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RESEARCH ARTICLE

Effect of vermicompost soil additive on growth performance, physiological and biochemical responses of tomato plants (*Solanum lycopersicum* L. var. Firenze) to salt stress

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ABSTRACT

Vermicomposting is increasing used to process food, sewage and other organic wastes through the breakdown by earthworms. Vermicompost addition to soils can improve plant growth through increasing the accessability of nutrients and lowering levels of contaminants found in other compost products. This study examins the effect of vermicompost on salinity tolerance in tomato plants (*Solanum lycopersicum* L., var. Firenze) via greenhouse pot experiments. Plants were grown on 4 substrates designated by letter identifiers: A control, "T" with 100% organic soil; a vermicompost treatment "Vc" was 80% organic soil + 20% Vermicompost; a compost treatment "C" was 80% organic soil + 20% Compost; and a mixture treatment "M" was 80% organic soil + 10% Vermicompost + 10% Compost. The four treatment groups were exposed to 3 NaCl concentrations (0, 50 and 150 mM); the experimenta; design within the greenhouse was complete randomized block. The plants' response to salinity stress was evaluated through morphological (shoot length, stem diameter, leaves number, root length, shoot and root fresh and dry weight), physiological (Chla, Chlb and Carotenoid) and biochemical (malondialdehyde (MDA) and catalase (CAT)) parameters. All measured parameters were significantly different between the four soil treatments. Plants grown on Vc substrate showed an improved growth and a better resistance to salinity stress. Analyzed parameters were positively influenced by the contribution of the organic matter (Vermicompost, compost and a mixture of the two) which plays a role in the slow, consistent release of mineral elements and provides soluble nutrients to reduce abiotic stresses. In conclusion, vermicompost could be a relevant method for reducing salt stress on tomato plants growth, addressing the challenges of growing food crops in drier, more saline contaminated environments.

Keywords: Vermicompost; NaCl; Plant growth; Chlorophyll content; antioxidant activity

INTRODUCTION

In nature, plants are challenged by abiotic and biotic stresses that can limit growth and reproduction; agricultural crops stressed by increasingly saline soil face significant yield losses. Agricultural yield increases are critical for future food crops production for global food security (Zörb et al., 2019). Increasingly saline soils resulting from overuse of groundwater resources is major environmental stressor in arid and semi-arid ecosystems; this stress is excerbaged by climate change (Lal, 2015; Zamin et al., 2019; Saleem et al., 2020). Increased soil salinity results from moisture evaporation from soil with fluctuating rainfall. Increasingly saline soils expose crops to higher salinity water, which stresses plants. Globally, 77 million hectares (5%) of the 1.5 billion hectares under cultivation are affected by high salinity attribueted to the poor quality of irrigation water (Sheng et al., 2008; R'him et al., 2013; İmamoglu and Dengiz, 2019).

In soil, high concentration of NaCl induces, directly or indirectly, leads to morphological, physiological, biochemical and metabolic adaptations in plants; the severity of these adaptations depends on environmental conditions such as light intensity, soil conditions and severity of the stress (Kamran et al., 2019). Salinity elevates

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osmotic pressure in the plant root-microrrhizza complex resulting in less water available to the plant. Water stress affects plant development and agricultural production worldwide (Kamran et al., 2019; Adhikari et al., 2020). Salinity stress reduces leaf biomass, stem length and roots length (Pradi-Vendruscolo and Seleguini, 2020); salinity stress also induces changes in the photosynthetic process (Adnan et al., 2020). Greenhouse plants placed under salinity stress show changes in gene expression. Salt-stressed planted produce reactive oxygen species (ROS); elevated formation of different ROS can lead to molecular damage. ROS can serve as indicator molecules signaling osmotic tolerance. The increased accumulation of ROS damages cell wall and enzymes and causes a breakdown of chlorophyll. To confront this oxidative stress, plants launch detoxification mechanisms by regulating antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and L-ascorbate peroxidase (APX) to support cellular homeostasis (Rehman et al., 2019). Past studies have shown that antioxidants increased their activities with salt stress in soybeans (Kataria et al., 2019) and wheat (Shafiq et al., 2020).

Remediation of increased soil salinity could be managed using different strategies such as soil leaching with water, chemical remediation and phytoremediaton via salt-resistant cultivars (Qadir et al., 2007). The addition of organic matter has been an effective practice against soil salinization globally (Tejada et al., 2006). Saline soils have less structural stability with low organic content. Past research has supported the use of organic materials such as compost and food processing wastes as soil additives (Tejada et al., 2006). This research examines the application of vermicompost (Vc) in mitigating the negative impacts of high salinity irrigation water on plants. Vermicompost added to soil has been shown to increase the growth of horticultural crops such as tomato (Atiyeh et al., 2000; Gutiérrez-Miceli et al., 2007), pepper (Arancon et al., 2005) and sweet corn (Lazcano et al., 2011). These findings have shown that vermicompost can significantly modulate the adverse impact of saline soil on plant morphological and physiological parameters by reducing the harmful effects of toxic elements and creating an anti-stress effect (Bidabadi et al., 2017; Benazzouk et al., 2018; Zhang et al., 2019; Moghdam and Reza, 2020).

Effects of salt stress on antioxidative enzymes responses have been studied in some plant species (Libertad and Manuel, 2014). Bidabadi et al. (2017) showed that Vc could have an anti-stress impact in pomegranate (*Punica granatum*) grown in saline soil by minimizing the toxic elements. Relationship between plant antioxidative response, as well as susceptibility and tolerance to salt stress is still being investigated for many plant species.

Tomato (*Solanum lycopersicum* L.) is the most widely cultivated vegetable in the world for their economic and nutritional value (Zhang et al., 2017). In Tunisia, the tomato plant is the main horticultural crop, with production between 1 and 1.3 million tons per year (GICA, 2020). This relatively low yield is mainly due to the saline soil conditions in the country. Salt stress affects growth parameters of tomato plants at seed germination, shoot and root extension, and fruit production (Cuartero and Fernandez-Munoz, 1998). Considering growing threat of increasing soil salinity combined with the the socio-economic importance of tomato in Tunisia, this study aimed to evaluate the potential for salt stress mitigation by additing vermicompost to the soil for the tomato plant variety "Firenze", a common variety cultivated in Tunisia.

MATERIALS AND METHODS

Plant material and experimental design

Tomato seeds (*S. lycopersicum* L.) variety "Firenze" were surface-disinfected with 5% sodium hypochlorite solution and rinsed with sterile distilled water. Seeds were then sown individually into cell plug trays filled with commercial peat (P) (Sphagnum peat) (KLASMANN Potgrond H80) and installed in a ventilated experimental greenhouse. The greenhouse was located at the Experimental Station of the High Institute of Agronomy of Chott-Mariem, Tunisia and was maintained at a temperuatre of 22 ± 1°C and 60 ± 10% relative humidity with a 16-h day/8-h night photoperiod.

