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ORIGINAL ARTICLE

The healing effect of liquid vermicompost against Kathon CG application in *Pisum sativum* spp. *arvence*

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Abstract

Kathon CG, which has a wide area of usage, is one of the representatives of the isothiazolinone group. Considering the application areas in industrial and cosmetic products, it can be a contaminant to water and soil, and it can be toxic to organisms. Vermicompost, known as the organic matter of worms, has important features in terms of increasing the production of plant biomass as well as increasing the soil content. In this study, Kathon CG was applied on the forage pea (*Pisum sativum* spp. *arvence)*, which is an important food source for humans and animals, and it was treated with liquid vermicompost to reduce the stress caused by the Kathon CG chemical on the plant. As a first step, the EC50/2, EC50, and EC50 × 2 values of Kathon CG were determined. These concentrations were applied to the plant, and the biochemical effects (total protein, H_2O_2 and MDA amounts, SOD activity) were examined at the end of 48 and 72 h. In the second stage of the experiment, the same chemical was applied with the same amount of liquid vermicompost by keeping the conditions constant, and the same biochemical parameters were examined. The healing properties of vermicompost were compared with and without liquid vermicompost. When statistical data are compared with the control group, the results revealed a significant difference between these two conditions ($p < 0.05$).

Keywords Antioxidant · *Pisum sativum* · Plant stress · Liquid vermicompost

Introduction

Kathon CG is a cosmetic preservative that contains a CMIT (5-chloro-2-methyl-4-isothiazolin-3-one/MIT (2-methyl-4-isothiazolin-3-one) (3: 1) blend and consists of magnesium salts such as 23% chloride and nitrate (Park et al. 2020) Kathon CG, which has a wide range of uses, is used as a preservative with antifungal and antibacterial properties in many areas such as hand, face, foot creams and shower gels, shampoos, detergents, air fresheners, wet wipes, toilet paper, metalworking fluids, wall paints, industrial products (Aerts et al. 2017; Chang and Nakrani 2014; Gardner et al. 2010; Geier and Lessmann 2020; Hoa et al. 2019; Johnsson et al. 2011; Park and Kwon 2016; Uter et al. 2014; Wang et al. 2020). Even if Kathon CG has such a wide range of uses in daily life, it also has toxic effects. Concentrated Kathon CG has been reported to cause a kind of irritation due to contact allergic accompanied together with local dermal irritation and delayed bullous dermatitis following accidental interaction with the skin or mucous membranes (Kujala and Niinimäki 1999; Schwensen et al. 2017). In cell culture studies, it was observed that sudden apoptotic cell death increased, the number of living cells decreased very much, and it was reported to cause DNA fracture due to having high toxicity (Anselmi et al. 2002). According to the results of the patch tests performed between 1991 and 2000 years, it was determined that the sensitivity rates to the Kathon CG mixture increased over time (Wilkinson et al. 2002). Since the 2000s, allergies have increased substantially (Urwin and Wilkinson 2013). MIT, which is included in the Kathon CG blend, was selected as the allergen of the year by the American Contact

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Dermatitis Association (ACDS) in 2013 (Militello et al. 2020). CMIT in Kathon CG has the highest sensitization potential among the isothiazoline group (Aerts et al. 2017). In addition, there is Kathon CG between the rates of 0.05% and 0.1% in the Plant Protection Mix used to protect plants from fungus and bacteria while growing plants in different agar media (Niedz 1998).

The process where worms are used to transform organic matter (commonly waste) into similar to humus material known as vermicompost, is called vermicomposting (Asgari and Moradi 2014). The general appearance of the vermicompost is similar to black soil, and it has no repellent odour; it contains all the enzymes necessary for plant growth, as well as soil antibiotics, vitamins, and growth hormones, and does not contain disease-causing substances, parasite eggs, weed seeds and heavy metals (Basak et al. 2013). Worm castings are important both for reprocessing the increased measure of organic waste and for lessening fertilizer consumption (Edwards et al. 2004; Blouin et al. 2019). Moreover, the use of vermicompost in large areas is effective to advance soil content in organic matter (Abraham and Lal 2004). First of all, worm castings, in particular, make it likely to decrease the amount of organic waste; secondly, alleviate organic matter and thirdly, augment plant biomass amount by various processes. Also, it boots the soil amount in organic matter that reduces the soil bulk concentration and amplifies the disposal of water and mineral nutrients, increases hormone-like impact, and reduces the effects of pests and pathogens (Edwards et al. 2004). Worm castings are a promising solution to increase the fertility and viability of agriculture. Aqueous solutions of vermicompost can be defined as aqueous excerpts of solid vermicompost where such microorganisms, soluble nutrients, and plant-beneficial elements are transformed into a liquid form (Salter and Edwards 2010). The most important properties of liquid vermicompost (LVC) are that, unlike its solid counterpart, it can be applied directly to plant leaves and used to wet the soil (Salter and Edwards 2010). There are studies showing that when liquid worm casting is applied on soils exposed to pesticides, it is effective in soil bioremediation, which neutralizes the pesticide in the soil by breaking it and increases the enzymatic activity in the soil (Sanchez et al. 2019). With the increasing industrialization, more productive and improved soil can be obtained by using worms in polluted lands around the industrial zone and turn the harmful wastes into better and fertile soils (Bhat et al. 2018). Therefore, it can be said that vermicompost, which is defined as worm compost, is a promising solution to increase the sustainability of agriculture.

