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Side-Effect of Soil Fumigation and Soil Drench by some Methyl Bromide Alternatives on three Soil Enzyme Activities

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ABSTRACT

There is an argent need to find safer, environmentally friendlier as well as efficient alternatives. Effect of some fumigants on activities of dehydrogenase (DHase), peroxidase (POD), and polyphenol oxidase (PPO) in soil was carried out. Three types of fumigants were tested: true-fumigants; dimethyl disulfide (DMDS), fumigant generators, phosphine (PH_3), 1,4-Dichlorobenzene (1,4-D), and formaldehyde (CH_2O), and bio-fumigants an essential oil of garlic, jojoba, citrus, and argan. Mini-atmosphere method was used for fumigant generators and soil drench method was used for true- and bio-fumigants. The results revealed that DMDS at concentration of 2000 and 20 mg/kg inhibited soil enzymes within the first four days, then reach the level of the enzymatic activity of a control soil sample. Almost the same trend of the effect of phosphine was obtained from either aluminum phosphide (AlP) or zinc phosphide (Zn_3P_2). Phosphine at 5 mg/L caused an increase in the DHase, POD and PPO activities while, at 150 mg/L, it caused inhibition approximately of 25%. Various essential oils proved to be harmless or very low effect for the soil enzymes. For the effect of all fumigants, the maximum enhancement percentage in the DHase was 37.5% while the maximum inhibition was 25%. Also, the maximum induction was 30% while the maximum reduction in POD activity was 42%. In addition, 48% and 40% were the maximum of stimulation and reduction, respectively in the PPO. Therefore, the activities of DHase, POD, and PPO found to be sensitive to fumigants and proved to be a good indicator of the degree of soil contamination with tested fumigants.

Keywords: soil fumigation, fumigants, soil enzymes, phosphine, dehydrogenase, peroxidase, polyphenol oxidase

INTRODUCTION

Pesticides are commonly used to control pests and diseases that attack various crops consequently it causes economic losses. However, pesticides have serious drawbacks, including reduced profits from high costs, destruction of natural enemy populations, resistance development for several compounds, and accumulation of pesticide residues on the food (Aly et al., 2016; Bawa, 2016). Hazard of pesticides has caused great concern, prompting to the search for new, effective, environmentally friendly and safer alternatives (Chacón et al., 2021). Therefore, these new approaches are important for agricultural production efficiency, global balance, and are continuously being sought to overcome the dangers caused by the synthetic pesticides. Healthy and sustainable agricultural production can be achieved with an integrated pest management approach that can reduce pesticide use by up to 75% for some crops (Farag, 2010).

The soil fumigation is the most effective management method for soil-pests. Because, methyl bromide, which was highly effective to control various soil-pests, has been banned (Noling, 2002), that attributed to environmental hazardous. Researchers have evaluated other alternatives (Mahmoud et al., 2008) but until now, there has been no impressive alternate fumigant proven to be effective against many pests (Rahman et al., 2021). Soil fumigation can control a wide spectrum of pests that find together at the same time (Thompson, 2022). Preplant soil fumigation is the most direct and effective way to guarantee production quality of high-value cash crops (Chellemi, 2014). When properly applied, fumigants deliver a high effective on pests and leave no chemical residue in products to pose a health concern. Therefore, the fumigation is the most effective management method for soil-pests.

Soil microbiological and soil biochemical properties are sensitive to both environmental stress and changes in management practices. Any disturbance to the soil due to the detrimental effects of pollutants on soil biochemical activities affecting soil health and affecting soil functions. Therefore, they are regarded as useful indicators of soil quality (Burylo et al., 2007). Nutrient

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cycling in soils involves reactions of chemical, physicochemical, and biochemical that being by enzymes, thus, soil enzymatic activity is of primary interest in plant litter decomposition, energy transfer, environmental quality and crop productivity (Tabatabai, 1994). Enzymes in soil can be generally classified as cellular and extracellular. Cellular enzymes such as DHase include those associated with viable proliferating cells, whereas extracellular enzymes such as urease, invertase and phosphatase are those released into the environment by secretion or lysis and active enzymes associated with dead cells and other non-living soil fractions (Dick, 1994). It was confirmed that enzyme activities are a useful tool for the detection of pesticide side effects in soil and they can be used as indicators of soil pollution (Richardson et al., 2009; Hou et al., 2011).

Among the important soil enzymes, DHase that is linked with microbial respiratory processes. The activity of DHase in the soil system is an indicator of overall microbial activity of the soils (Bolton Jr *et al.*, 1985). POD (E.C. 1.11.1.7) is an important class of enzymes in soil and plant litter, as various types of PODs are released by fungi during the decomposition of lignin in soil organic matter. It is the antioxidant enzyme (Mi et al., 2014). PPO (EC 1.10.3.1.), plays an important role in respiration, catalyzing the aerobic oxidation of polyphenols and their derivatives (Rocha and De Morais, 2005). PPO is produced in almost all living organisms, including animals, plants and microorganisms (Tan *et al.,* 2010).

