Biologia (2022) 77:1109–1119
https://doi.org/10.1007/s11756-021-01001-7

ORIGINAL ARTICLE

The impact of Vermicompost on *Pisum sativum* spp. *Arvence* L exposed to methylisothiazolinone

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Received: 12 April 2021 / Accepted: 21 December 2021 / Published online: 18 January 2022
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Abstract

2-methyl-2H-isothiazol-3-one (MIT), widely used in industrial and cosmetic products, is one of the significant representatives of the isothiazolinone group. Due to widespread application fields of MIT, it can be easily mixed with water and soil. Vermicompost (VCT), which has an enriching effect on the properties of the soil, provides nutrients to the plants and can transform the soil into a more livable form for plants. In this study, MIT was applied to the *P. sativum*, which has high nutrition content and is a significant food source for animals. Also, VCT was applied to decrease the stress stemming from the MIT chemical on the plant. In order to achieve this, the effective concentration (EC50) value of MIT was determined, and the EC50/2 and EC50x2 concentrations were used on the plant, and the biochemical effects (i.e., total protein, MDA and H_2O_2 amounts, SOD activity) at the end of the 48th and 72nd hours were examined. Then, the same chemical was applied with VCT, keeping all processes constant, and the same biochemical parameters were investigated. When the results were compared with the group treated with VCT, it was observed that there was a difference, especially in Total protein, MDA amounts, and SOD activity. When statistical data was compared with the control group, it was significantly different at the $p < .05$ level. This result implies that VCT can be a good solution for recyclings increased the amount of organic waste, and reducing the use of artificial fertilizers.

Keywords *Eisenia foetida* · Methilizothiazolinon · *Pisum sativum* · Superoxide dismutase · Vermicompost

Abbreviations

EC50	Effective Concentration
EC50/2	Half of EC50 volume
EC50x2	Twice EC50 volume
H_2O_2	Hydrogen peroxide
MDA	Malondialdehyde
MIT	2-methyl-2H-isothiazol-3-one
$O_2^{\cdot-}$	Superoxide radical
·OH	Hydroxyl radical
P.sativum	*P. sativum* spp. *Arvence* L
ROS	Reactive Oxygen Species
SOD	Superoxide Dismutase
VCT	Vermicompost

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Introduction

Isothiazolinones, which have a wide range of uses, are antibacterial and antifungal preservatives that have been used since the 1970s (De Groot and Herxheimer 1989; Fewings et al. 1999). One of the best representatives of this group, 2-methyl-2H-isothiazol-3-one (MI or MIT), is used in hand, face, foot creams and shower gels (Uter et al. 2014), shampoos (Hoa et al. 2019), detergents (Yazar et al. 2011), air fresheners (Park and Kwon 2016), wet wipes (Chang and Nakrani 2014), toilet paper (Gardner et al. 2010), industrial products (Aerts et al. 2017) in metalworking fluids (Geier and Lessmann 2020), as a wood preservative (Wang et al. 2020), in the paint (Thyssen et al. 2006), and water-based poster paints used by children (Filippi et al. 2020). Since the 2000s, there has been a great increase in contact dermatitis allergy, and MIT isothiazolinone sensitivity has been named as "new epidemic" or "second epidemic" (Gameiro et al.

2014; Urwin and Wilkinson 2013). In addition, MIT was chosen as the allergen of the year by the American Contact Dermatitis Association (ACDS) in 2013 (Militello et al. 2020). In February 2017, the European Commission banned the use of MIT in non-rinsing cosmetic products and limited the use of MIT in rinse-off cosmetics to 15 ppm in 2018 (Schwensen and Johansen 2020).

Considering MIT's wide range of application areas (Nielsen 1994), many ways allow isothiazolinones to enter water or soil environments, so they are frequently detected in wastewater and surface waters (Nowak et al. 2020; Bollmann et al. 2017; Li 2019). In spite of their extensive use, there is limited research on the formation of these materials in natural environments. Besides, high biocide concentrations can be hazardous for microorganisms found in sewage treatment plants (Rafoth et al. 2007). When the degradation times of MIT are examined after mixing with the soil, it has been determined that it decays rapidly in the soil by decreasing at the rate of 50% in 7 h (Bollmann et al. 2017). Residues of biocides such as MIT are likely to be constantly present due to repeated inputs and can be considered a "pseudo-persistent" pollutants in this context (Bollmann et al. 2017).