Plantlets with 4−5 true leaves transplanted (one plantlet per pot) into pots filled with one of the four substrates introduced:

Substrate 1 was the control (T): 100% organic soil (soil from a certified organic plot).

Substrate 2 was the vermicompost treatment (Vc): made of 80% organic soil and 20% Vermicompost that has been prepared from a mixture of vegetable bark (kitchen wastes) and leaf litter (collected on lawns).

Substrate 3 was the compost treatment (C): made of 80% organic soil and 20% Compost, obtained from an aerobic fermentation of four kinds of manure (25% bovine, 25% sheep, 25% poultry and 25% horses).

Substrate 4 was the mixture treatment (M): made of 80% organic soil; 10% Vermicompost and 10% Compost.

Physicochemical characteristics of used substrates are presented in Table 1.

Table 1: Physico-chemical characteristics of different substrates

	Peat	Organic soil	Vermicompost	Compost
pH	6	8.3	8.74	7.64
EC (ms/cm)	0.91	0.77	2.15	6.43
OM (%)	0.85	0.76	74	53
T.O.C (%)	0.49	0.44	43.02	30.8
Salinity (g/l)	0.6	0.53	1.5	4.5
Porosity (%)	85	55	62.5	35

EC: electrical conductivity; OM: organic matter; TOC: total organic carbon

Table 2: Significant multivariate effects (at $P<0.05$)

	Trace of Pillai	ddl	F	Sig
Salinity Stress (SS)	1.880	26.000	15.717	0.000**
Substrate (Sub)	2.600	39.000	7.005	0.000**
Sub × SS	4.109	78.000	2.842	0.000**

**Highly significant

Plantlets were watered every two days with tap water to field capacity. The Hoagland nutrient solution was added prior to salt stress application (Hoagland, 1933). One week after transplanting, plantlets were subjected to salt stress by appliying three levels of NaCl (0, 50 and 150 mM) over a 6 week period.

The experiment followed a factorial design with two factors randomized. Factors were the substrates type (T, Vc, C and M) and the stress factor represented by three levels of salt (C1 = 0 mM; C2 = 50 mM and C3 = 150 mM of NaCl). Growth was monitored during the experiment period by measuring shoot length (SL), stem diameter (SD) and leaves number (LN). The experiment was ended after about 12 weeks (85 days) after sowing. The tomato plants were uprooted, and then the root length (RL), shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), root dry weight (RDW), chlorophyll (Chla and Chlb), carotenoid content (Car), malondialdehyde (MDA) and catalase (CAT) were measured.

Growth parameters

Growth parameters included shoot and root length (cm), stem diameter (mm), leaves number, fresh and dry weights of aerial and root part (g) were measured on three plants chosen randomly per treatment. Shoot length was determined using a ruler; stem diameter was measured by digital caliper. Leaf number was counted for each plant. After washing, fresh shoot and root weights were recorded. For dry weight measurement, shoots and roots were dried at 80 °C for 48 hours.

Physiological Parameters

Extraction of leaf chlorophyll and carotenoid contents was conducted via the methods in Lichtenthaler (1987). The optical density (OD) was measured using spectrophotometer at 663.2 nm for the Chlorophyll a (Chla) and 646.8 nm for the Chlorophyll b (Chlb) and 470 nm for carotenoids (Car). Chlorophyll and carotenoid pigments contents (mg g^{-1} FW) were estimated using the formula:

$$Chla = \left(12.25 \times A_{663.2}\right) - \left(2.79 \times A_{646.8}\right)$$

$$Chlb = \left(21.50 \times A_{646.8}\right) - \left(5.1 \times A_{663.2}\right)$$

$$Car = \frac{\left(1000 \times A_{470} - 1.8 \times Chla - 85.02 \times Chlb\right)}{198}$$

Biochimical Parameters

Lipid peroxidation was measured by determining the MDA amount (Ortega-Villasante et al., 2005). Results were expressed as nmol of Thiobarbituric Acid Reactive Substances (TBAR's) per g of fresh weight (FW).

CAT activity was determined by the decomposition of H_2O_2 at 240 nm according to the method of Claiborne (1984) and was expressed as µmol H_2O_2/min/mg protein.

Statistical analysis

Results were analyzed in SPSS software (version 20.0). A multivariate analysis of variance (MANOVA) was performed to evaluate the effect of substrate types on alleviating the salt stress effects on tomato plants. Plant resistance was determined as quantitative measures of plant growth, physiological parameters and biochemical parameters. Data were further analyzed in a One-Way Analysis of variance (ANOVA) ($p < 0.05$) followed by Turkey's test at 5% of probability. For the Vc treatment group under salt stress, pearson correlation analysis was used to evaluate the relationship between experimental parameters (significance levels at $p \leq 0.01$ and $p \leq 0.05$).

RESULTS

Statistical results of MANOVA test shows that salt stress (SS), substrate (S) and the interaction (SS × S) all have a highly significant effect on all measured parameters ($p = 0.000$ at $\alpha < 0.05$); thus plants repsonded to salt stress across all treatment groups (Table 2). ANOVA statistical analysis revealed a highly significant effect of salt stress and substrate on the expression of measured parameters (Shoot length, stem diameter, leaves number, root length, aerial and root fresh and dry weight, Chla, Chlb, Car, MDA and CAT). The effect of the interaction (SS × S) is significant in LN, SFW, SDW, RFW and RDW except for SL, SD and RL (Table 5 and 6).