Various fumigants are being evaluated against pests to obtain the effective fumigant to become valuable replacements for methyl bromide. Phosphine (PH_3) is a colourless gas which is odourless when pure, but the technical product usually has a foul odour, described as "fishy" or "garlicky" (WHO, 1988). Aluminum, magnesium and zinc phosphides are the most commonly used metal phosphides. Metal phosphides hydrolyze in acids to yield phosphine (Union, 2012). The antimicrobial activity of phosphine varies depending on the microbial species (WHO, 1988). Formaldehyde (CH_2O) is uniquely important because of its widespread use and toxicity (Fagnani et al., 2003). Various approaches have been used to release the formaldehyde gas, which is usually generated by adding formalin to potassium permanganate (Mitchell et al., 2000). Paradichlorobenzene (1,4-dichlorobenzene, 1,4-D) is a chlorinated organic substance that, when exposed to air, 1,4-D slowly sublimates from a solid to a vapor. Dimethyl disulfide DMDS provides effective control of soil-borne pathogens and nematodes in the production of high-value crops (Dugravot et al., 2003; Gautier et al., 2008). Plant essential oils are concentrated volatile hydrophobic liquids extracted from different parts of the aromatic plants (Sharma et al., 2017). They are complex mixtures of volatile terpenoids, mainly monoterpenes and sesquiterpenes, and aromatic phenols (Figueiredo et al., 2008). Phytochemicals are considered to be relatively economical, non-hazardous and environmentally safe as they are biodegradable and have little or nil toxicity to non-target organisms (Chandel and Deepika, 2010; Javaid and Rauf, 2015).

The insecticidal activity evaluation of pesticides and other pest control agents have received a great deal of study. However, pesticide effects on soil enzymatic activities have so far received little attention (Omar *et al.,* 2001). Therefore, the aim of the study is to evaluate the side-effect of fumigants on the activities of plant enzymes (DHase, POD, PPO), to determine the potential of using the safe fumigant in IPM program.

MATERIAL AND METHODS

Tested chemicals

Fungicide: Thiophanate-methyl (Tobist)

A commercial fungicide Tobist 70% WP was tested as a standard fungicide, dimethyl 4,4′-(*o*-phenylene)bis(3-thioallophanate). It was obtained from the Egypt Chem Co., Egypt. Water solubility 18.5 mg/L at 20 °C, M.W. 342.

Fumigants

True fumigants

Dimethyl disulfide (DMDS): Composition technical is ≥ 99% pure. M.W. 94, B.P. 109.6°C, water solubility 2.7 g/L (20°C).

Fumigant generators

Phosphine (PH_3) is used_as a fumigant, M.W. 34, B.P. –87.4°C, water solubility 26 cm^3/100 mL at 17°C. It was generated from Aluminum phosphide (AlP), Phostoxin Tablets 55%. Phosphine also was generated from Zinc phosphide (Zn_3P_2), Powder 80%.

1,4-Dichlorobenzene (1,4-D): It is called Paradichlorobenzene, is an organic compound, M.W. 147 g/mol, B.P. 174°C. It turns directly from a solid into a gas.

Formaldehyde (HCHO): It was obtained as a product from the reaction between formalin 40% with $KMnO_4$. M.W. 30, B.P. -19.5 °C, water solubility 55% (30 °C).

Bio-fumigants (Essential oils)

Four essential oils were purchased from Pure Life Co., Cairo, Egypt. All samples were stored in hermetically sealed vials at 4 ^{0}C in the dark until use.

Oil of jojoba S*immondsia chinensis*, density 0.863-0.873 g/cm^3. Its composition in fatty acids is palmitic acid 35%, oleic acid 15%, gadolic acid 65-80%, erucic acid 10-20%.

Oil of garlic *Allium sativum*, density 0.863-0.873 g/cm^3. Its composition in fatty acids is palmitic acid 10-14%, oleic acid 20-30%, palmitoleic acid 1%, stearic acid 4%, linoleic acid 5-6%, arachidic acid 4%, linolenic acid 1%, behenic acid 3.5%, gadolic acid 1%, and erucic acid 1.5%.

Oil of argan *Argania spinose*, density 0.888-0.898 g/cm^3. Its composition in fatty acids is saturated fat is 10%, mono- unsaturated fat 73%, poly unsaturated fat 1%, fatty acids omega-6 3.6%.

Oil of orange *Citrus sinensis*, density 0.858-0.890 g/cm^3. Lemonene 93.7%, α-pinene 0.65%, sabinene and β-pinene 1%, myrecen 2.09%, octanal 0.41%, linalool 0.31%, δ-3-carene 0.31%, and dicanal 0.27%.