Worms expand the physical characteristics of the soil by transforming many solid wastes in the soil into enriched fertilizers (vermicompost, VCT), significantly accelerating nutrient mineralization and carbon release. Due to these properties, agents are used as live biological means for vermicomposting technology (Roy et al. 2020; Hussain et al. 2021. VCT is obtained by subjecting the organic residues/wastes to fermentation by the microorganisms in the environment and then subjecting them to a rapid humification and detoxification process in the digestive system of the earthworms (Domínguez and Edwards 2011; Lin et al. 2018). VCT, which has a healing effect on soil properties, provides nutrients to the plants and can be applied to all areas where organic cultivation is done (Demir et al. 2010). It has been detected that the increase in VCT dose in loamy and clay soils has a positive impact on plant growth and soil properties, the content of P, Mg, Cu, Zn has increased, and there is an increase in plant growth (Kim et al. 2015; Aktaş 2018). Blouin et al. (2019), in their meta-analysis data on plant growth of VCT, stated that it provided an average increase of 26% in commercial yield, 13% in total biomass, 78% in shoot biomass, and 57% in root biomass. Studies are showing that VCT reduces the number of heavy metals in the soil, increases the uptake of metals such as cadmium, sodium, magnesium, iron, zinc, manganese, and copper into the soil and plant, thus transforming the soil into a more livable form for plants (Jadia and Fulekar 2008; Ali et al. 2013; Hussain et al. 2021). Moreover, it has been reported that worm species such as *Eisenia fetida* and *Lampito mauritii* can effectively consolidate the coal ashes coming from coal-fired factories in the soil, and VCT they create dramatically increases the potentially toxic metals (Pb, Cd and Cr) in the coal ash (Mondal et al. 2020).

Under these perspectives in this study, E50/2 (half of the EC50 volume), EC50 (effective concentration), and EC50x2 (twice EC50 volume) concentrations of MIT were applied on the *P. sativum* spp. *Arvence* L (*P.sativum)* is high in protein, carbohydrate, and fibre content and is an important food source for animals. Then, at the end of the 48th and 72nd hours, changes in total protein amount, hydrogen peroxide ($H_2O_{2)}$ concentrations, and superoxide dismutase (SOD) enzyme activities were examined. In addition, the amount of malondialdehyde (MDA), one of the final products of lipid peroxidation, was determined from the effects of stress on membranes, which is the primary target of stress in plants. Then, by applying the same chemical together with VCT, keeping the conditions constant in the same amounts, it was investigated whether there was any change in the determined biochemical parameters.

Regarding the chemical of MIT, there is no study in the literature on whether it influences the biochemical parameters studied on *P.sativum*. As a result of this study, the biochemical effects of MIT, widely used in various fields, on *P.sativum*, a model organism, have been investigated for the first time. Besides, it was observed whether the toxic effect on the plant changed or not by applying VCT together with this chemical, whose use in high doses caused the formation of stress factors in the plant. It is the first study to show whether vermicompost reduces the toxic effect of a chemical or not. The study aimed to identify some biochemical execution of MIT material used in the industry and cosmetic industry on *P.sativum*, which is the model organism in this study. It also aimed to expose the possible traces of oxidative stress that may occur in the plant, the plant's stress response, lipid peroxidation, and its impact on plant growth with the addition of VCT.

Materials and methods

Plant material, the 2-methyl-2H-isothiazol-3-one (MIT) and vermicompost

The selected plant material *P.sativum* as commercial seeds were obtained from the Central Research Institute of Turkey. Liquid manure vermicompost (Rivermar Inc., Tekirdag / Turkey) and MIT was obtained commercially. The 2-methyl-2H-isothiazol-3-one (MIT) was commercially purchased (Sigma). The chemical structure of MIT can be seen in Fig. 1:

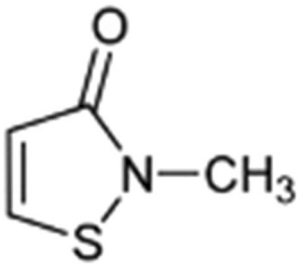


Fig. 1 Chemical Structure 2-methyl-2H-isothiazol-3-one (MIT)

Determination of EC50 values

Commercially purchased MIT (Sigma) was prepared freshly before each trial with distilled water at 16 different concentrations in glass bottles. These solutions were applied after mixing in a magnetic stirrer for at least 15 min and a maximum of 30 min. After the *P. sativum* was mechanically washed three times with distilled water, 20 pieces were placed in the petri dishes to determine the EC50 value, and 16 different concentrations were applied for MIT. After applying MIT, the concentration that reduced the root length by half compared to the control group was accepted as the EC50 value (Yuksel and Aksoy 2017; Roy et al. 2021). The concentration that cut the root length in half was considered the EC50 value. This study used EC50/2, EC50, and EC50x2 concentrations for both chemicals. Each application was carried out in replicates.

