11 under both non-saline and salt stress. Salt stress decreased the fresh weight and germination rate from 137 ± 3 to 90 ± 6 mg and from 87 to 70% on the 11th day ($p < 0.01$), respectively. However, the levels alleviated by FF-, V-alone and especially FF+V-combined pretreatment under non-saline and salinity conditions.

Table 1 applied treatment in the order of increasing fresh weights and germination percentage of the wheat seedlings were 1:9 < 9:1 < 2:1 < 1:2 < 1:1 FF:V combined pre-treatment under both non-saline and salinity conditions. It was found that the application of fish flour and vermicompost led to continues increases in the root and shoot lengths during the experimental period. Application of salt stress decreased the root and shoot length from 4.9 ± 0.8 to 3.6 ± 0.6 cm and from 6.8 ± 0.8 to 4.7 ± 0.6 cm on the 11th day, respectively. However, the lengths in wheat seedlings grown from FF+V combined pretreatment induced significantly under salt stress ($p < 0.05$). Combined treatment in the order of increasing root and shoot lengths of the wheat seedlings were 1:9 < 9:1 < 2:1 < 1:2 < 1:1 FF:V combined pretreatment under salt stress.

Table 2 the total phenolic and flavonoid contents of the wheat seedlings organs (seed, shoot and root) increased significantly in the entire treatment group ($p < 0.05$) (Table 3). Generally, total phenolic and flavonoids increased more under salt stress when compared to non-saline conditions. Applied treatment in the order of increasing total phenolic and flavonoid contents of the wheat seedlings were 1:9 < 1:2 < 9:1 < 2:1 < 1:1 FF:V combined under salt stress. The maximum increase of the total phenolic contents were 1.5–, 1.4- and 1.6 folds while for flavonoid 1.7-, 2- and 2-fold in shoot, root and seed of FF:V combined pretreatment under salt stress as compared to non-saline control respectively ($p < 0.01$).

Salt stress markedly increased PAL, POD enzyme activities and also proline, LPO levels ($p < 0.01$) (Table 4). It is considered that these enzymes activities increase as a result of exposure to salt stress. The 0.5 g FF: 0.5 g V group of FF:V combined pretreatment appeared to be the most effective treatment in counteracting the hazardous effects of salt stress on PAL and POD enzyme activities. The fish flour and vermicompost applied alone increased PAL and POD

Table 3: Total phenolic and flavonoid contents of wheat for different treatments under salt stress 150 mM NaCl