Results of growth parameters of tomato plants (shoot length, stem diameter, leaves number, root length, fresh

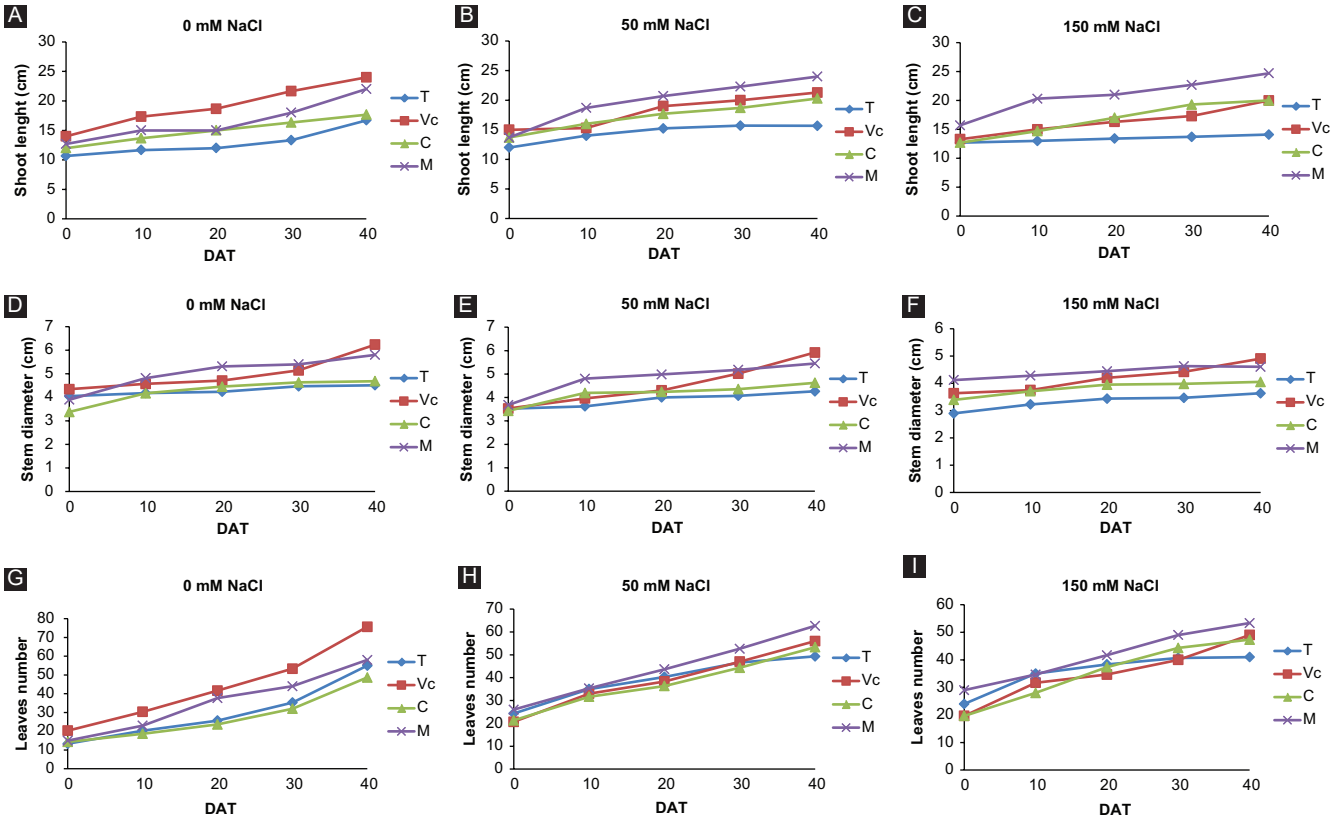

Fig 1. Kinetics of evolution of shoot length (A, B, C), stem diameter (D, E, F) and leaves number (G, H, I) of tomato plant by the substrates under different NaCl levels; T: control soil (100% organic soil); Vc: 80% soil + 20% vermicompost; C: 80% soil + 20% compost and M: 80% soil + 10% vermicompost + 10% compost and DAT: days after transplanting.

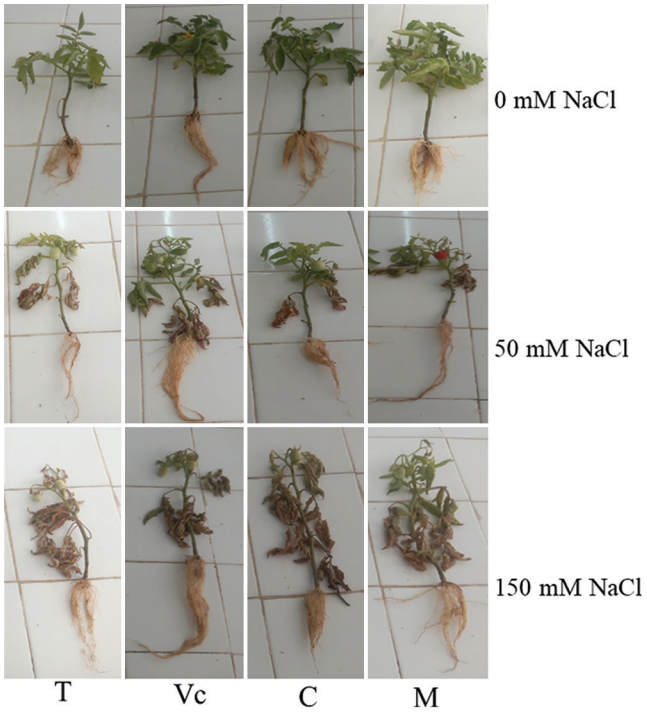

Fig 2. Effect of salinity leaves on aerial and root part of tomato plants grown in different subtraces. T: control soil (100% organic soil); Vc: 80% soil+20%compost; M: 80% soil + 20%compost; M: 80% soil+10% vermicompost + 10% compost.

and dry weight of shoots and roots) receiving different NaCl concentrations are given in Table 3.

Effect of substrate and SS on Growth parameters

SL, a growth parameter, was different between treatment groups control and treated plants (Fig. 1 A, B and C). In unstressed conditions (0 M NaCl), the greatest SL was observed on substrate Vc (24 ± 1.00 cm). All growth parameters were greater in substrate Vc compared to other treatment groups. (Fig. 1 A). SL in different substrates was slightly influenced with the gradual increase in salt stress (Figs. 1 B and C and Fig. 2). According to the variance analysis, salt stress caused a not significatif decrease of shoot length for all plants except for M substrate (Table 3). This result is statistically translated by the existence of a non-significant negative correlation between the two variables ($r = -0.045$, Table 7). The decrease of SL was found in substrate T (14.1 ± 0.90 cm), while the lowest decrease occurred in the mixture M (24.7 ± 2.08 cm) at the same level of NaCl (150 mM) (Table 3).