Vermicompost efficiency ratio

VCT was commercially available, and dilution was done with distilled water. VCT was applied to seeds at different concentrations (100%, 75%, 50%, 25%, 20%, 15%, 10%, 7.5%, and 5%). The concentration (or % (v/v) ratio) at which the highest root length was observed in *P. sativum* was accepted as the VCTproductivity ratio and applied as the healing concentration (Chaichi et al. 2018; Arancon et al. 2007) According to the data obtained, the concentration plants developed best was applied as a healing agent for toxic substances. The content of VCT (w/w) was: 5.2% organic matter; 1.1% total nitrogen (N), 1.1% organic nitrogen; 0.2% total phosphorus pentaoxide (P_2O_5); 1% water-soluble potassium oxide (K_2O); 1.2% total humic + fulvic acid; EC is 2.8 dS / m (pH 7).

Biochemical analysis

Total protein determination

The protein content of lentil seeds was identified based on the study of Bradford (1976). In order to calculate the protein determination, a standard graph was prepared. Accordingly, 3 mg/mL BSA (Bovine Serum Albumin) stock solution was prepared and diluted at different concentrations. After measuring in the spectrophotometer at 595 nm, the standard graph was determined. 200 µl of the sample was added into 3 ml of Bradford Reagent and calculated at 595 nm in a spectrophotometer (UV mini 1240, Shimadzu), each of which was repeated three times. A total of approximately 50-80 fresh root samples were used for each concentration. The amount of protein in the plant extract was calculated with the prepared standard graph.

Measurement of malondialdehyde amount

The samples were identified by calculating the MDA amount content based on the method of Heath and Packer (1968). It was modified by the study of Cakmak and Horst (1991). 0.3 g root tip was crushed after 3 mL of 0.1% TCA (Trichloroacetic acid) was added. It was centrifuged at 15000 g for 10 min at room temperature. The supernatant was combined with equal parts of 20% TCA containing 0.5% TBA (Thiobarbutyric acid) and then left at 100 °C for 20 min until coloured reddish-pinkish. The samples were immediately placed on ice and centrifuged at 15000 g for 5 min after reaching room temperature. The supernatant was collected; the amount of malondialdehyde was determined at 532 and 600 nm in the spectrophotometer. TBA-TCA mixture was used as a blank. The calculation formula was 155 $m^{-1}c^{-1}$ (Cakmak and Horst 1991).

It was measured at 532 and 600 nm. As a blind sample, a 20% TCA mixture containing 0.5% TBA was used. The calculation was carried out according to the formula 155 mM^{-1} cm^{-1}.

MDA amount (µM) = (A532 − A600)/155 x 1000 x 3

Measurement of H_2O_2 concentration

P. sativum root samples weighing 0.5 g were crushed in a mortar with 5 mL of 0.1% TCA. The crushing was carried out on the ice. The homogenate was centrifuged for 30 min at a+4 °C speed of 10,000 g (Doupis et al. 2011). A total of 3 mL of a prepared by adding 0.5 mL of supernatant, 0.5 mL of 100 mM potassium phosphate buffer solution (pH 7), and 2 mL of 1 M potassium iodide (KI) was produced. The reaction with KI, the crude extract, was stored in the dark at +4 °C for 1 h. After 1 h, the samples were measured at 390 nm in a spectrophotometer. 0.1% TCA was used as a blank (Alexieva et al. 2001).

Superoxide dismutase (SOD) activity

The SOD activity in the samples was photochemically calculated based on the method used in Mishra et al. (2006)

and Tripathi and Gour (Tripathi and Gaur 2004). Based on this process, 2 g of fresh *P.sativum* root samples were crushed in a mortar in 100 mM potassium phosphate buffer solution (pH 7) containing 5 mL of 0.1 mM EDTA and 1% PVP. This procedure was carried out on the ice. The homogenate was centrifuged at 15,000 g at +4 °C for 15 min. As substrate buffer for SOD analysis, 50 mM potassium phosphate buffer (ph 7.8), 9.9 mM L-methionine, 57 mM NBT (Nitroblue tetrazolium chrorid), 0.025% (w/v) Triton X-100, 0% .0044 (w/v) riboflavin was weighed and prepared. The solution was thawed by stirring in a mixer for 30 min and then brought to +4 °C (Dixit et al. 2001) and prepared fresh for each trial. The substrate buffer and the plant-derived homogenate were mixed. The mixture was incubated for 15 min under two 21-watt fluorescent lamps. The substrate buffer, which does not contain enzymes, was placed in a flask and kept under the light for 15 min and used as a blank (Mishra et al. 2006; Tripathi and Gaur 2004). After the reaction under the light, the beakers were covered with aluminium foil and one unit of SOD activity was determined as the amount of enzyme required to reduce 50% of the NBT nitro blue tetrazolium kept under light at 560 nm.

Statistical analysis

The statistical analyses of data were completed using SPSS for Windows version 22.0 statistical software (SPSS Inc., Chicago, USA). Statistically significant differences between the groups were compared using one-way ANOVA. After identifying the conformity of the data to a normal distribution (Test of Normality - Shapiro-Wilk), the test of homogeneity of variances (Levene Statistic) was used, and Tukey heterogeneous ones were also tested for the data with homogenous variances. The data were displayed by mean ± standard deviation (SD), and p values less than .05 were considered statistically significant.