In this study, some biochemical effects of Kathon CG on *P. sativum*, which is an appropriate experimental model (Salazar-Mercado et al. 2019) that can be used in the laboratory to evaluate the cytotoxic effect of the toxic substance, were examined. In the first stage, EC50 values were determined by applying Kathon CG chemical in different doses on *P. sativum*. Then, E50/2, EC50, and EC50 × 2 were prepared, and the total protein, Measurement of Malondialdehyde amount (MDA), and H_2O_2 levels were determined at the end of the 48th and 72nd hours, and the activities of superoxide dismutase and catalase enzymes were observed. Then, the same biochemical parameters were measured again by applying vermicompost tea (called VCT). Thus, it was determined whether VCT has a healing effect on the stressed plant due to the toxic effect.

Materials and methods

Sample preparation

Commercially purchased Kathon CG (Cayman Chemical, USA) was prepared freshly in glass bottles with distilled water for each run. It was applied to the seeds after being mixed in a magnetic stirrer for a minimum of 15 min and a maximum of 30 min. Liquid vermicompost commercially (Riverm Ltd., Tekirdag/Turkey) was obtained, and the dilution process was performed with distilled water. Also, *Pisum sativum* ssp. *sativum var. arvence* was obtained commercially (HK Seeds, Kutahya/Turkey).

Determination of EC50 value

The pea seeds were washed three times using distilled water, then kept under UV light, and 20 pieces were placed on 22 × 25 cm Petri dishes with paper towels (El-Amier et al. 2019). To determine the EC50 value of Kathon CG, eight concentrations were applied to it. Distilled water was used in the control group. Peas were germinated in a dark environment at 23.5 °C in a drying-oven. Each application was done in 3 repetitions. The concentration that halved the root length at the end of the 48th hour was accepted as the EC50 value. During this study, EC50/2, EC50, EC50 × 2 Kathon CG concentrations were used in each experimental group.

Determination of LVC efficiency ratio

Liquid vermicompost fertilizer was applied to *P. sativum* seeds at the rates of 100%, 25%, 20%, 15%, 10%, 7.5%, 5%, 4%, 2%, 1%, and 0.5%. At the end of the 48th hour, *P. sativum* root length measurement was taken, and the concentration with the best *P. sativum* germination rate was applied as the healing concentration. The content of LVC is as follows; 5.2% Organic matter, 1.1% Total nitrogen (N), 1.1% Organic nitrogen, 1% P2O5, 1.2% K2O, 1.2% humic + fulvic acid.

Total protein determination

All of the biochemical analyses were performed at the times when mitosis occurred in the roots, between 11:00 and 12:00 when cellular activity was at its highest (Bonjoch and Tamayo 2001). 200 mg root sample was homogenized in 0.05 g PVP (Polyvinylpyrrolidone) and 0.05 M Tris buffer solution (pH 8). The supernatant was taken by centrifuging at 15,000 g for 20 min at + 4˚C (Acemi et al. 2020). The supernatant was diluted with the rate of 1: 2 Tris buffer solution (pH 8). According to the study of Bradford (1976), 200 µl of the sample was added into 3 ml of Bradford Reagent, and each was measured at 595 nm spectrophotometer (UV mini 1240, Shimadzu) as three replicates. A total of approximately 50–80 fresh root samples were used for each concentration. After the measurements were taken at 595 nm, the standard graph was prepared, and the total protein amounts were determined.

Determination of hydrogen peroxide (H_2O_2)

Root samples were taken from *P. sativum* as 0.5 gr were crushed in a mortar and pestle in 5 mL 0.1% TCA (Trichloroacetic acid). The crushing session was carried out on the ice. The homogenate was centrifuged at 10,000 g for 30 min at + 4 °C (Doupis et al. 2011). A total of 3 ml of a solution containing 0.5 ml of supernatant, 0.5 ml of 100 M potassium phosphate buffer solution (pH 7), and 2 ml of 1 M potassium iodide (prepared with fresh bidistilled water) was prepared in glass bottles, mixed and the bottles were covered with aluminium foil to protect from light. Samples were preserved in the dark at + 4˚C for 1 h to react. After 1 h, samples were measured at 390 nm in the spectrophotometer. 0.1% TCA was used as a blind sample (Alexieva et al. 2001). H_2O_2 amounts were determined by creating standard graphics. Since H_2O_2 values decreased, each dose was measured within 50 min at the latest.