Tested soil

Soil samples were taken from the top 15 cm in a field at Agricultural Experiment Station, Faculty of Agriculture, Alexandria University. Soil samples have been no history of pesticide or fumigation treatment. The soil analysis indicated that the texture was sandy loam soil (30% clay, 44% silt, 26% sand), pH 8.1, EC 680 ppm, organic carbon content 0.92%, HCO_3^- 245 ppm and total cations 338 ppm. The soil samples were passed through a 4-mm sieve and stored at room temperature before being used in the experiments.

Soil treatment

The experiments were conducted using PVC jars 1L volume contained 250 g soil moistened with tap water to achieve 60% water holding capacity.

For soil fumigation method (Niu et al., 2013), small vials 10 mL volume containing the source of fumigants AlP, Zn_3P_2, formalin and $KMnO_4$ and 1,4-D were hanged into the closed jars. The soils were exposed to 150 mg PH_3/L from AlP or Zn_3P_2, and 500 mg/L of 1,4-D or formaldehyde.

For soil drench method, quantities of tested essential oils and DMDS were added to jars to obtain 2000 mg/kg soil and thiophanate-methyl at 200 mg/kg soil.

Also, the concentrations equivalent to EC_{50} values of different compounds that obtained from other experiments against *F. oxysporum* were tested (data un-published). The EC_{50} values were 5 mg/L for PH_3 from AlP or Zn_3P_2, 24 mg/L for 1,4-D, 39 mg/L for formaldehyde, 20 mg/kg for DMDS, and essential oil garlic at 52 mg/kg, jojoba at 43 mg/kg, citrus at 19 mg/kg, and argan at 39 mg/kg, and at 1 mg/kg for thiophanate-methyl. Jars of control were drenched with tap water. The treated jars were maintained for 28 days under 25°C. Three jars (as three replicates) were taken from each treatment at different time intervals 1, 4, 7, 14, 21, 28 days after treatment and the activity of the tested enzymes was determined.

Determination of DHase activity

The DHase activity in soil was determined calorimetrically by the reduction of 2,3,5-triphenyltetrazolium chloride (TTC, colorless) to triphenylformazan (TPF, red color) which is extracted by methanol and measured at 490 nm according to (Casida Jr et al., 1964; Tabatabai, 1994) with slightly modifications. At each sampling time, five grams of the treated soil sample were inserted into a test tube (10 mL capacity) and addition of 1 mL of 1% aqueous solution of TTC and 2 mL of distilled water. The tubes were tightly covered with parafilm paper to ensure anaerobic condition and then incubated in the dark at 37°C for 24 h. After incubation, the TPF produced was extracted by using 4 mL of methanol for each tube and the contents were shaken vigorously, stirred for one minute and filtered through Whatman filter paper No. 1. Extraction was repeated three times and the extracts were combined together. The absorbance of TPF in the filtrate was determined at 490 nm.

Determination of POD activity

Sample suspensions were prepared by adding 1 g of soil to 5 ml of 50 mM acetate buffer, pH 5.8. Suspension was homogenized with a Vortex Mixer for 5 minutes. Enzyme activities were measured by combining 2 mL of soil suspension with 2 mL of catechol solution (5 mM) at room temperature for 1h. Each sample, including controls, received 0.2 mL of 0.3% hydrogen peroxide. After centrifugation at 10000 rpm for 30 minutes. The supernatants were determined spectrophotometrically at 460 nm (Bach, 2013).

Determination of PPO activity

The reaction mixture consisted of 5 mL of 50 mM acetate buffer, pH 5.8 and 2 mL of the soil suspension. Then, 2 mL catechol solution (5 mM) was added to start the reaction. The developing color was measured at 412 nm (Burns *et al.,* 2013).

Statistical analysis

The statistical analysis was performed using the SAS software (SAS Institute Inc., Cary, NC, USA). One-way analysis of variance (ANOVA) was used to analysis the data. The Least Significant Difference (LSD) test at $P \leq 0.05$ was used for mean comparison. Also, data were analyzed by Finny test to obtain the EC_{50} (Finney, 1971).