Table 1 Results of concentrations used to determine EC50 value for MIT chemical

Used Dose MIT (g/L)	Root Length (cm) $\overline{X}$ +*SD*
0	1.37 + 0.35
0.18	1.18 + 0.17
0.2	1.03 + 0.11
0.5	0.78 + 0.07
0.8	0.63 + 0.24
0.9	0.54 + 0.13
1	0.53 + 0.19
1.6	0.34 + 0.03

Results

At the end of the 48th hour, the concentration MIT that halved the root length was regarded as the EC50 value. EC50/2, EC50, and EC50x2 concentrations were used for both chemicals throughout this study. According to these results, EC50 values 0.8 g/L; EC50 / 2 concentration 0.4 g/L and EC50x2 1.6 g/L are presented in Table 1.

Vermicompost ratio was determined in 10% that the highest yield was obtained with an average of 1.7 ± 0.24 cm root length on *P.sativum* as presented in Table 2.

Total protein determination

In total protein determination analysis, distilled water and 0.4 /L (EC50/2); 0.8 g/L (EC50); 1.6 g/L (EC50x2) MIT concentrations were applied to *P.sativum* as a control group. At the end of the 48th and 72nd hours, the total protein amount increased with the increase in MIT concentration compared to the control group (see Table 3 for details). Moreover, statistically significant differences were observed in the application of 1.6 g/L at the end of the 48th hour (see

Table 2 The efficiency rate of vermicompost applied to *P.sativum*

Vermicompost(%)	Root Length (cm) $\overline{X}$ +SD
0	1.43 + 0.25
1	1.36 + 0.16
5	1.41 + 0.11
10	1.7 + 0.24
15	1.56 + 0.08
20	1.46 + 0.16
25	1 + 0.08
100	0.8 + 0.09

Table 3 The mean values and statistical data of total protein amounts for 48th and 72nd hours of MIT applied control group, and MIT + 10% VCT applied control group to *P.sativum* seeds

Time (Hours)	Applied Dose MIT	mg/mL protein $\overline{X} \pm SD$	p value	MIT + 10% Vermicompost	p value
	Control	1.169 ± 0.07	–	1.245 ± 0.05	–
48 h	0.4 g/L	1.364 ± 0.08	.477	1.367 ± 0.04	.018*
	0.8 g/L	1.552 ± 0.1	.112	1.563 ± 0.01	.013*
	1.6 g/L	1.884 ± 0.12	.034*	1.52 ± 0.02	.014*
	Control	0.556 ± 0.03	–	0.539 ± 0.02	–
72 h	0.4 g/L	0.571 ± 0.03	.478	0.492 ± 0.001	.010*
	0.8 g/L	0.816 ± 0.01	.070	0.602 ± 0.04	.738
	1.6 g/L	0.941 ± 0.01	.060	0.787 ± 0.02	.002*

*$p < .05$

Table 3). At the end of the 48th hour, there was an increase in both the total protein amount and the concentration of VCT added MIT in the experimental group prepared as 10% VCT and 0.4 g/L; 0.8 g/L; 1.6 g/L MIT concentrations compared to the control group (1.169 mg/mL). At the end of the 72nd hour, the total protein amount increased proportion to the increasing concentrations of MIT compared to the control group. All MIT + 10% VCT concentrations at the end of both 48th and 72nd hours were found to be significantly different ($p < .05$).

Measurement of malondialdehyde (MDA) amount

In the determination of the amount of MDA, it was found that the MDA level decreased at the end of the 48th hour when the amount of MIT concentrations increased in the experimental group using only MIT compared to the control group. However, at the end of the 72nd hour, an increase was detected in the MIT applied seeds compared to the control group, while the most significant increase in the amount of MDA was found in the EC50x2 concentration of 1.6 g/L MIT (see Table 4 for details). In the MIT + 10% VCT applied experimental group, a decrease in the amount of MDA with the increasing amount of concentrations of MIT was observed at the end of the 48th hour, but MDA amounts increased significantly at 1.6 g/L concentrations. In the determination of MDA analysis, all the data in the experimental group were statistically significant compared to the control group ($p < .05$).