Determination of malondialdehyde (MDA)

300 mg of root sample was crushed in a mortar and pestle with liquid nitrogen. 3 mL 0.1% TCA was added and taken into falcon tubes. It was then centrifuged at 15,000 g for 10 min at room temperature. The supernatant was mixed in equal proportions in a beaker with 20% TCA containing 0.5% TBA (Thiobarbituric acid). The mixture in the beaker was left on the heating plate (MR Hei-Standard, Heidolph) at 100˚C for 20 min until it turned reddish. Samples were taken immediately on ice and, after reaching room temperature, centrifuged at 15,000 g for 5 min. The supernatant was taken, and the amount of malondialdehyde was measured at 532 and 600 nm in the spectrophotometer. A 20% TCA mixture containing 0.5% TBA was used as a blind sample. The calculation was based on Lighter and Horst's 155 $m^{-1}c^{-1}$ formula (Cakmak and Horst 1991; Heath and Packer 1968).

Superoxide dismutase (SOD) activity determination

0.2 g of fresh *P.sativum* root sample was crushed in a mortar in 100 mM potassium phosphate buffer solution (pH 7) including 5 mL of 0.1 mM EDTA and 1% PVP. This process was carried out on the ice. The homogenate was prepared by weighing 0.025 (w/v) Triton X-100, 0.0044% (w/v) riboflavin at 15,000 g RPM. The solution was dissolved under stirring for 30 min and then used at + 4° C (Dixit et al. 2001) and prepared freshly for each run. 2.95 ml of substrate buffer and 0.05 ml of the supernatant prepared as 21 ml in total were taken into a 25 ml conical flask and mixed. The mixture was left under two 21 Watt fluorescent lamps for 15 min. Substrate buffer without enzymes was placed in a flask, kept under light for 15 min, and used as a blind sample (Mishra et al. 2006; Tripathi and Gaur 2004). After the reaction under light, the beakers were covered with aluminium foil and measured at 560 nm in the spectrophotometer. Each dose was measured within 15 min at the latest. The SOD value was calculated as U/mg.

Statistics

ANOVA (one-way analysis of variance) was performed using the SPSS program (version 22) to detect differences between data. After determining the conformity of the data to a normal distribution (Test of Normality-Shapiro–Wilk), the test of homogeneity of variances (Levene Statistic) was applied, and Tukey test was performed for the data with homogenous variances and Dunnett T3 test for the heterogeneous ones.

Results

EC50 value

The EC50 value for Kathon CG was determined as the value that halved the *P. sativum* root lengths compared to the control. While root length was determined as 1.4 cm in the control group, 3 mL/L Kathon CG concentration was determined as the value that halved this root length. Approximately, 0.74 cm root length was measured. As indicated in Fig. 1, it was found that root elongation decreased in *P. sativum* seeds at increasing Kathon CG concentrations. According to these results, the EC50 value for Kathon CG was determined as 3 mL/L, the EC50/2 value as 1.5 mL/L, and the EC50 × 2 value as 6 mL/ L (Fig. 1). Our experiments were conducted on these concentrations.