SD evolution varies according to salt stress and culture substrate type (Figs. 1 D, E and F). Under control conditions, Vc substrate shows a maximum collar growth of 6.23 ± 0.53 mm. This value drops to 5.8 ± 0.46 mm,

Table 3: Effects of salt stress (SS) and substrate on SL, SD, LN, RL, SFW, SDW, REW and RDW of tomato plants

SS	Substrate	SL	SD	LN	RL	SFW	SDW	RFW	RDW
(mM)		(cm)	(mm)		(cm)	(g plant^{-1})	(g plant^{-1})	(g plant^{-1})	(g plant^{-1})
0	T	16,7 ± 1,52^{bcd}	4,51 ± 0,73^{cde}	55 ± 4,04^{bc}	14 ± 3,46^{b}	6,15 ± 0,93^{de}	2,06 ± 0,51^{de}	0,75 ± 0,05^{de}	0,17 ± 0,08^{e}
	Vc	24 ± 1,00^{a}	6,23 ± 0,53^{a}	75,66 ± 6,65^{a}	19 ± 0,70^{ab}	15,30 ± 1,53^{a}	4,52 ± 0,60^{bc}	1,77 ± 0,14^{a}	0,52 ± 0,005^{abcd}
	C	17,6 ± 2,08^{bcd}	4,68 ± 0,56^{abcde}	48,66 ± 4,72^{bc}	16 ± 2,87^{ab}	9,35 ± 1,50^{bcd}	3,46 ± 0,20^{bcde}	1,27 ± 0,05^{abc}	0,33 ± 0,04^{bcde}
	M	22 ± 2,64^{ab}	5,8 ± 0,46^{abc}	58 ± 2,64^{b}	26 ± 1,52^{a}	11,15 ± 0,45^{abc}	6,45 ± 0,27^{a}	1,52 ± 0,05^{ab}	0,78 ± 0,04^{a}
50	T	15,7 ± 1,52^{cd}	4,26 ± 0,62^{cde}	49,33 ± 2,51^{bc}	11 ± 1,29^{b}	6,025 ± 0,61^{de}	2,80 ± 0,46^{cde}	0,92 ± 0,05^{cde}	0,3 ± 0,04^{bcde}
	Vc	21,3 ± 2,08^{abc}	5,92 ± 0,22^{ab}	56 ± 2,82^{bc}	13 ± 2,94^{b}	11,2 ± 1,01^{abc}	3,91 ± 0,93^{bcd}	1,38 ± 0,06^{abc}	0,27 ± 0,04^{bcde}
	C	17 ± 2,30^{abc}	4,62 ± 0,26^{bcde}	53,33 ± 1,15^{bc}	14 ± 2,16^{b}	5,6 ± 0,63^{de}	2,12 ± 0,71^{de}	0,9 ± 0,12^{cde}	0,22 ± 0,05^{de}
	M	24 ± 1,00^{a}	5,45 ± 0,30^{abcd}	62,66 ± 5,03^{ab}	26 ± 3,87^{a}	12,64 ± 0,1^{ab}	3,89 ± 1,08^{bcd}	1,77 ± 0,14^{a}	0,54 ± 0,02^{abc}
150	T	14,1 ± 0,90^{d}	3,64 ± 0,39^{e}	41 ± 3,00^{c}	9,75 ± 1,70^{b}	4,75 ± 0,94^{e}	1,71 ± 0,4^{e}	0,5 ± 0,12^{e}	0,23 ± 0,09^{cde}
	Vc	20 ± 2,64^{abc}	4,90 ± 0,44^{abcde}	49 ± 6,24^{bc}	13,25 ± 2,87^{b}	6,525 ± 0,52^{de}	2,94 ± 0,81^{cde}	1,05 ± 0,13^{bcd}	0,17 ± 0,08^{e}
	C	16,7 ± 2,64^{abc}	4,05 ± 0,85^{de}	47,33 ± 2,51^{bc}	13,25 ± 2,62^{b}	4,225 ± 1,55^{e}	2,56 ± 0,07^{de}	0,6 ± 0,13^{de}	0,22 ± 0,01^{de}
	M	24,7 ± 2,08^{a}	4,6 ± 0,61^{bcde}	53,33 ± 1,15^{bc}	17 ± 2,12^{b}	7,425 ± 0,35^{cde}	5,00 ± 0,34^{ab}	1,05 ± 0,07^{bcd}	0,57 ± 0,09^{ab}

Means indicated with different letters show significant differences among treatments following Tukey's test (p<0.05); Values represent means ± standard deviation (n=3); T: control soil (100% organic soil); Vc: 80% soil+20% vermicompost; C: 80% soil+20% compost; M: 80% soil+10% vermicompost+10% compost; SL: shoot length; SD: stem diameter; LN: leaves number; RL: root length; SFW: shoot fresh weight; SDW: shoot dry weight; RFW: root fresh weight and RDW: root dry weight

Table 4: Effects of salt stress (SS) and substrate on Chla, Chlb, Carotenoids, MDA and CAT of tomato plant

SS	Substrate	Chl a	Chl b	Car	MDA	CAT
(mM)		(mg g^{-1} FW)	(mg g^{-1} FW)	(mg g^{-1} FW)	(nmol g^{-1} FW)	(µmol mg^{-1} protein)
0	T	665 ± 22,02^{bcd}	474 ± 14,01^{cd}	99 ± 6,67^{abc}	0,56 ± 0,08^{bc}	162 ± 3,15^{de}
	Vc	1643 ± 75,19^{a}	825 ± 26,24^{a}	189 ± 6,41^{a}	0,41 ± 0,02^{c}	144 ± 3,88^{e}
	C	932 ± 30,50^{b}	557 ± 21,77^{bcd}	136 ± 2,18^{abc}	0,64 ± 0,58^{bc}	172 ± 6,20^{de}
	M	820 ± 25,42^{bc}	539 ± 18,48^{bcd}	143 ± 1,77^{abc}	0,56 ± 0,004^{bc}	192 ± 6,20^{cde}
50	T	477 ± 3,56^{de}	463 ± 10,54^{cd}	73 ± 2,46^{c}	1,30 ± 0,01^{bc}	253 ± 1,57^{bc}
	Vc	1511 ± 21,10^{a}	760 ± 18,82^{ab}	158 ± 3,70^{abc}	0,57 ± 0,02^{bc}	187 ± 5,80^{de}
	C	691 ± 16,53^{bcd}	688 ± 15,58^{abc}	116 ± 4,47^{abc}	0,87 ± 0,01^{bc}	220 ± 8,70^{bcd}
	M	739 ± 11,33^{bcd}	676 ± 1,65^{abc}	117 ± 4,50^{abc}	0,73 ± 0,03^{bc}	222 ± 8,64^{bcd}
150	T	266 ± 20,45f	303 ± 2,982^{d}	80 ± 1,44^{bc}	2,47 ± 0,03^{a}	366 ± 8,35^{a}
	Vc	849 ± 4,83^{b}	461 ± 12,91^{cd}	188 ± 4,91^{a}	0,80 ± 0,01^{bc}	215 ± 7,96^{bcd}
	C	423 ± 12,53^{de}	399 ± 8,16^{d}	104 ± 8,16^{abc}	1,39 ± 0,02^{b}	265 ± 7,00^{b}
	M	503 ± 15,61^{cde}	451 ± 19,73^{cd}	174 ± 6,23^{ab}	1,00 ± 0,001^{bc}	243 ± 9,93^{bc}

Means indicated with different letters show significant differences among treatments following Tukey's test (p<0.05); Values represent means ± standard deviation (n=3); T: control soil (100% organic soil); Vc: 80% soil+20% vermicompost; C: 80% soil+20% compost; M: 80% soil+10% vermicompost+10% compost; Chla: chlorophyll a; Chlb: chlorophyll b; Car: carotenoid; MDA: malondialdehyde and CAT: catalase.