Table 4 The mean values and statistical data of 48 and 72 h MDA amounts of MIT applied control group, and MIT + 10% VCT applied control group to *P.sativum* seeds

Time (Hours)	Applied Dose MIT	MDA µM $\overline{X} \pm SD$	p value	MIT + 10% Vermicompost	p value
48 h	Control	5.717 ± 0.02	–	2.622 ± 0.02	–
	0.4 g/L	3.368 ± 0.03	.000*	1.695 ± 0.02	.018*
	0.8 g/L	2.838 ± 0.02	.000*	1.852 ± 0.01	.000*
	1.6 g/L	3.657 ± 0.03	.000*	4.601 ± 0.02	.000*
72 h	Control	1.097 ± 0.01	–	2.177 ± 0.02	–
	0.4 g/L	1.031 ± 0.02	.000*	1.542 ± 0.02	.000*
	0.8 g/L	1.854 ± 0.02	.000*	1.291 ± 0.005	.000*
	1.6 g/L	3.566 ± 0.02	.000*	1.221 ± 0.007	.000*

*$p < .05$

Measurement of H_2O_2 concentration

In the experimental where the amount of H_2O_2 was measured in the plant, it was found that H_2O_2 level increased compared to the control group at the end of the 48th and 72nd hours with the increasing amount of MIT concentrations (see Table 5 for details). Except for the results obtained from seeds treated with 0.4 g / L MIT at the end of the 72nd hour, all other results were significant ($p < .05$) in the experimental group (Table 5). In the group in which MIT + 10% VCT was applied, a slight decrease was observed in the number of H_2O_2 (5.77 µM/g) in the concentrations of EC50 / 2 MIT added VCT at the end of 48th hour but an increase in the number of H_2O_2 (8.01 µM/g, 8.46 µM/g) revealed in EC50 and EC50x2 applications. Moreover, data in 0.8 g / L (EC50) and 1.6 g / L (EC50x2) MIT + 10% VCT applications at the end of 48th and 72nd hours in the experimental group were statistically significant compared to the control group.

(SOD) activity

In the experimental group prepared to investigate the SOD activity, the SOD activity increased with the applied MIT at the end of the 48th and 72nd hours compared to the control group. Except for the results obtained from seeds treated with 0.4 g/L MIT at the end of the 48th hour, all results were significant in the experimental group (see Table 6 for details). In the experimental group in which MIT + 10%

Table 5 The mean values and statistical data of 48th and 72nd hours H_2O_2 amounts of MIT applied control group, and MIT + 10% VCT applied control group to *P.sativum* seeds

Time(Hours)	Applied Dose MIT	H_2O_2 µM/g $\overline{X} \pm SD$	p value	MIT + 10% Vermicompost	p value
48 h	Control	5.81 ± 0.02	–	6 ± 0.08	–
	0.4 g/L	6.07 ± 0.02	.002*	5.77 ± 0.05	.507
	0.8 g/L	8.52 ± 0.05	.000*	8.01 ± 0.02	.000*
	1.6 g/L	10.31 ± 0.08	.000*	8.46 ± 0.01	.000*
72 h	Control	3.85 ± 0.03	–	3.95 ± 0.01	–
	0.4 g/L	3.93 ± 0.04	.240	4.27 ± 0.06	.068
	0.8 g/L	4.61 ± 0.03	.003*	4.88 ± 0.02	.000*
	1.6 g/L	5.23 ± 0.03	.003*	5.15 ± 0.07	.008*

*$p < .05$

Table 6 The mean values and statistical data of 48th and 72nd hours of SOD activity in MIT applied control group, and MIT + 10% VCT applied control group to *P.sativum* seeds

Time(Hours)	Applied Dose MIT	SOD activity U/mg $\overline{X} \pm SD$	p value	MIT + 10% Vermicompost	p value
	Control	2.33 ± 0.28	–	13.57 ± 0.37	–
48 h	0.4 g/L	3.29 ± 0.28	.198	17.06 ± 0.35	.000*
	0.8 g/L	6.92 ± 0.27	.044*	24.12 ± 0.3	.000*
	1.6 g/L	10.37 ± 0.42	.000*	14.63 ± 0.36	.086
	Control	7.82 ± 0.24	–	5.1 ± 0.77	–
72 h	0.4 g/L	11.49 ± 0.23	.000*	6.77 ± 0.49	.074
	0.8 g/L	10.95 ± 0.23	.000*	10.56 ± 0.46	.000*
	1.6 g/L	9.99 ± 0.24	.007*	3.39 ± 0.30	.060

*$p < .05$

VCT was applied, an increase in the amount of SOD activity was observed in the MIT group that added VCT at the end of 48th and 72nd hours compared to the control group for all concentrations except for 1.6 g/L. For this concentration, at the end of 72 h, a decrease was detected. In this experimental group, the results obtained from the EC50/2 and EC50 groups at the end of the 48th hour and the EC50 group at the end of the 72nd hour were significant ($p < .05$).