RESULTS AND DISCUSSION

Effect of tested fumigants on DHase activity

As seen in Table (1), the two essential oils garlic and jojoba at 2000 mg/kg soil have a similar influence on soil DHase activity, exerting an increase about of 30% at 14th day after which the DHase begins to decrease to reach 100% activity. At the same time a positive effect can be observed between the influence of citrus and argan oils on DHase activity at 1st day and 14th day after treatment. About 11% and 29% positive influence on DHase activity after application of thiophanate-methyl fungicide at recommended dose was observed at 1st and 14th day. DMDS at 2000 mg/kg soil inhibited DHase activity to about 10% in the first week of treatment, and then the activity increased by 20% in the second week, after which the activity was remained at the normal level until 28th day. Regarding fumigant generators, data presented in Table (1) showed that the activity of DHase depressed to 75% at 4th day while increased to 119% at 14th day affected by phosphine at 150 mg/L of space. The fumigant 1,4-D did not effect on the DHase activity through the first week, while after that it enhanced the activity to 124% after 14 days and to 110% after 21 and 28 days. DHase activity was increased in formaldehyde treatment, the increase of activity was of 29% at 1st day, 14% at 4th day, 27% at 14th day and 20% after three and four weeks from treatment. The DHase activity found to be constant after three and four weeks for all fumigant treatments Table (1). It was observed that the responses of DHase to fumigant types, exposure time, and concentration had different patterns.

The effect of essential oils on the DHase activity, Figure (1) showed that, in the first week, the activity of DHase in the treatment of garlic and jojoba oil decreased of 10%. After two weeks, the activity of DHase significantly increased then, decreased with the increases of exposure time. The DHase activities were high at the first day, about 17 and 30% and at 14th day, about 29 and 12% then, to be in the range of 5-10% up for the 28 days in treatments of citrus and argan oil, respectively. DMDS incorporated with the soil slightly induced the activity of DHase at the first day while it reduced the activity of 10% within the first week followed by inducing to 117.62% at 14th day. Also, thiophanate-methyl induced DHase activity within 28 days after soil treatment, the range of induction was 2.5-46.0% (Figure 1).

Table 1. Effect of tested fumigants on soil dehydrogenase activity over 28 days

Dehydrogenase activity % (as control activity 100%)								
Treatment		Concentration (mg/kg) or (mg/L)	Time (day) 1	4	7	14	21	28
Soil drench method								
Bio-fumigants (Essential oils)	Garlic	2000	102.19^{c}	96.86^{c}	101.92^{a}	129.66^{c}	99.76^{d}	99.76^{d}
	Jojoba	2000	106.31^{b}	95.02^{d}	100.36^{b}	130.47^{b}	102.74^{b}	101.74^{b}
	Citrus	2000	125.47^{a}	99.44^{c}	106.05^{a}	137.8^{a}	109.76^{b}	109.76^{b}
	Argan	2000	130.89^{a}	102.21^{b}	97.57^{c}	119.04^{d}	111.95^{a}	111.95^{b}
True	DMDS	2000	90.31^{c}	93.36^{b}	92.43^{c}	120.16^{d}	99.08^{d}	99.14^{d}
Thiophanate-methyl		200	111.48^{b}	101.98^{c}	99.56^{b}	129.43^{d}	104.76^{c}	104.76^{c}
Mini-atmosphere method								
Fumigant generators	Zn_3P_2	150	106.00^{b}	75.34^{f}	97.73^{bc}	119.25^{d}	95.70^{e}	95.68^{e}
	AlP	150	104.00^{b}	79.84^{f}	101.75^{ab}	118.28^{d}	101.72^{c}	101.72^{c}
	1,4-D	500	105.03^{b}	101.22^{c}	103.34^{ab}	124.34^{c}	110.15^{b}	110.15^{b}
	Formaldehyde	500	129.42^{a}	114.54^{a}	105.44^{a}	127.07^{d}	120.62^{a}	120.62^{a}

Means in the column followed by different letters indicate significant differences among treatments at $P \leq 0.05$
Concentration is (mg/kg) for essential oils and DMDS, concentration is (mg/L) for fumigant generators