Characteristics	Salinity	Treatments	Wheat Organs		
	%	(g +g FF+V)	Shoot	Root	Seed
Total soluble phenolic content		Control	14.7 ± 1.4	8.6 ± 0.8	9.6 ± 0.2
(mg g^{-1} FW)		0.1 g FF +0.9 gV	16.2 ± 2.0$^{\varepsilon}$	11.6 ± 0.6$^{\varepsilon}$	11.4 ± 0.5$^{\varepsilon}$
		0.5 g FF + 1 g V	15.9 ± 0.7$^{\varepsilon}$	14.7 ± 1.1$^{\varepsilon}$	11.3 ± 0.2$^{\varepsilon}$
		0.9 g FF+ 0.1 g V	19.9 ± 0.7$^{\varepsilon}$	11.9 ± 0.9$^{\varepsilon}$	13.5 ± 0.1$^{\varepsilon}$
		1 g FF+ 0.5 g V	18.3 ± 0.7$^{\varepsilon}$	12.6 ± 0.9$^{\varepsilon}$	12.4 ± 0.1$^{\varepsilon}$
		0.5 g FF +0.5 gV	18.9 ± 0.7$^{\varepsilon}$	11.9 ± 0.9$^{\varepsilon}$	12.8 ± 0.1$^{\varepsilon}$
	100	Control	15.1 ± 2.4	11.5 ± 1.2	11.2 ± 0.6
		0.1 g FF +0.9 gV	15.6 ± 3.2$^{\varepsilon}$	9.6 ± 1.8	13.8 ± 0.5$^{\varepsilon}$
		0.5 g FF + 1 g V	18.0 ± 2.2$^{\varepsilon}$	11.9 ± 0.9$^{\delta}$	9.8 ± 0.4
		0.9 g FF+ 0.1 g V	20.1 ± 1.6$^{\varepsilon}$	11.0 ± 1.5$^{\delta}$	14.2 ± 0.7$^{\varepsilon}$
		1 g FF+ 0.5 g V	21.6 ± 2.3$^{\varepsilon}$	11.9 ± 0.7$^{\delta}$	14.6 ± 1.2$^{\varepsilon}$
		0.5 g FF +0.5 gV	22.6 ± 2.3$^{\varepsilon}$	13.9 ± 0.7$^{\delta}$	15.2 ± 1.2$^{\varepsilon}$
Total flavonoid content					
(µg g^{-1} FW)		Control	175 ± 12	150 ± 03	723 ± 25
		0.1 g FF +0.9 gV	182 ± 11$^{\varepsilon}$	211 ± 11$^{\varepsilon}$	886 ± 25$^{\delta}$
		0.5 g FF + 1 g V	185 ± 13$^{\varepsilon}$	139 ± 13$^{\delta}$	104 ± 12$^{\varepsilon}$
		0.9 g FF+ 0.1 g V	168 ± 15$^{\varepsilon}$	132 ± 11$^{\varepsilon}$	986 ± 41$^{\varepsilon}$
		1 g FF+ 0.5 g V	196 ± 13$^{\varepsilon}$	175 ± 13$^{\delta}$	119 ± 12$^{\varepsilon}$
		0.5 g FF +0.5 gV	203 ± 15$^{\varepsilon}$	192 ± 11$^{\varepsilon}$	122 ± 11$^{\varepsilon}$
	100	Control	186 ± 13	153 ± 10	986 ± 26
		0.1 g FF +0.9 gV	204 ± 11	188 ± 11	924 ± 35$^{\delta}$
		0.5 g FF + 1 g V	219 ± 12$^{\delta}$	205 ± 12$^{\delta}$	984 ± 14$^{\varepsilon}$
		0.9 g FF+ 0.1 g V	243 ± 1.1$^{\varepsilon}$	233 ± 11$^{\varepsilon}$	1162 ± 11$^{\varepsilon}$
		1 g FF+ 0.5 g V	271 ± 13$^{\varepsilon}$	276 ± 10$^{\varepsilon}$	1357 ± 11$^{\varepsilon}$
		0.5 g FF +0.5 gV	288 ± 13$^{\varepsilon}$	299 ± 11$^{\varepsilon}$	1458 ± 12$^{\varepsilon}$

Data are the mean ± SD of three independent experiments
$^{\delta}p<0.05$ (probably significant); $^{\varepsilon}p<0.01$ (definitely significant)

Table 4: PAL and Gua-dep POD activities, LPO and proline contents under salt stress 150 mM NaCl on the 11th day

Parameter	No salinity		Salinity stress, 150 mM NaCl		
	Control	Non-treatment	Combined pretreatment		
			0.1gFF+0.9gV	0.5gFF+0.5gV	0.9gFF+0.1gV
PAL (U.mg^{-1} protein)	22 ± 1	38 ± 3$^{\varepsilon}$	38 ± 18$^{\varepsilon}$	44 ± 12$^{\varepsilon}$	38 ± 14$^{\varepsilon}$
Gua-dep POD (U.mg^{-1} protein)	0.5 ± 0.1	2.5 ± 0.3$^{\varepsilon}$	4.4 ± 0.3$^{\varepsilon}$	5.9 ± 0.2$^{\varepsilon}$	4.9 ± 0.4$^{\varepsilon}$
LPO (nmol MDA.g^{-1})	0.15 ± 0.1	0.22 ± 0.0$^{\varepsilon}$	0.1 ± 0.0$^{\delta}$	0.08 ± 0$^{\delta}$	0.1 ± 0.0$^{\varepsilon}$
Proline (µmol g^{-1} FW)	2.2 ± 0.1	4.5 ± 0.1$^{\varepsilon}$	6.8 ± 0.1$^{\varepsilon}$	5.9 ± 0.1$^{\varepsilon}$	4.5 ± 0.1$^{\delta}$