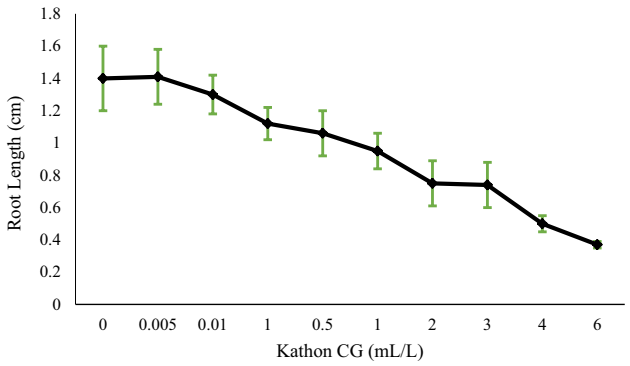


Fig. 1 EC50 value for Kathon CG applied on *P. sativum*

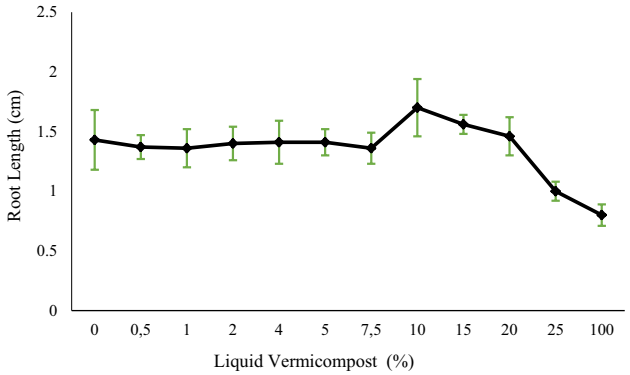


Fig. 2 The efficiency rate of LVC applied to *P. sativum*

Liquid vermicompost productivity amount

By applying different amounts of liquid vermicompost, the amount of liquid vermicompost forming the greatest root growth was determined (Fig. 2). Especially in 0–7.5% applications, no significant change was observed in root growth, while a significant growth was observed in 10% LVC (1.7 ± 0.24 cm) application. It was determined that applications above 10% LVC decreased the growth rate of root lengths. According to these results, it was concluded that the most suitable LVC concentration for root growth was 10% application. In our experiments, 10% LVC was used as the optimum quantity.

Total protein determination

In the determination of total protein, distilled water was applied from *P. sativum* seeds to the control group, and Kathon CG concentrations prepared as EC50/2, EC50, EC50 × 2 were applied to the experimental groups. Total protein amounts measured after 48 h in control group was 0.88 mg/mL. In the experimental group following concentrations were determined: 0.94 mg/mL at 1.5 mL/L (EC50/2), 1.05 mg/mL at 3 mL/L (EC50) and 1.24 mg/mL at 6 mL/L (EC50 × 2) in Kathon CG concentration.

After Kathon CG + 10% LVC was applied, the measured total protein amounts were 1.06 mg/mL in the control group. EC50/2, EC50, EC50 × 2 Kathon CG + 10% LVC group were determined as at amounts of 0.96 mg/mL, 1.21 mg/mL 1.32 mg/mL of total protein, respectively. When the total amount of protein measured after 72 h was evaluated, in the control group 0.47 mg/mL was determined. 0.75 mg/mL at 1.5 mL/L (EC50/2), 0.83 mg/mL at 3 mL/L (EC50) and 0.91 mg/mL at 6 mL/L (EC50 × 2) were detected in the experimental group. After Kathon CG + 10% LVC was applied, total protein amounts were 0.58 mg/mL in the control group. On the other hand, EC50/2, EC50, EC50 × 2 Kathon CG + 10% LVC group were determined 0.67 mg/mL, 0.72 mg/mL and 0.91 mg/mL, respectively (Table 1). The statistically significant and insignificant results based on our analysis are shown in Table 1 ($p < 0.05$).

Table 1 Kathon CG applied control group and the mean values and statistical data of 48 and 72 h total protein amounts in *P. sativum* seeds treated with Kathon CG + 10% LVC

Time (hours)	Applied dose Kathon CG (mL/L)	mg/ml protein $\overline{X} \pm$ SD	*P* value	Kathon CG + 10% LVC (mL/L)	mg/ml protein $\overline{X} \pm$ SD	*P* value
	Control	0.88 ± 0.05	–	Control	1.06 ± 0,01	–
48	1.5	0.94 ± 0.03	0.877	1.5	0.96 ± 0.01	0.020*
	3	1.05 ± 0.03	0.158	3	1.21 ± 0.01	0.005*
	6	1.24 ± 0.01	0.044*	6	1.32 ± 0.02	0.001*
	Control	0.47 ± 0.02	–	Control	0.58 ± 0.03	–
72	1.5	0.75 ± 0.01	0.001*	1.5	0.67 ± 0.02	0.156*
	3	0.83 ± 0.003	0.003*	3	0.72 ± 0.01	0.063
	6	0.91 ± 0.01	0.001*	6	0.91 ± 0.01	0.004*

*$p < 0.05$

Measurement of H_2O_2 concentration

H_2O_2 measured at the end of 48 h in the experimental group applied Kathon CG, 7.07 µM/g at 1.5 mL/L (EC50/2), 7.29 µM/g at 3 mL/L (EC50), 11.25 µM/g in the control group. It was determined as 16.48 µM/g at Kathon CG concentration in mL/L (EC50 × 2). At the end of 72 h, while the H_2O_2 measured in the control group was 3.19 µM/g, in the experimental group effective concentration volumes of 1.5 mL/L (EC50/2), 3 mL/L (EC50), 6 mL/L (EC50 × 2) and Kathon CG concentrations of 4.02 µM/g, 4.14 µM/g and 5.83 µM/g were established, respectively.

The H_2O_2 amounts obtained at the end of 48 h in the Kathon CG + 10% LVC group were 6.28 µM/g in the control group, and 5.55 µM/g at 1.5 mL/L (EC50/2), at 3 mL/L (EC50) 8.00 µM/g and at 6 mL/L (EC50 × 2). 9.02 µM/g was determined in the experimental group. After 72 h, in the control group following amount were determined: 4.48 µM/g, at 1.5 mL/L (EC50/2) 4.76 µM/g, at 3 mL/L (EC50) 5.20 µM/g and at 6 mL/L (EC50 × 2) 5.76 µM/g Kathon CG + 10% LVC in application.