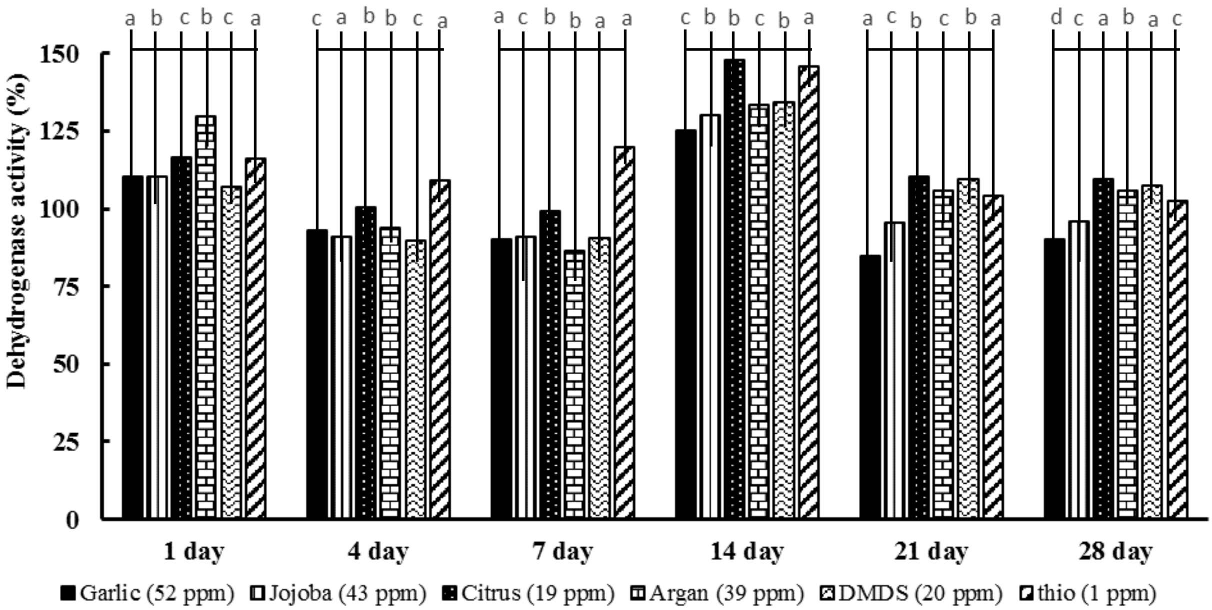

Fig. 1. Effect of essential oils and DMDS at their EC_{50} concentrations on dehydrogenase activity using soil drench method

The columns in each time interval followed by different letters indicate significant differences among treatments at $P \leq 0.05$

As shown in Figure (2), in the soil exposed to phosphine at its EC_{50}, the activity of DHase was remained constant up for the first week after that, the activity was increased to 37.5% on day 14 then, it slightly decreased compared to the DHase activity in the control. The activity of DHase was slightly inhibited with 1,4-D in the first four days then, increased at 7th day to about 119% after that, it gradually decreased up to about 109% in the second, third and fourth week after exposure. Formaldehyde enhanced the activity of DHase, the enhancement ranged 8-26% throughout 28 days except at 4th day the activity was about 93% compared to the control.

DHase enzyme is considered to play a significant role in the biological oxidation of soil organic matter by donating hydrogen from organic substrates to inorganic acceptors (Zhang et al., 2021; Moeskops et al., 2010). Also, the DHase activity is considered to be an indicator of the oxidative metabolism in soils and thus of the microbial activity (Trevors, 1984). Our results showed that fumigants tested can reduce soil DHase activity, but this effect is transient. This can be attributed to the ability of microorganisms to overcome the stress caused by the chemicals by developing the capability to utilize chemicals as a nutrient source and proliferating in such an environment (Vasic et al., 2022).

Several environmental factors, including heavy metal contamination and soil fertilization or pesticide use can affect significantly DHase in the soil environment (Wolińska and Stępniewska, 2012). DHase activity proved to be a good indicator of the degree of soil contamination with agrochemicals. Our result is in agreement with results obtained from various studies that indicated the activity of DHase significantly increased with the increases of exposure time (Liu et al., 2006; Niu et al., 2013). In addition, various essential oils proved to be harmless for the soil enzymatic. Overall, this observation provides solid bases for the development of novel biopesticides for sustainable crop protection (Campolo et al., 2020).

Effect of tested fumigants on POD activity

The results showed that phosphine produced from AlP and Zn_3P_2 at concentration of 150 mg/L space, exhibited an obvious inhibitory effect on POD activity over the first four days after exposure. The inhibition percentage was about 25% within the first four days and about 10% after three weeks. Also, the results indicated that 1,4-D and formaldehyde at 500 mg/L space, and thiophanate-methyl at 200 mg/kg soil inhibited the POD enzyme within the first four days and at 21st day after treatment, the inhibition percentage ranged from 8 to 25% (Table 2). The activity of POD was reduced within the first four days of 4-42% with all treatments except garlic and jojoba oil that enhanced the POD activity in the range of 4-16%. Garlic oil at 2000 mg/kg soil decreased the activity of POD at 14th day to 81.72% and at 21st day to 87.90%. The POD activity was depressed in the treatment of citrus oil at 2000 mg/kg soil within the first week and the activity was recovered to the normal level until 28 days. Also, data in (Table 2) indicated that argan oil (2000 mg/kg soil) increased the POD activity to 129.28% at 1st day then it decreased the activity to 93.80, 90.18, and 83.40% at 4th, 7th, and 14th day, respectively. The results revealed that no significantly negative effect on the activity of POD at 7th day was detected in all treatments. Also, no significantly inhibition of POD enzyme was recorded at 28th day after treatment by all fumigants while, about 5% inhibition was produced at the same time in the treatment of thiophanate-methyl.