Discussion

Recently, the application of cosmetic-based personal care and beauty products has increased more than ever worldwide. Today, since there are preservatives, fragrances and surfactants to increase the performance, quality, value, and lifespan of cosmetic products, various types and numbers of compounds are used to formulate cosmetic products. However, most of these chemical additives can lead to health problems by causing toxic effects on the human body. Therefore, the haphazard application of cosmetic materials has recently become a major problem threatening public health (Bilal and Iqbal 2019). In this study, some biochemical effects of (MIT) compound, which is in the group of isothiazolinones and has a very wide area of use, on forage pea (*P.sativum*), which is an important food source, have been detected. Subsequently, vermicompost was added to the MIT applied seeds, and the healing results on their biochemical effects were examined.

In the present experiment investigating the EC50 value, there was a decrease in the root length of *P.sativum* in the MIT chemical applied group compared to the control group. It can be said that there is a significant decrease in EC50x2 MIT compared to the control group. Moreover, it can be implied that the root growth slows down compared to the control group in increasing concentrations of MIT. As a result, when the MIT concentration increased during the stages of germination and the control group values were compared, it was determined that the root lengths were decreased in parallel with the increase in the active substance concentrations. It can be concluded that MIT acts as a cytostatic agent that can delay and inhibit germination and root length. In response to the cytotoxic data obtained, it is possible to say, according to the experiments used to determine the productivity of vermicompost, the application of 10% vermicompost has a positive effect on root elongation. The addition of vermicompost significantly increased the root growth rate (see Table 2). This difference observed is most likely the effect of organic matter, namely vermicompost, processed by worms. Vermicompost increases soil fertility and promotes plant growth, making them more resistant to stress and healthier. In addition, it improves and renews the microbial community structure of the soil while achieving higher yields with the superior nutritional quality of the harvested food and feed (Bhadauria et al. 2014; Zhen et al. 2014;). Another study on *P. sativum* cv stated that VCT has a significant positive impact on growth and yield, improving root length, promoting root meristem development, and activating growth points in seedlings (Maji et al. 2017). These data also support studies on vermicompost productivity (Atiyeh et al. 2000; Bachman and Metzger 2008; Blouin et al. 2019).

Total protein content, which is an important indicator of living metabolism, is known as an important sign of the organism's response to adverse conditions (Nagajyoti et al. 2010). Plants activate their metabolic activities, including hormone induction, kinase cascade signalling, and gene expression regulation, and amino acid metabolism against various stress conditions, among others (Wasternack and Hause 2013; Michaletti et al. 2018). Physiological, biochemical and genetic activities produce an upsurge in protein concentration in the plant. The stress that plants experience under inappropriate circumstances leads to the structure of a protein becoming misfolded or an increase in the quantity of protein (Yuksel et al. 2021; Aksoy et al. 2022). In this study, at the end of the 48th and 72nd hours, the total

amount of protein increased with the increase in the MIT concentration applied compared to the control group. The observed increase in total protein amount in seeds treated with MIT compared to the control group may be the metabolic response of the plant to stress conditions. Researchers have reported protein degradation of storage proteins during seed germination (Palma et al. 2002; Bartels and Sunkar 2005). An increase in the total amount of protein at the end of the 72nd hour compared to the 48th hour may affect. It is observable that protein degradation is not fast at increasing MIT concentrations; therefore, the germination rate is low, and root elongation is less. As a result, the amount of protein may increase due to the increase in the synthesis of enzymes related to the antioxidant system.

On the other hand, at the end of the 48th and 72nd hours, an increase was observed in the amount of total protein in the experimental groups treated with vermicompost and MIT compared to the control group. These effects have been interpreted in terms of stimulating protein catabolism and/or interference with MIT-induced protein biosynthesis. In the present study, the increase in the amount of soluble protein observed in MIT concentrations in root samples compared to control can be evaluated as the plant's reaction under stress conditions. An example of this is the increase in proline amino acids, which increases the solubility of soluble proteins in the plant under different stress conditions (Miller et al. 2010). The main reason for the changes in soluble protein amounts and protein profiles can be attributed to the stress of the seeds and the physiological, biochemical, and genetic changes that occur accordingly. It can be considered important data that the less increase in the total amount of protein in the VCT applied group than the group not applied. Adding organic matter to a growth medium has positive effects on improving soil functioning (Murphy 2014; Blouin et al. 2019). The reason for the low increase in the VCT applied group may be the effect of the applied vermicompost. VCT may have reduced stress conditions by affecting plant metabolism. There are studies that state vermicompost has such properties (Blouin et al. 2019). All results of experimental groups were found to be statistically significant in determining the total protein amounts.