In the experimental group formed to measure the amount of H_2O_2 in the plant, both an increase in the amount of H_2O_2 and the increase in Kathon CG concentration applied group was observed compared to the control group at the end of 48 h. At the end of 72 h, an increase in the amount of H_2O_2 with increasing Kathon CG concentrations was detected compared to the control group. At the end of 48 h in the Kathon CG + 10% LVC group, a slight decrease was found in the amount of H_2O_2 in EC50/2 concentrations added LVC compared to the control group (6 µM/g), while an increase in the amount of H_2O_2 was detected in EC50/2, EC50, and EC50 × 2 applications, even if not much. The statistically significant and insignificant data in this experimental group are presented in Table 2 ($p < 0.05$).

Determination of MDA

Various data were taken from the experimental groups in determining the amount of MDA. MDA levels measured at the end of 48 h in the control group using only Kathon CG were 4.37 µM, in the experimental group at 1.5 mL/L (EC50/2),3.62 µM, at 3 mL/L (EC50) 2.92 µM and at 6 mL/L (EC50 × 2) 5.31 µM were determined. MDA amounts measured at the end of 72 h were 2.62 µM in the control group, 2.14 µM at 1.5 mL/L (EC50/2), 1.73 µM at 3 mL/L (EC50) and 6.21 µM at 6 mL/L (EC50 × 2). In particular, the decrease in the amount of MDA in the 48-h experimental group treated with LVC was quite significant compared to the control group.

After the application of Kathon CG + 10% LVC, the amount of MDA taken at the end of 48 h was determined as 3.28 µM in the control group, EC50/2, EC50, EC50 × 2 Kathon CG + 10% LVC concentrations in the experimental group, 2.60 µM, 2.35 µM and 2.74 µM, respectively. The amount of MDA taken at the end of 72 h was 1.29 µM in the control group, and 0.91 µM in EC50/2, 1.69 µM in EC50 and 2.72 µM in EC50 × 2 concentrations. The average values and statistical data of 48 and 72 h MDA in *P.sativum* seeds treated with Kathon CG + 10% LVC and only Kathon CG are given in Table 3. In the determination of MDA analysis, all the data were found to be statistically significant compared to the control group ($p < 0.05$).

SOD activity

SOD enzyme activity measured at the end of 48 h in applied Kathon CG was 10.77 U/mg in the control group. In the experimental group, 7.04 U/mg at 1.5 mL/L (EC50/2), 6.86 U/mg at 3 mL/L (EC50) and 1.60 U/mg 6 mL/L at (EC50 × 2) Kathon CG concentrations were used. At the end of 72 h, 4.10 U/mg was determined in the control group. Following concentrations were used in the experimental group:

Table 2 Kathon CG applied control group and the mean values and statistical data at 48 and 72 h of H_2O_2 amounts in *P. sativum* seeds treated with Kathon CG + 10% LVC

Time(hours)	Applied dose Kathon CG (mL/L)	H_2O_2 µM/g $\overline{X} \pm$ SD	*P* value	Kathon CG + 10% LVC (mL/L)	H_2O_2 µM/g $\overline{X} \pm$ SD	*P* value
	Control	7.07 ± 0.02	–	Control	6.28 ± 0.02	–
48	1.5	7.69 ± 0.06	0.002*	1.5	5.55 ± 0.05	0.240
	3	11.25 ± 0.13	0.000*	3	8.00 ± 0.03	0.003*
	6	16.48 ± 0.01	0.000*	6	9.02 ± 0.01	0.003*
	Control	3.19 ± 0.01	–	Control	4.48 ± 0.06	–
72	1.5	4.02 ± 0.02	0.507	1.5	4.76 ± 0.02	0.068
	3	4.14 ± 0.04	0.000*	3	5.20 ± 0.05	0.000*
	6	5.83 ± 0.01	0.000*	6	5.76 ± 0.03	0.008*

*$p < 0.05$

Table 3 Kathon CG applied the Control group and the mean values and statistical data of 48 and 72 h MDA amounts in *P. sativum* seeds treated with Kathon CG + 10% LVC