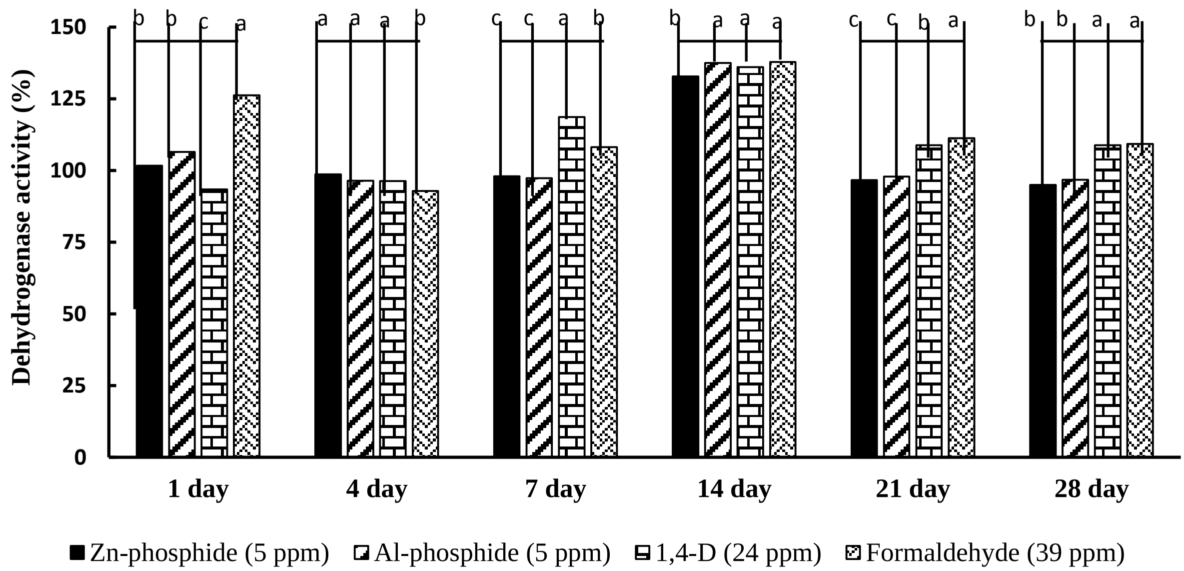

Fig. 2. Effect of fumigant generators at their EC_{50} on dehydrogenase activity using soil fumigation method
The columns in each time interval followed by different letters indicate significant differences among treatments at $P \leq 0.05$

Table 2. Effect of tested fumigants on soil peroxidase activity over 28 days

Peroxidase activity % (as control activity 100%)								
Treatment		**Concentration (mg/kg) or (mg/L)**	**Time (day)**					
			1	**4**	**7**	**14**	**21**	**28**
Soil drench method								
Bio-fumigants (Essential oils)	Garlic	2000	116.98^{a}	98.73^{a}	98.42^{ab}	81.72^{b}	87.9^{ab}	103.9^{a}
	Jojoba	2000	107.4^{a}	104.55^{a}	101.29^{ab}	94.03^{a}	99.04^{a}	103.89^{a}
	Citrus	2000	96.48^{b}	82.31^{a}	104.65^{ab}	99.08^{a}	104.41^{a}	105.74^{a}
	Argan	2000	129.28^{a}	93.80^{a}	90.18^{b}	83.40^{b}	102.4^{a}	99.18^{a}
True fumigant	DMDS	2000	58.11^{c}	96.09^{a}	108.05^{a}	107.39^{a}	104.95^{a}	98.76^{a}
Thiophanate-methyl		200	82.41^{c}	75.52^{c}	113.51^{a}	112.57^{a}	84.16^{ab}	94.60^{b}
Mini-atmosphere method								
Fumigant generators	Zn_3P_2	150	78.48^{b}	71.44^{c}	101.29^{ab}	95.51^{a}	88.62^{ab}	105.10^{a}
	AlP	150	74.05^{b}	75.4^{c}	103.85^{a}	100.24^{a}	91.09^{ab}	105.56^{a}
	1,4-D	500	81.44^{b}	76.52c^{c}	115.17^{ab}	111.88^{a}	77.94^{b}	100.66^{a}
	Formaldehyde	500	79.09^{c}	90.25^{b}	101.01^{b}	107.08^{a}	92.00^{ab}	112.40^{a}

Means in the column followed by different letters indicate significant differences among treatments at $P \leq 0.05$
Concentration is (mg/kg) for essential oils and DMDS, concentration is (mg/L) for fumigant generators

On the other hand, the effect of fumigants at their EC_{50} concentrations on the activity of soil POD showed in Figure (3 and 4). The enzyme activities were inhibited by all the treatments in different degrees at 4th day except garlic oil (52 mg/kg soil) and phosphine (5 mg/L space) that did not affect on the POD activity at this time. The activities of the enzyme increased with all treatments from 107.07% to 138.13% compared to the control on 1st day. The statistical analysis revealed that the lowest enhancement of POD activity was produced by jojoba followed by phosphine while, the highest enhancement of the enzyme was obtained from treatment of DMDS followed by thiophanate-methyl. At concentration of 5 mg/L space of phosphine treatment, resulted in an increase in the POD activity to the maximum level, which was then maintained at a steady level.