Table 5: Results of variance analysis for SL, SD, LN, SFW, SDW, RFW and RDW

Variation parameters	df	SL	SD	LN	RL	SFW	SDW	RFW	RDW
SS	2	ns	**	**	*	**	*	**	*
Substrate	3	**	**	**	**	**	**	**	**
SS × Substrate	6	ns	ns	*	ns	**	*	**	*
Error	24	0,33	0,09	0,91	0,62	0,21	0,1	0,02	0,02
CV(%)		9,58	10,18	6,54	14,63	10,09	17,82	8,23	13,54

ns, * and **: not significant and significant at 5% and 1% levels of probability; SS: salinity stress; df: degree of freedom; SL: shoot length; SD: stem diameter; LN: leaves number; RL: root length; SFW: shoot fresh weight; SDW: shoot dry weight; RFW: root fresh weight and RDW: root dry weight.

Table 6: Results of variance analysis for Chla, Chlb, Carotenoid, MDA and CAT

Variation parameters	df	Chla	Chlb	Car	MDA	CAT
SS	2	**	**	*	*	**
Substrate	3	**	**	**	**	**
SS×Substrate	6	*	**	ns	**	**
Error	24	27,53	16,57	4,83	0,06	3,07
CV(%)		21,59	14,24	4,41	0,07	14,03

ns, * and **: not significant and significant at 5% and 1% levels of probability; SS: salinity stress; df: degree of freedom; Chla: chlorophyll a; Chlb: chlorophyll b; Car: carotenoid; MDA: malondialdehyde and CAT: catalase.

4.68 ± 0.56 mm and to 4.51 ± 0.73 mm, respectively in M, C and T substrates. Substrate is not significantly correlated to SD (r = 0.299, Table 7).

Salt stress reduced significantly stem diameter of plants cultivated on all substrate types (Vc, M, C and T) especially at 150 mM of NaCl with 4.90 ± 0.44; 4.6 ± 0.61; 4.05 ± 0.85 and 3.64 ± 0.39 respectively. This result is translated by highly significant negative correlation existence between salt stress and stem diameter (r = −0. 439**, Table 7).

Table 7: Pearson correlation between measured parameters

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 SS	Pearson Correlation	1														
	Sig. (2-tailed)															
2 Sub	Pearson Correlation	0,000	1													
	Sig. (2-tailed)	1,000														
3 SL	Pearson Correlation	-,045	,665**	1												
	Sig. (2-tailed)	,797	,000													
4 SD	Pearson Correlation	-,439**	,299	,529**	1											
	Sig. (2-tailed)	,007	,076	,001												
5 LN	Pearson Correlation	-,496**	,219	,498**	,713**	1										
	Sig. (2-tailed)	,002	,200	,002	,000											
6 RL	Pearson Correlation	-,315	,593**	,482**	,433**	,424**	1									
	Sig. (2-tailed)	,061	,000	,003	,008	,010										
7 SFW	Pearson Correlation	-,518**	,398*	,524**	,761**	,723**	,562**	1								
	Sig. (2-tailed)	,001	,016	,001	,000	,000	,000									
8 SDW	Pearson Correlation	-,262	,588**	,545**	,617**	,473**	,651**	,692**	1							
	Sig. (2-tailed)	,123	,000	,001	,000	,004	,000	,000								
9 RFW	Pearson Correlation	-,428**	,519**	,643**	,705**	,631**	,559**	,892**	,601**	1						
	Sig. (2-tailed)	,009	,001	,000	,000	,000	,000	,000	,000							
10 RDW	Pearson Correlation	-,166	,661**	,581**	,445**	,405*	,533**	,639**	,753**	,685**	1					
	Sig. (2-tailed)	,334	,000	,000	,007	,014	,001	,000	,000	,000						
11 Chla	Pearson Correlation	-,498**	,067	,427**	,728**	,583**	,335*	,651**	,456**	,581**	,234	1				
	Sig. (2-tailed)	,002	,697	,009	,000	,000	,046	,000	,005	,000	,169					
12 Chlb	Pearson Correlation	-,482**	,196	,405*	,565**	,535**	,315	,471**	,241	,482**	,157	,754**	1			
	Sig. (2-tailed)	,003	,252	,014	,000	,001	,061	,004	,157	,003	,362	,000				
13 Car	Pearson Correlation	-,211	,406*	,566**	,545**	,409*	,397*	,579**	,601**	,578**	,502**	,559**	,415*	1		
	Sig. (2-tailed)	,217	,014	,000	,001	,013	,017	,000	,000	,000	,002	,000	,012			
14 MDA	Pearson Correlation	,521**	-,284	-,423*	-,583**	-,477**	-,195	-,499**	-,366*	-,573**	-,231	-,502**	-,409*	-,385*	1	
	Sig. (2-tailed)	,001	,094	,010	,000	,003	,256	,002	,028	,000	,174	,002	,013	,020		
15 CAT	Pearson Correlation	,716**	-,159	-,353*	-,571**	-,573**	-,250	-,507**	-,332*	-,542**	-,181	-,658**	-,497**	-,438**	,768**	1
	Sig. (2-tailed)	,000	,353	,035	,000	,000	,141	,002	,048	,001	,290	,000	,002	,008	,000	

* significant, ** highly significant; SS: salinity stress; Sub: substrate; SL: shoot length; SD: stem diameter; LN: leaves number; RL: root length; SFW: shoot fresh weight; SDW: shoot dry weight; RFW: root fresh weight; RDW: root dry weight; Chla: chlorophyll a; Chlb: chlorophyll b; Car: carotenoid; MDA: malondialdehyde and CAT: catalase.

Despite the severity of NaCl levels, the evolution kinetics of stem diameter of the plants showed that Vc substrate proved to give suitable evolution followed by M substrate compared to the T control (Fig. 1 D, E and F).