Time (hours)	Applied dose Kathon CG (mL/L)	MDA µM $\overline{X} \pm$ SD	*P* value	Kathon CG + 10% LVC (mL/L)	MDA µM $\overline{X} \pm$ SD	*P* value
	Control	4.37 ± 0.04	–	Control	3.28 ± 0.01	–
48	1.5	3.62 ± 0.04	0.000*	1.5	2.60 ± 0.03	0.002*
	3	2.92 ± 0.06	0.000*	3	2.35 ± 0.02	0.000*
	6	5.31 ± 0.03	0.000*	6	2.74 ± 0.02	0.002*
	Control	2.62 ± 0.02	–	Control	1.29 ± 0.01	–
72	1.5	2.14 ± 0.02	0.000*	1.5	0.91 ± 0.001	0.000*
	3	1.73 ± 0.02	0.000*	3	1.69 ± 0.02	0.002*
	6	6.21 ± 0.03	0.000*	6	2.72 ± 0.01	0.000*

*$p < 0.05$

11.64 U/mg at 1.5 mL/L (EC50/2), 8.36 U/mg at 3 mL/L (EC50) and 0.93 U/mg at 6 mL/L (EC50 × 2).

SOD enzyme activity obtained at the end of 48 h in the Kathon CG + 10% LVC group was 20.52 U/mg in the control group. In the experimental group, 12.62 U/mg at 1.5 mL/L (EC50/2), 19.92 U/mg at 3 mL/L and 14.03 U/mg at 6 mL/L (EC50 × 2) were determined. At the end of 72 h, 15.70 U/mg was established in the control group whereas 21.90 U/mg at 1.5 mL/L (EC50/2), 14.49 U/mg at 3 mL/L (EC50) and 21.28 U/mg at 6 mL/L (EC50 × 2) were used in the Kathon CG + 10% LVC application.

In the experimental group formed to determine the SOD activity, it was found that the SOD activity significantly decreased with the Kathon CG application in relation to the control group after 48 h. On the other hand, while an increase was observed in the EC50/2 and EC50 Kathon CG applications in 72 h data, it was determined that the EC50 × 2 Kathon CG concentration decreased significantly (Table 4). In the group treated with Kathon CG with 10% LVC, decreasing SOD activities was detected 48 h compared to the control group. Also, in the same group, an increase was observed in the amount of EC50/2 and EC50 × 2 at the end of the 72-h experiment, while a slight decrease was reported in the EC50 group. The values and statistical results are taken in this experimental group are given in Table 4.

Discussion

The usage in cosmetics such as personal care and beauty products has boosted worldwide more than ever, and a large number of chemicals are used to increase some features of these products such as their performance, property, significance, and durability. Most of these chemical additives cause health risks by creating toxic effects on the human body, and at the same time, they easily reach the plants by mixing with irrigation water and soils and polluting the environment. Therefore, cosmetic products' indiscriminate use has become a major problem threatening both public and environmental health (Bilal and Iqbal 2019). In light of this information, some biochemical effects of Kathon CG, which has been used in many cosmetic and personal care products, on *P. sativum* were examined in this study. For this purpose, EC50/2, EC50, and EC50 × 2 doses of Kathon CG chemical, whose EC50 value was determined and applied on *P. sativum* seeds. Moreover, the total protein, MDA, H_2O_2

Table 4 Kathon CG applied Control group and the mean values and statistical data of 48 and 72 h SOD activity in *P. sativum* seeds treated with Kathon CG + 10% LVC

Time(hours)	Applied dose Kathon CG (mL/L)	SOD activity U/mg $\overline{X} \pm$ SD	*P* value	Kathon CG + 10% LVC (mL/L)	SOD activity U/mg $\overline{X} \pm$ SD	*P* value
	Control	10.77 ± 0.23	–	Control	20.52 ± 0.63	–
48	1.5	7.04 ± 0.26	0.001*	1.5	12.62 ± 0.69	0.000*
	3	6.86 ± 0.44	0.000*	3	14.03 ± 0.68	0.000*
	6	1.60 ± 0.28	0.000*	6	19.92 ± 0.63	0.993
	Control	4.10 ± 0.44	–	Control	15.70 ± 0.71	–
72	1.5	11.64 ± 0.39	0.000*	1.5	21.90 ± 0.85	0.000*
	3	8.36 ± 0.42	0.000*	3	14.49 ± 0.76	0.417
	6	0.93 ± 0.46	0.000*	6	21.28 ± 0.68	0.000*

*$p < 0.05$

amounts were determined, and the activities of superoxide dismutase and catalase enzymes were stated. The same biochemical parameters were then measured again by applying 10% LVC while keeping the conditions constant. Thus, it was examined whether LVC has a healing effect on the plant against Kathon CG substance, which can be toxic when used in high amounts.