The compounds most affected by reactive oxygen species are thought to be lipids that form the main structure of the cell membrane (Cai et al. 2004). Reactive oxygen species oxidize the lipids found in the membranes in the cell, disrupting the membrane structure and thus reducing the selective permeability properties of the membranes in ion uptake. The intermediate product malondialdehyde, which occurs in the oxidation reactions of lipids in cell membranes due to stress factors in the plant, is one of the decomposition products of unsaturated fatty acids in the biomembrane. For this reason, the amount of MDA is used to determine the extent of stress damage in stress studies (Parida and Das 2005; Gill and Tuteja 2010; Yuksel and Aksoy 2019). Lipid peroxidation begins rapidly in the presence of a sufficient amount of reactive atoms or molecules in the organism. The resulting MDA impacts the ion exchange in cell membranes, leading to cross-linking of membrane compounds. Thus, it causes negative consequences such as a change in ion permeability and an increase or decrease in enzyme activities. Due to this feature of MDA, it can react with nitrogen-bearing groups of DNA so, it can be said to be mutagenic and genotoxic (Halliwell and Chirico 1993; Valavanidis et al. 2006). In the present study, when the MDA amounts formed in the control group and *P.sativum* seeds that were applied MIT, while the amount of MDA decreased. This situation may be due to the germination process. Proteins formed during germination may prevent the formation of reactive oxygen species and consequently the formation of MDA.

At the end of the 72nd hour, significant growth was found in MDA quantity, particularly in the EC50x2 concentration, compared with the control group. This implies that the plant may have been stressed by MIT and amplified lipid peroxidation. There was a decline in the amount of MDA in the VCT and MIT applied seeds at the end of both the 48th hour and 72nd hour compared to the control group. This shows that the decrease in MDA amount in the germinated and grown *P.sativum* under the same conditions was because of the vermicompost application. Also, as indicated in the total protein assays, there was a significant decrease in MDA amount in the group with VCT compared to the group without added. It can show the effect of VCT on this data. It has been stated that the MDA level, that is, lipid peroxidation, increases with stress in many plants (Yuksel and Aksoy 2019). On the other hand, recent data show that non-enzymatic membrane peroxidation is not always harmful to the cell and that oxidative fatty acids and carotenoids act as ROS scavengers (Farmer and Mueller 2013). However, protein and membrane structure in plants can be modified and impaired by ROS formed due to high-stress conditions. As a result, membrane permeability may increase, and electrolytes may spread out of the cell. Therefore, it can be said that it has mutagenic and genotoxic properties (Aksoy et al. 2022). In the determination of MDA analysis, all the data were found to be statistically significant compared to the control group ($p < .05$).

H_2O_2 occurs due to the catalysis of superoxide by the SOD, which has high catalytic activity in aerobic organisms. Having a long life cycle (1 ms) compared to other (ROS) types, H_2O_2 is a moderately reactive molecule (Das and Roychoudhury 2014). While H_2O_2 causes oxidative damage and cell death in peroxisomes by oxidizing thiol groups, they act as signal molecules in programmed cell death when they are produced at low levels. (Gratão et al. 2005; Livanos et al. 2012; Ren et al. 2014). The dangerous nature of H_2O_2 is due to its ability to diffuse easily

through the cell membrane and its readiness to transform into the OH radical (Asad et al. 2004). In the present study, it was determined that the amount of H_2O_2 increased with MIT in the 48th and72nd hours compared to the control group. Increased H_2O_2 amounts compared to the control group may indicate the toxic effect of MIT on the plant that may have caused stress. The use of H_2O_2 as a signal molecule in biochemical stresses (Valko et al. 2007) and the increase in the amount at the moment of oxidative stresses highlight the idea that it is a sign of stress in plants. The results of a previous study highlighting that the amount of H_2O_2 increased under stress conditions in plants have been supported by the findings in this study (Yuksel and Aksoy 2019). In the present study, the linear increase in the amount of MDA and the amount of H_2O_2, especially in the increasing MIT concentration, support related literature findings. In the experimental group where VCT and MIT were applied, at the end of the 48th hour, a decrease in EC50/2 MIT concentration was observed, but there was an increase in EC50 and EC50x2 MIT concentrations. In plants, H_2O_2 plays many beneficial roles by regulating many basic physiological processes at low concentration, while at high concentration, it causes stark cellular damage through oxidative modification of DNA and proteins. But because of its moderate reactivity, H_2O_2 causes cellular damage after converting to more reactive species such as ·OH, which has a single unpaired electron (Farooq et al. 2019). The reason for the decrease in the desired level compared to the experimental group with VCT added may be due to the effect of a signal molecule of H_2O_2. When the germination time is considered, a sufficient decrease was not observed. However, it can be concluded that there is a decrease compared to the 48-h periods of the group without VCT. Especially, the increase in EC50x2 MIT concentration of the group without VCT was significant, while this rate remained at a similar level in the 48-h group with VCT added. At the end of the 72nd hour, while there was an increase in the number of H_2O_2 at increasing concentrations of MIT + 10% VCT compared to the control group, there was no significant change in the 72-h experimental group without VCT added. As the amount of MIT increases, the plant may be unable to cope with stress. It also modulates O_2 radicals of metabolic reactions catalyzed by three types of SOD in plants, producing H_2O_2, which functions as an essential metabolite and universal signalling molecule under biotic and abiotic stresses (Del Río et al. 2018). Considering the effect of the SOD enzyme on forming the amount of H_2O_2, increased SOD activity may have caused an increase in the amount of H_2O_2. As a matter of fact, VCT added the experimental group used to the SOD activity determination, especially the EC50 MIT concentration increased considerably in 72 h. This process can be tested again with other study groups by increasing the VCT ratio.