The effects of substrate and SS on leaves number (LN) of tomato plants are presented in Fig. 1 (G, H and I) and table 2. Substrate T is the least favorable medium for plants development under all salt conditions. Plants grown in Vc and M substrates showed a better growth in terms of leaves number with 75.66 ± 6.65 and 58 ± 2.64 respectively under unstressed conditions. With increasing levels of NaCl, this number decreases and reaches 53.33 ± 1.15 for M; 4.90 ± 0.44 for Vc and 47.33 ± 2.51 for C while the control substrate T recorded only 41 ± 3.00 at 150 mM of NaCl (Table 3). A highly significant negative correlation is recorded between salt stress and leaves number ($r = -0.496^{**}$, Table 7).

The effect of SS and substrate on root length (RL) is shown in Table 2 and Fig. 2. Under control conditions, substrate T (the control) had the lowest root development (14 ± 3.46 cm). Highest values were found in culture substrates M and Vc with respectively 26 ± 1.52 and 19 ± 0.70 cm. Under SS, the RL was significantly reduced in all tomato plants. The lowest value of RL, was recorded in substrate T with 9.75 ± 1.70 cm. A negative correlation is recorded between RL and SS (r = −0.315, Table 7) while a positive and highly significant correlation is reported between RL and substrate, SL, SD and LN.

SS significantly reduced shoot fresh (SFW) and dry weight (SDW) of all treated tomato plants (Table 3) compared to control. SFW and SDW recorded high significant positive correlations on the one hand with SL; SD; LN, RL and on the other hand between themselves (r = 0.692**) (Table 7).

The effect of SS on root fresh (RFW) and dry weight (RDW) is shown in Table 3. In unstressed plants, the highest value of RFW was recorded in substrate Vc (1.77g ± 0.14). However, the lowest value was noted in substrate T (0.75g ± 0.05). Salt stress reduced significantly RFW of all plants. This reduction is very clear for plants developed on substrate C, which recorded the highest percentage of damage (52.76%) compared to control. Variance analysis showed that experimental factors and their interaction (SS × Substrate) have a high significant effect on RFW. A highly significant negative correlation is recorded between RFW and SS (r = −0.428**, Table 7).

Effects of salt stress on physiological parameters

Results of leaves Chlorophyll content and carotenoids of salt-stressed plants are given in Table 4. Plants exposed to NaCl stress exhibited a significant decrease in chlorophyll a (Chla) and chlorophyll b (Chlb) and the highest damage was caused by 150 mM NaCl level. The application of 20% of vermicompost, without a salt stress, had positive effect on Chla and Chlb compared to control substrate. As well, data mean comparison indicated that the highest levels of Chla and Chlb respectively with the means of 1643 and 825 mg g^{-1} FW were related to vermicompost treatment (0 mM NaCl) and the lowest Chla and Chlb respectively with 266 and 303 mg g^{-1} FW were obtained from T treatment (150 mM NaCl) (Table 4). Current results demonstrated that addition of vermicompost significantly increased the levels of chlorophyll a and chlorophyll b even though expanding levels of salt stress is exerted. The effects of SS and Substrate interaction on Chla and Chlb contents were found to be significant ($p < 0.05$) (Table 6) and a highly significant negative correlation is recorded between salt stress and Chla and Chlb (r = −0.498**; r = −0.482** respectively) (Table 7).

Result of statistical analysis (ANOVA) showed a significant effect of SS and substrate on the expression of carotenoids (Table 4). Indeed, carotenoids content in tomato plants was significantly decreased by rising levels of salinity ($p < 0.05$). The highest values were found in substrates Vc with 189 ± 6.41 mg g^{-1}FM (0 mM NaCl) while the lowest values were acquired in substrates T with 73 ± 2.46 mg g^{-1} FM (50 mM NaCl). Nonetheless, results of variance analysis of carotenoids showed a non-significant effect of the interaction (SS × Substrate) (Table 6).

Effects of salt stress on biochemical parameters

MDA level is usually used as an indicator of cell oxidative damage. Results given in Table 4 showed that moderate salinity (50 mM of NaCl) could apply a significant modification in MDA concentration in leaves system of tomato plants. At a severe level of NaCl stress (150 mM), accumulation of MDA was significantly stimulated. As forecast, SS increased MDA contents of tomato leaves (Table 4). The lowest concentrations of MDA were measured in plants grown in substrate Vc (0.41 ± 0.02 nmol g^{-1} FW) under unstressed conditions. Whereas, highest concentrations of MDA were measured in plants grown in substrate T followed by substrate C (2.47 ± 0.03 and 1.39 ± 0.02 nmol g^{-1} FW respectively) under SS condition (150 mM NaCl).

MDA contents of tomato leaves were strongly affected by both substrate and the interaction (SS × Substrate) ($p < 0.01$) and in lesser degree by salinity ($p < 0.05$) (Table 6). In fact, MDA content of plants grown in Vc substrate were 26.78% and 67.61% lesser than those in substrate T under untreated conditions and in elevated NaCl level (150 Mm) respectively (Table 4).

CAT activity in tomato leaves were significantly affected by SS and different treatments (Table 4). Expanded salinity has caused an increasing in the activation of CAT enzymes. Results showed that in leaves of plants grown in Vc, CAT raised under SS but these results remain nonetheless lower than those generated by the substrate T under all conditions of the experiment. According to this, the highest CAT value was recorded by T substrate as 366 ± 8.35 µmol mg^{-1} Prot with the highest salinity level (150 mM) and the lowest value was recorded by Vc substrate as 144 ± 3.88 µmol mg^{-1} Prot in control conditions.

Application of Substrates, SS and their interaction (SS × Substrate) ($p < 0.001$) significantly affected CAT enzyme activities in tomato leaves (Table 6). Additionally, CAT activity showed highly significant positive correlation with SS (r = 0.716**) (Table 7).

Considering the substrate treatments under saline conditions, correlation analysis was performed to assess the

relationship between different parameters of tomato plant (Table 7). Relationships between all studied parameters were evaluated in terms of statistical significance levels by depending on the error limit of $p \leq 0.01$ and $p \leq 0.05$. High positive correlations were found between substrate and SL (0.665**); RL (0.593**); SDW (0.588**), RDW (0.519**) and Car (0.406*). As well, results showed high positive correlations between SS MDA (0.521**) and CAT (0.716**). Negative correlations were found between SS and SD (−0.439**); LN (−0.496**); SFW (− 0.518**); RFW (−0.428**), Chla (−0.498**) and Chlb (−0.482**) (Table 7). Alternatively, applications effects on the relationship between other parameters of tomato plant were found insignificant. SS can cause a multitude of complex interactions involving plant metabolism or liability to injury. High levels of NaCl usually leads to hyperosmotic and oxidative stress, which can impede plant growth and development, and even lead to death (Xiong et al. 2002; Fan et al. 2011).