In the experimental group, which was carried out to determine the EC50 value, a decrease in root length occurred in the increasing concentrations of Kathon CG chemical applied on *P. sativum* in relation to the control group (Fig. 1). With this experimental group, both the EC50 value, which reduces the root length to half, was determined, and the inhibitory feature of the root elongation of the increasing concentrations of Kathon CG was demonstrated. As a result, when the values of Kathon CG concentration increased during germination and the control group were compared, decreases in root lengths were detected due to increased active substance concentrations. On the other hand, it was observed that 10% LVC application had a positive effect on root elongation in the experimental group was formed for determining the productivity of vermicompost (Fig. 2). These growth responses are likely because of the hormone-like activity of humic acids from the LVC. Because vermicompost, which is occurred as a result of worm activity, increases the presence of auxins and hormones such as gibberellin thanks to the humic acid it contains. (Atiyeh et al. 2002). It can be concluded that vermicompost has hormone-like activity, and this causes more root initiation, increased root biomass, improved plant growth and development. These data give parallel results with the studies on productivity increase on plants due to the application of vermicompost (Atiyeh et al. 2002; Blouin et al. 2019; Edwards et al. 2010; Guo et al. 2019; Ievinsh 2011).

Proteins are multifunctional macromolecules that constitute the main workloads of living cells (Hasan et al. 2017). They operate in areas such as cellular signalling, regulation, catalysis, intracellular and intercellular movement of nutrients and various molecules, membrane fusion, and structural support and protection (Amm et al. 2014; Hasan et al. 2017). The protein's function is characterised by its structure obtained by transcription, translation, and post-translational modifications in the cell. The stress that plants are exposed to under unsuitable conditions causes the protein to be folded incorrectly or increase the protein amounts while disrupting the structure of a protein (Amm et al. 2014; Yuksel and Aksoy 2019; Zhou et al. 2016). In the present study, at the end of 48 h, the total protein amount increased with the increase in Kathon CG concentration compared to the control group (Table 1). At the end of 72 h, the increasing Kathon CG concentrations and total protein amounts boosted compared to the control group. The increase of proteins that play a very important role in the reaction of plant and adaptation to changing environmental conditions in the first group without LVC added may be the metabolic reaction of the plant to stress conditions after exposure to Kathon CG chemical. It is believed to be one of the important indicators that there is a growth in the expression of genes of related proteins under stress conditions, and they act in the regulation of stress tolerance (Hasan et al. 2017). Similar results were observed in the present study's experimental group with 10% LVC added compared to the group without LVC. An increase in the number of proteins was found at the end of both 48 and 72 h of the experiment. The increase in the synthesis of enzymes related to the antioxidant system, the increase of the proline amino acid, and the increased biochemical molecules of the plant under stress conditions can be shown as examples of it. However, when the data in groups with and without LVC added were compared, the amount of protein obtained at the end of 72 h in the group with LVC was less than in the group without added LVC was observed as important data. Similar results after 48 h can be attributed to the proteolytic degradation of storage proteins during seed germination (Yuksel 2017). Physiological, biochemical, and genetic changes that occur due to the stress of the seeds at the specified concentrations of Kathon CG material may have caused an increase in the amount of soluble protein. Including organic matter to the plant's development medium positively affects soil functioning (Blouin et al. 2019; Murphy 2014). The reason for the low increase in total protein in EC50/2 and EC50 concentrations in the 72-h LVC applied group may be the effect of the vermicompost application. It can be concluded that the applied LVC reduces stress conditions in plant metabolism because other variable parameters (e.g., temperature, light, and water) have not been changed, and the result of the present data is also supported by other related studies.

H_2O_2, which is formed from catalysis by the enzyme superoxide dismutase (SOD), is a moderately reactive molecule (Das and 2014). Increasing production of reactive oxygen species such as H_2O_2 under stress can cause many damages, ranging from lipid peroxidation, protein oxidation, nucleic acid damage, enzyme inhibition to programmed cell death (apoptosis) (Farooq et al. 2019; Khan et al. 2017). When H_2O_2 is produced at low levels, they serve as signal molecules in programmed cell death (Ren et al. 2014). Therefore, it can be said that reactive oxygen species (ROS) are produced in wealth by photosynthesis, providing abundant redox signals, and acting as fundamental regulators of energy and metabolic flows (Farooq et al. 2019; Foyer 2018). In the current study, the amount of H_2O_2 increased with the increase in Kathon CG concentrations applied compared to the control group after of the 48th hour. At the end of 72 h, an increase in the amount of H_2O_2 with increasing Kathon CG concentrations was also detected compared to the control group. The related literature supports the result

that the increased H_2O_2 amounts after the plant's exposure to stress conditions (Farooq et al. 2019; Khan et al. 2017; Yuksel and Aksoy 2019). It is noteworthy that the increase in the amount of H_2O_2 remained limited compared to the non-applied group in the second experiment group, which was conducted to determine whether the applied LVC removes H_2O_2 from the environment. The difference in the 48-h group was especially striking (see Table 2), and this change may be due to the effect of the applied LVC. Some factors such as soluble mineral nutrients, humic and fulvic acids, and plant growth hormones, among others, in LVC can also be key factors in healing stress conditions while positively affecting plant growth.