Data are the mean ± SD of three independent experiments
$^{\delta}p<0.05$ (probably significant); $^{\varepsilon}p<0.01$ (definitely significant)

Table 5: Non-treatment (-FF-V) and 0.5g FF+ 0.5 g V combined treatment under non-saline and salt stress on the content of individual phenolic compounds in the shoot of wheat

Phenolic compounds (mM)	No salinity		Salinity stress, 150 mM NaCl	
	-FF-V	+FF+V	- FF-V	+ FF+V
Rutin	78.3 ± 1.4	61.9 ± 12	31.9± 2	98.0 ± 9$^{\varepsilon}$
Scopelletin	60.9 ±1.8	15.4 ± 0.5$^{\varepsilon}$	41.1 ± 5$^{\varepsilon}$	39.4 ± 7$^{\varepsilon}$
Syringic acid	58.6 ± 5	59.7 ± 11$^{\delta}$	56.2 ± 11$^{\delta}$	124.7 ± 15$^{\varepsilon}$
Gallic acid	58.7 ± 11	255.8 ± 1$^{\delta}$	81.5 ± 1$^{\delta}$	328.8 ± 10$^{\varepsilon}$
Salicylic acid	6.0 ± 1.2	7.4 ± 0.3	21.7 ± 3	6.79 ± 0.5

Data are the mean ± SD of three independent experiments
$^{\delta}p<0.05$ (probably significant); $^{\varepsilon}p<0.01$ (definitely significant)

activity but increases were not significantly from each other.

Scopoletin, rutin and the phenolic acids such as syringic, gallic, salicylic acids were identified in the wheat shoot and quantified using HPLC (Table 5). FF+V combined priming did not change significantly the endogenous levels of rutin and scopoletin under non-saline conditions. Thus, exogenous FF+V may be metabolized as precursors for syringic acid, gallic acid and salicylic acid under non-saline conditions. However, all the individual phenolic compounds such as scopoletin and rutin in the shoot of wheat seedlings increased significantly under salt stress ($p < 0.01$).

Discussion

In this study, there were high synergistic influences between fish flour and vermicompost on wheat quality with increasing strongly phenolic biosynthesis, while vermicompost-alone, fish flour-alone treatment did not induce phenolic compounds effectively. As seen from the results vermicompost or fish flour alone failed to meet all the requests of wheat phenolic production for available growth parameters. Fish flour source provide proline and proline precursors and these amino acids were effectively used to stimulate the phenolic biosynthesis. The proline-linked pentose phosphate pathway could stimulate shikimate and phenylpropanoid pathways and therefore a modulation of this pathway can cause to the phenolic phytochemicals production. Vermicompost is a large quantity of nutrient and resource compost (Song *et al.,* 2014). In addition, its abundant resources could provide for phenolic biosynthesis as energy and carbon sources. It has been reported about benefits of vermicompost on plant growth (Song *et al.,* 2015). Our studies explored the combination of vermicompost and fish flour influences on plant growth performance especially under salt stress. We hypothesized that vermicompost and fish flour could lead beneficial effects of phenolic biosynthesis on wheat but such effects were dependent on synergistic interactions between them and their doses (1:1) under salt stress. It is reported that vermicompost could effectively support plant productivity (Kalra *et al.,* 2010; Singh *et al.,* 2012).

Germination percentage and fresh weight were decreased under salt stress (Kasim *et al.,* 2016). The root and shoot lengths decreased also under salt stress during the treatment period. Fish flour and vermicompost act as signal molecules, showed positive effects of plant adaptation against salt stress.