At 7th day, all treatments at their EC_{50} concentrations, stimulated the POD activity, the higher activity was 117.76% from argan treatment while the lower activity was 108.57% from phosphine. On experimental day 14, however, only 1,4-D slightly reduced the enzyme activity of 5%. The POD activity found to be stimulated in different degrees after three and four weeks for all fumigant treatments except jojoba that inhibited the POD enzyme at the same time (Figure 3 and 4).

It was observed that POD activity in soil has been measured in a small number of studies (Bach, 2013). Regarding the POD tested and the applied fumigant concentration, our results exhibited that the effects on soil enzymes activities can be positive or negative. Consequently, the effect of fumigants could be due to many factors among them the chemical structure as well as their concentrations. This result is in agreement with previous studies (Jastrzębska, 2011; Micuti et al., 2018). Phosphine caused a general increase in POD activity in response to both phosphine concentration and the exposure time. This result supported, phosphine treatment caused an increase in the POD activity to the maximum level, which was then maintained at a steady level (Mi et al., 2014).

In our study, it was found that the activity of POD has been recovered from the inhibitory or stimulative effect and reach the level of the enzymatic activity of a control soil sample after 28 days. This result harmony with results of previous studies that found that the POD activity, analyzed after agrochemical pesticide treatments for 28 days, no significant differences were obtained compared to control (Shiyin et al., 2004). The xenobiotics in soil could affect on the activity of soil enzymes, in contrast POD can oxidize the toxic substances (Erman and Vitello, 2002). Consequently, POD has an important role in the removal of toxic substances from the environment.

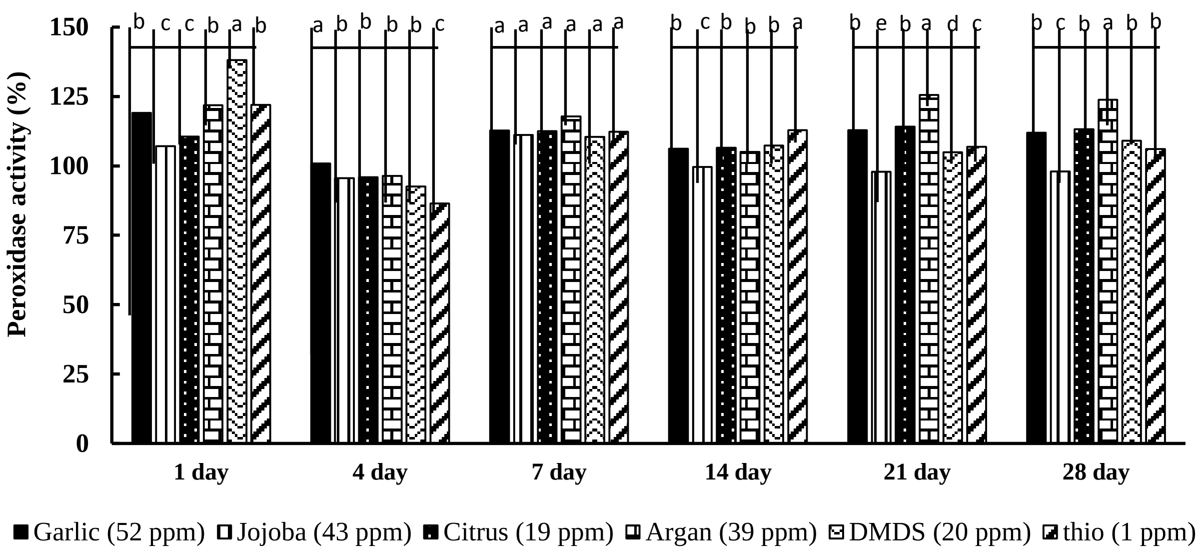

Fig. 3. Effect of essential oils and DMDS at their EC_{50} concentrations on peroxidase activity using soil drench method

The columns in each time interval followed by different letters indicate significant differences among treatments at $P \leq 0.05$