DISCUSSION

Tomato plants response to vermicompost use in growth medium under salinity stress conditions were assessed. It is evident that salt stress decreases plant growth (Evelin et al., 2012) and that vermicompost enhances plant development (Blouin et al., 2019) which were confirmed by the results of this study.

Statistical results of the combined effect of SS and culture substrate on some growth, physiological and biochemical parameters of tomato plant (*Solanum lycopersicum* L.) showed a variable response. Plants SL, SD, RL, SFW, SDW, RFW and RDW significantly decreased with the rising levels of NaCl (Table 3). However Vc substrate mitigates salt stress effects, so that at 150 mM of NaCl, SL; SD; SFW; SDW and RFW were greater than that of the control substrate treatment, having undergone no salt stress (19.76%; 8.64%; 6.01%; 42.71% and 40% respectively). The recording of a negative correlation between SS and all tomato growth parameters are mainly due to the inhibition of food reserves mobilization and the suspension of cell division (Belaqziz et al., 2009). Identical outcomes have been reported for basil and marjoram (Ahl and Omer 2011). Under saline conditions, Hajer et al. (2006) reported a reduction in seed germination of three tomato cultivars. This effect can be partially osmotic or ion toxicity that may change photosynthetic process including enzyme activation (Croser et al., 2001). In this study, improved RL in Vc treated plants could have led to enhancement of plant growth parameters under different levels of NaCl. Vermicompost attenuates the impact of SS in plant organs, confirming its effectiveness on resistance to this abiotic stress in *S. lycopersicum* (Chinsamy et al., 2014). Same results have also been recorded for sunflower (Jabeen and Ahmad, 2017) and peppermint (Xu et al., 2016). Ekinci et al. (2012) noted a 60% decrease in lettuce shoot dry weight (*Lactuca sativa* L.) in saline condition (100 mM of NaCl) compared to the untreated control. In response of lettuce to SS, a drastic decrease in dry weight and even lower salt concentrations affecting membrane stability is recorded (Hniličková et al., 2019). Present findings of SDW effectively in accordance with the findings of those previous studies. As well, there was an increase in the density of the aerial and root part (indicated by the fresh and dry weight) in tomato plants treated with Vc and M compared to control plants. This involves an increase in aerial and root surface area, which attributed to the absorption of nutrients, thus stimulation of plants growth and development. Indeed, under control conditions, substrates Vc and M maintained the highest values of all measured growth parameters. This could be explained by the increase of nutrient content and porosity in the soil, which improves soil structure, fungal and bacterial population and biological activity (Durak et al., 2017; Dhen et al., 2018) and at the same time, by its raising of humic acid content which gives a high adsorption capacity thanks to its large surface area and its eleveted cation exchange capacity (Atiyeh et al., 2000a, 2000b). Throughout vermicomposting process, plant growth hormones, symbiotic microorganisms, and other plant growth regulators are enhancned by humic acid (Atiyeh et al., 2002; Arancon et al., 2004) which positively affects plant growth when adding this vermicompost to the soil. Moreover, it mitigates the harmful effects of irrigation water salinity, not sternly damaging the height; stem diameter and biomass production of noni plants, as revealed by Santos et al. (2019). This is in accord with the results of Chinsamy et al. (2014) which revealed an enhancement in the morphological attributes of tomato seedlings cultivated with Vc under high SS (100 mM). Ayyobi et al. (2014) and Libutti et al. (2020) reported that using vermicompost as organic amendment generated an enhancement of plant growth in peppermint and in Swiss chard plants respectively. The use of Vc was optimal for ameliorating other soil properties thanks to increased organic matter, nutrient contents, soil aeration and vital role of vermicompost on soil enzyme action (Makkar et al., 2017; Nurhidayati et al., 2018). The beneficial effect of vermicompost on growth and yield characteristics may be due to improving soil structure conditions, which promoted the plant to have a good root development by ameliorating soil water holding capacity and this permitted favorable plant supply with water and nutrients which in turn, increases the amount of plant biomass produced (Joshi et al., 2014). In comparison with traditional compost, vermicompost has a higher nutritional value thanks to the

role of earthworms in increasing mineralization rate and humification degree, which classifies vermicompost as an ideal organic manure for better growth, yield and quality of numerous plants (Azarmi et al. 2009). Existence of microbiota particularly fungi, bacteria and actinomycetes makes it suitable for plant growth (Joshi et al., 2014; Dhen et al., 2019).

In this study, Chla and Chlb were significantly reduced under SS in all treated-plants. Indeed, a negative correlation between SS and photosynthesis are recorded. Chlorophyll a and b as well as Carotenoid content were reduced in tomato leaves. This correlation is mostly due on the one hand to inhibition of chlorophyll synthesis and on the other hand to its degradation (Banerjee and Roychoudhury, 2017). This disturbance in chlorophyll metabolism leads to suppression of photosynthesis, a shortage of energy equivalents and eventually leads to plant death. Besides, inhibition of chloroplasts development and degradation of plastids is sometimes observed in plants such as fennel (Abd EL-Wahab 2006). Further, damage to chlorophyll content may due to accumulation of toxic ions in leaves. Decreasing chlorophyll when plants face stress conditions may be due to an alternation in N metabolisms in relation to the production of compositional compounds such as proline, which enter into the osmosis regulation, since an expansion in proline production results in less involvement of glutamate in the chlorophyll biosynthetic pathways (Håkanson and Eklund, 2010). Further, some growth regulating agents such as abscisic acids and ethylene promote chlorophyllase enzyme activity (Ali et al., 2004). Adamipour et al. (2019) showed that increasing levels of salinity caused a decline in chlorophyll and carotenoid contents. Vermicompost treatments reversed the adverse effects of NaCl and generated a significant increase in plants chlorophyll content in condition of SS. As well, plants leaves treated with Vc recorded the highest value of chlorophyll content over control plants. In the same approach, Afkari (2018) showed that SS has different negative effects on plant physiological processes. On the one hand, increasing SS reduced the number of photosynthetic pigments. On the other hand, increasing vermicompost application enhanced the activity level of Chla, Chlb, total chlorophyll and carotenoids. Vermicompost application on tomato plants resulted in the increase in photosynthetic pigments compared to the control. This increase caused by organic matter supplementation has also been reported on several other plants (Bidabadi et al., 2017). This beneficial impact of vermicompost on photosynthesis pigments can be attributed to photosynthetic rate inflation and CO_2 assimilation which ameliorate mineral uptake by the plant (Ayyobi et al., 2014). As well, resistance to chlorophyll pigment degradation by Vc supplementation can also be attributed to improved water productivity. Amiri et al. (2017) recorded that Vc treatments defends the chlorophyll content proportion to carotenoid content. Thus, the rise in carotenoid content after Vc addition can due to the increase in chlorophyll. In the current study, the using of vermicompost significantly increased carotenoid content synthesis in tomato leaves. This result could be explained by the high capacity of vermicompost to suppling nutritious elements like Fe, Zn, Mg, and N directly and indirectly (Narkhede et al., 2011). However, Pant et al. (2009) reported a decrease of carotenoid activity in both antioxidant enzymatic and non-enzymatic systems by reason of the oxidative stress caused by SS in plant tissues. Related findings were showed in marjoram (*Origanum majorana*) (Baatour et al., 2010).Moreover, Ayyobi et al. (2014) declared that Vc increased carotenoid content in peppermint (*Mentha piperita* L.) leaves. Results of variance analysis demonstrated the significant impact of SS and vermicompost on the photosynthetic pigments at a significance level of 1% and the interaction of these two factors on the total chlorophyll at the significance level of 1%, but not on chlorophyll a, chlorophyll b and carotenoids (Afkari, 2018). Earlier investigation demontrated that the modification in chlorophyll content was additionally dependent on salt concentration in the soil (Chen et al., 2014). Extended exposure to high salinity concentrations generally induces in drastic changes in chlorophyll content. For a short time of SS (2 weeks), Chla and b did not differ between salt treatment and the control on blessed thistle and peppermint (Xu et al., 2016). This can be partially explained by the short duration of the analysis.