SOD (E.C.1.15.1.1) is one of the most effective intracellular enzymatic antioxidants belonging to the family of metalloenzymes found in all aerobic organisms. SOD are enzymes that form the first line of defence against ROS-induced damage under stress and convert O^{2-} and H_2O_2 to water and molecular oxygen. It has been found that SOD is regulated by abiotic stress conditions (Boguszewska et al. 2010). Also, it has been stated by some researchers that superoxide anion leads to membrane damage, cellular toxicity, single-chain breaks in DNA, lipid peroxidation. (Turrens 2003; Halliwell 2006; Sharma et al. 2012). In some researches, it has been stated that the increases in gene expression of superoxide dismutase enzymes have important roles in countering reactive oxygen species caused by biotic and abiotic stress, coping with stress, and contributing to the survival of plants under stress conditions (Hoogewijs et al. 2008; Fischer et al. 2013; Yuksel and Aksoy 2019). The present study's findings showed that the activity of SOD increased at the end of the 48th hour and 72nd hour in the experimental group, where only MIT was applied compared to the control group. Moreover, these findings support the studies from literature reporting the increase in SOD activity under stress. This may indicate by the MIT chemical that the plant activates the control of reactive oxygen species to survive under oxidative stress conditions and the antioxidative defence systems to survive.

At the end of the 48th hour, an increase in SOD activity was observed in the experimental groups to which MIT and 10% VCT were added, compared to the control group. After a slight increase in EC50/2, the sudden increase in EC50 and the close return of EC50x2 MIT concentration in the control group may be evidence that the plant can cope with stress. The sudden decrease in SOD activity at the EC50x2 MIT concentration supports this theory. In this case, it can be said that the addition of VCT is effective. At the end of 72 h, the same results were observed in the samples of this study. At the end of the 48th hour, significant antioxidative responses also occurring in SOD activity suggest that VCT may be functional in the plant's stress mechanism. According to the data published in the literature, SOD, whose activity is dose and time-dependent, is present extensively in the plant cell, in chloroplasts, cytosol, mitochondria, peroxisomes and apoplast. Many studies have stated that stress factors trigger SOD activation, but we can also find conflicting data (Sharma et al. 2012; Szőllősi 2014). These results can be further elaborated by investigating the activity of isozymes along with the total SOD activity. Our findings are that VCT reduces SOD activity, which increases stress in plants. Increasing air pollution and drought, UV-B radiation, heavy metal pollution of water and soil are problems that need to be

solved for the environment and people. It is difficult for plants exposed to abiotic and biotic stresses due to their passive life to be tolerant of unfavourable conditions and get used to these conditions. It has been argued in previous research that metabolic, genetic and physiological disorders caused by biotic and abiotic stress factors cause slow growth of plants and low crop yields (Szőllősi 2014). It is necessary to recycle organic wastes and reduce the use of artificial fertilizers to understand and develop stress tolerance, grow more efficient products, and reduce environmental pollution.

Conclusion

The findings of this study will provide a general evaluation, and the results of the analysis will also give the producers and consumers an idea about the advantages and disadvantages in the areas where MIT is used. According to the data obtained by determining the biochemical effects of the MIT substance on *P.sativum*, it is thought that it can contribute to the researchers working with plant physiology to cover the deficiency on this subject to a certain degree in the literature to clarify some gaps. In light of these data, it can be concluded that vermicompost is a good solution for both recyclings the increased amount of organic waste and reducing the use of artificial fertilizers. Besides, VCT can positively reduce the damage in nature, even to a certain extent, due to increased chemical use and can be used as a support to improve plant stress metabolism. In a nutshell, MIT creates changes in some biochemical parameters on the forage pea (*P.sativum*), and these changes caused by stress in the plant are improved with the application of vermicompost.

Acknowledgments We would like to express our gratitude to Kocaeli University [BAP- 2019/019] for their financial support during this study.

Author contributions Burcu YUKSEL Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Roles/Writing - original draft; Writing - review & editing.

Duygu AYDIN Conceptualization; Data curation; Investigation; Methodology; Project administration; Resources; Software; Validation; Visualization.

Ozlem AKSOY Methodology; Project administration; Resources; Supervision; Validation; Visualization.

Funding This study's financial support is provided by Kocaeli University [BAP- 2019/019].

Declarations

Ethics approval No need for ethics approval.

Conflict of interest The authors have declared no conflict of interest.

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