PAL activity is a key enzyme of secondary metabolism and its activity was more induced under high salt stress. Therefore, FF+V combined treated wheat seedlings indicated higher PAL activity under salt stress than non-saline control. Similarly, Abu El-Soud *et al.* (2013) reported that PAL activity of the chickpea seedlings treated with ellagic acid under osmotic stress was higher as the untreated seedlings. In consistent with PAL activity, according to our results, FF+V combined treated wheat seedlings could accumulate larger amount of total phenolics and flavonoids under stress than the untreated seedlings. The accumulation of flavonoids was induced in non-treated wheat seedlings under salt stress. Moreover, FF+V combined treated wheat seedlings accumulated efficiently higher flavonoids content under salt stress. Accumulation of phenolics and flavonoids could prevent the lipid peroxidation, phenolic compounds were proven to trap the lipid alkoxy radicals according to the their structure (Milic *et al.,* 1998). Phenolic compounds prevent the diffusion of free radicals to the hydrophobic matrix of the membrane and limit the peroxidative reactions in the hydrophobic region of the bilayer (Verstraeten *et al.,* 2003).

Exogenous FF+V may be metabolized as precursors for some phenolic acids especially gallic acid (6-fold increase) and syringic acid (2-fold increase) under salt stress. It might be supported by the detected 6-fold accumulation of gallic acid in the shoot of wheat seedlings primed with FF+V. Therefore, it has been reported that gallic acid accumulated in strawberry under salt stress (Keutgen and Pawelzik, 2008). The major detected phenolic acids in wheat shoot are syringic and gallic acids, while salicylic acid was less abundant. Our results are in accordance with the previous studies on wheat phenolome (Saleh and Madany, 2015). In addition to phenolic acids, also rutin (1.3-fold increase) and scopoletin (1.6-fold increase) were increased in wheat seedlings against salt stress. Similarly, Saleh and Madany (2015) had declared that coumarin priming has resulted in accumulation of scopoletin in the leaves of wheat.

Regardless of salt stress, coumarin and FF+V priming has resulted in significant free phenolic acids accumulation. Saleh and Madany (2015) only found that chlorogenic and caffeic acids decreased after coumarin priming. When we looked at other studies FF+V-priming give the best results syringic acid as 2-fold and gallic acid as 6-fold increase to salt stress. These might be correlated with a role of FF and V in the regulation of shikimic acid pathway. In addition, accumulation of phenolic acids, flavonoids and flavonoids precursor in response to FF+V treatment, could improve the non-enzymatic antioxidant capacity as known for their free radical scavenging capacity. It is known that salicylic acid has a manifold role in modulating the growth and metabolism of plant suffering salinity. Therefore, the slightly salicylic acid accumulation the shoot of wheat seedling under salt stress was observed.

FF+V pretreatment stimulated more the POD activity than under non-saline control. Meanwhile, the stimulatory influence of FF+V on POD activity was more evident under high salt stress. The increase POD activity can contribute to the antioxidant storage by scavenging H_2O_2 and provide oxidized substrates for some physiological procedures.

This study results suggest the ability of wheat seed priming with FF+V to improve wheat resistance to salt stress. The growth mitigating effect of FF+V on wheat seedling grown under salt stress might be associated with the proline accumulation that could reduce the cellular osmotic potential (Rhandir and Shetty, 2003). The accumulation of phenolics and flavonoids increased the antioxidant defense system. They protect by acting as antioxidant molecules or by serving as substrates for peroxidase activity and allows in scavenging of H_2O_2.

Fish flour and vermicompost have a manifold role in plant growth regulation under salt stress. In the present study, fish flour and vermicompost are used for improving plant tolerance to salt stress the first time. The wheat seeds were treated with FF+V (0.5 g +0.5 g V) (1:1 FF:V) give the best results for increasing phenolic biosynthesis and plant growth.

Conclusion

In conclusion, the strongly synergistic influence by both organic vermicompost and fish flour rich from pentose phosphate pathway precursors revealed that environmentally approach for intensive agricultural lands is easy and possible against salt stress.

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