The impact of free oxygen radicals on the cell membrane is occurred by lipid peroxidation (Xu et al. 2019) Lipid peroxidation, which causes to the damage of the cell membrane, produces the final product malondialdehyde (MDA) due to several reactions occurring in a row (Cai et al. 2004; Gill and Tuteja 2010; Ighodaro and Akinloye 2018). Many previous studies reported that the level of MDA, namely lipid peroxidation, upsurges in many plants under stress (Nouairi et al. 2006; Popova et al. 2009; Pospíšil and Yamamoto 2017; Yuksel and Aksoy 2019). However, protein and membrane structure in plants are the two most important structures affected by ROS formed due to high-stress conditions. When ROS damages the membrane structure, membrane permeability increases, and electrolytes spread out of the cell (Nagajyoti et al. 2010; Narayanan 2021). MDA, which occurs as a result of lipid peroxidation, affects the ion exchange in cell membranes, causing cross-linking of membrane compounds. Therefore, it can be called mutagenic and genotoxic (Hill et al. 2021). In the current study, while determining the amount of MDA in the 48-h experiment group, there was a decrease in EC50/2 and EC50 Kathon CG concentrations compared to the control group. Also, it was found that there was an increase in the EC50 × 2 Kathon CG concentration (see Table 3). The results showed that the amount of MDA significantly increased in the EC50 × 2 Kathon CG concentration without LVC added group at the 72 h. These data are in line with the findings of related literature that reports the increased amounts of MDA in plants under stress conditions. In other words, cell membrane damage occurred in the plant as a result of stress at high Kathon CG concentrations. The decrease in the amount of MDA in the group with 10% LVC indicates that LVC positively affects stress metabolism. One of the important results of this research was that the MDA increase was not much in the 72-h experimental group, compared to the control, without the LVC added group (see Table 3). The findings stated that LVC impacted positively the stress metabolism of the plant while determining the amount of MDA. Parallel results have been found with studies on the productivity increase on plants stemming from the application of vermicompost (Blouin et al. 2019; Guo et al. 2019).

H_2O_2, produced in the peroxisome through the oxidation of fatty acids and other substrates, is catalyzed by superoxide dismutase forms containing copper/zinc (Cu/Zn-SOD) or iron (Fe-SOD) (Aghdam et al. 2020). The expression of SOD is regulated by abiotic stress conditions (Das and Roychoudhury 2014). Superoxide anion (O-), which is catalyzed by SOD and converted to water and molecular oxygen, causes membrane damage, cellular toxicity, single-chain breaks in DNA, and lipid peroxidation (Grime 2006; Kamran et al. 2020; Lambers et al. 2008; Nagajyoti et al. 2010; Yuksel and Aksoy 2019). In SOD activity determination studies, it can be stated that different results were observed in the experimental groups. In contrast, decreases were observed in the 48-h experimental group without LVC, while an increase was found in EC50/2 and EC50 applications in the 72-h experiment group, whereas a high amount of decrease in activity was detected in the EC50 × 2 application (see Table 4). These results indicate that the plant under stressed by Kathon CG activates antioxidative defence systems to control ROS. However, the effect of the sudden drop in the 72-h EC50 × 2 application can be explained by either a disturbance in the transcriptional system or inactivity in SOD proteins. This sudden decrease indicates that the plant cannot cope with stress, especially in relation to the control group. Instead, it is important to highlight that the enzyme activities in the group with added 10% LVC are higher than in the group without added. The fact that this increase is proportional to the change in H_2O_2 and MDA makes the data more meaningful. Significant antioxidative responses that also occur in SOD activity point out that LVC can positively affect the plant's stress mechanism.

Conclusion

Plant stress caused by the increase of chemicals used in the industry and cosmetics industry causes agricultural problems in many parts of the world. Temporary or permanent biotic and abiotic stresses cause physical and biochemical changes in plants, which in this case affect plant growth and development, and consequently lead to economic crop losses. In general, the present study tries to contribute to the related literature thanks to the data obtained to investigate the biochemical effects of Kathon CG substance on the model organism, *P. sativum*. All in all, LVC is a good solution that can be used in recycling the increased volume of organic waste and reducing the usage of artificial fertilizers; it can make agriculture more efficient and have positive effects on the health of living organisms.

Author contribution statement OA: conceptualization; data curation; investigation; methodology; project administration; resources; software; supervision; validation; visualization. DA: conceptualization; data curation; investigation; methodology; project administration; resources; software; supervision; validation; visualization. BY: conceptualization; data curation; formal analysis; investigation; methodology; project administration; resources; software; supervision; validation; visualization; roles/writing-original draft; writing-review and editing.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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