One of the causes of oxidative damage following the SS condition is the peroxidation of membrane lipids, which causes negative impacts like MDA, ion permeability and adjustment of enzyme activity (Distelbarth et al., 2012). Present study showed that by increasing of SS, MDA activity increases as well (Table 5). Indeed, MDA in tomato leaves were firmly influenced by substrate (Table 6). Expectedly, SS enhanced MDA content of plants leaves. This result is consistent with the findings of Butt et al. (2016) in chilli plant. Decline of membrane cell and its lipids in response to SS along with MDA production have been observed in corn (Gunes et al., 2007), in borage (Afkari, 2018) suggesting that borage may adopt an adequate measure to evaluate the plant reaction to SS. MDA content of Vc treated plants were lower than those of untreated plants under all salt stress levels. Chinsamy et al. (2014) reported the favorable effects of Vc on the physiological capacity of tomato plants which develops an adaptive mechanism in response to SS. However, this effect of Vc on MDA is found to be reduced when ambient salinity increases. It can be deduced that low amount of Vc is insufficient to overcome the detrimental effect of high salinity.

CAT is the most important enzyme clearing hydrogen guaiacol peroxidase (POD) and the most important antioxidant enzyme in the plants. As a concequence of the SS, antioxidant enzyme activities transform ROS into innocuous compound, which represent the ultimate relevant resistance mechanisms of plants in case of oxidative stress. SS increases the CAT activity in many plants, which was in accordance with our study. In fact, a considerable increase in CAT activities has appeared when salinity increases, in order to add plant tolerance to SS. In the same approach, CAT enzyme performs as an iron binding protein that takes action when POD hydrogen is high in plant cells (Asghari et al., 2016). Other researchers have also revealed the elevated activity of the CAT enzyme in response to salinity stress in pot marigold (*Calendula officinalis* L.) (Hemmati et al., 2018) and chickpea (*Cicer arietinum* L.) (Sadak et al., 2017). CAT enzyme contributes to plant survival through enzymatic reaction by eliminating the reactive oxygen species and preventing the cell wall destruction (Jiang and Zhang, 2001). Afkari (2018) reported that highest CAT enzyme activity was related to 15 wt % Vc treatment under SS (12 ds/m NaCl). This result highlights the important role of CAT in protect cells against the effects of hydrogen POD by converting hydrogen peroxide into water and oxygen (Garrat et al., 2002). In this study, our results differ from these previous reports (Table 4). CAT activities were effectually rationalized in the aerial tissues of tomato plant after addition vermicompost and/or compost in comparison with the control. Comparable results were recorded by Xu et al. (2016) in blessed thistle. Other interesting findings were recorded by Adamipour et al. (2019) who founded that CAT activity in pot marigold plants treated with Vc increased clearly with SS (50, 100 and 150 mM of NaCl) and then significantly decreased at 200 mM of NaCl. Plants treated with Vc exhibited significant CAT activities that actively participate in the extension of ROS compared to control (non-Vc) plants. Further, the increase in antioxidant enzymes activity succeeding Vc application may indicate the creation of a protective mechanism to decrease SS-induced oxidative harm (Khan et al. 2010). Bidabadi et al. (2017) showed that antioxidant enzyme mechanisms are excited in pomegranate plants treated with Vc and exposed to SS. These findings certify the effectiveness of Vc in generating antioxidant enzymatic mechanisms in plants.

Several studies have considered the use of vermicompost by vegetable producers as an alternative to crop management aimed to reducing synthetic inputs (Abawi and Widmer, 2000). This amendment improves soil quality and reduces losses due to salt stress, reduces environmental pollution and increases harvest and yields (Oka, 2010). It is important to look further into this aspect to highlight the potential for this amendment.

CONCLUSION

Salt stress significantly decreased growth and physiological parameters of tomato plants by affecting the SL, SD, LN, RL, SFW, SDW, RFW, RDW, chlorophyll content, and carotenoids of tomato grown under different NaCl levels (0, 50 and 150 mM NaCl). Further, biocehmical parameters changed significantly with increasing salinity levels. Vermicompost improved tomato growth, chlorophyll content and biochemical parameters. In the Vc substrate, plants grow well and achieved maximum growth compared to the other substrate treatment groups. The high organic content of humic substances allow the retention of water allow a well buffered ionic environment. The rhizosphere supply mechanisms as well as the transfers of mineral elements in the plant are thus facilitated. However, this aspect is more important under non-stress conditions than in crops subjected to salt stress. Further, vermicompost impact was also effective when combined with compost. Highest improvement was recorded when using vermicompost alone and the second-best by the synergistic use of mixture M. Plants treated with 20% compost (substrate C) having less interesting results under saline stress, this can be explained by its high electrical conductivity which favors an environment richer in salinity for the roots. However, substrate C was much more efficient than substrate T (control) under all conditions of the experiment.

Present study revealed advantageous effect of vermicompost on the improvement of plant growth, physiological and some biochemical parameters in addition to reducting the deleterious effect of salt stress.

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Availability of data and material

All data generated or analysed during this study are included in this published article.

Conflict of interest

The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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Author contributions

SAB and BAD designed the study and wrote the manuscript. SAB and SH performed the experiments. ND and IBA analyzed the data. All authors discussed the results and contributed to the final manuscript.

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