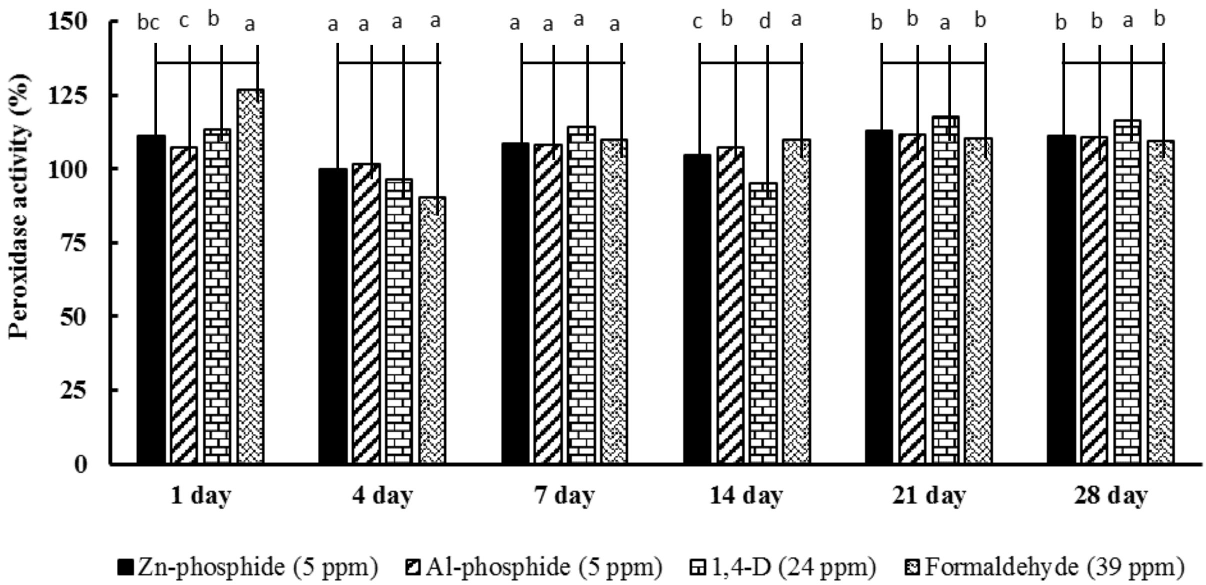

Fig. 4. Effect of fumigant generators at their EC_{50} on peroxidase activity using soil fumigation method
The columns in each time interval followed by different letters indicate significant differences among treatments at $P \leq 0.05$

Effect of tested fumigants on PPO activity

Our study showed different trends for the PPO activities investigated for different treatments and at different sampling times. As seen in Table (3), the essential oils (garlic, jojoba, citrus, and argan) with concentration of 2000 mg/kg soil, had a similar influence on soil PPO activity, exerting an increase of 6, 14, 24 and 21%, respectively at 1st day and an increase of 32, 34, 47 and 2%, respectively at 21st day after soil treatment. At 14th day, garlic oil decreased the activity of PPO to 92.5%, in contrast; jojoba oil stimulated the activity to 124.4%. Also, the results showed that DMDS (2000 mg/kg soil) significantly depressed the PPO activity at all time intervals except at 7th day. In addition, PPO activity was decreased in treatment of formaldehyde over the interval of experiment except at day 7 and 28. The reduction percentages were 25, 19, respectively at 1st and 4th day, the inhibition of the enzyme was < 11% during the second, third and fourth week. The activity of PPO was decreased in treatment of thiophanate-methyl over the interval of experiment except at 7th day.

Data presented in Table (3) revealed that both fumigants phosphine and 1,4-D reduced activity of PPO over the first four days then the activity was recovered to the normal level that similar to its level in control sample until 28 days. Statistical analysis showed that at 1st day, all treatments reduced the activity of PPO except the essential oils, also at 4th day all treatments decreased the activity except garlic oil. In addition, at 7th day, the response of PPO activity to the fumigants was vary, ranged of ± 10%.

Furthermore, data presented in Figure (5 and 6) exhibited the effect of fumigants at their EC_{50} concentrations on the activity of soil PPO. It was showed that the effect of fumigants investigated on PPO activity can be positive or negative. The results revealed that at 1st day, all treatments significantly stimulated the activity of PPO except garlic oil at concentration of 52 mg/kg soil that did not effect. The activity of PPO was affected by all treatments at 7th and 14th day; the varying in the activity was ±10%. The enzyme activity was reduced of 6% with citrus and argan oil at 21st day. Regarding thiophante-methyl treatment, the PPO was increased over the first four days then the activity was reduced over the following 10 days, after that the activity of PPO returned to normal level with slight increase of 4% until 28th day. Moreover, it was observed that the same trend of the effect of phosphine was obtained from either aluminum phosphide or zinc phosphide (Figure 6).

Our results are in close agreement to earlier studies by a number of workers, where it has been shown that depending on the chemical structure and concentration, the use of agrochemicals can provide various results (Jastrzębska, 2011; Micuti et al., 2018). PPO plays an important role in respiration, and its activity is high in cropland (Khosrozadeh et al., 2022). Atrazine significantly affected the activity of PPO in the soil at different periods (Yang et al., 2021). Hence, their activities may be a useful indicator for evaluating soil quality and health (Floch et al., 2007).

In general, the significant treatment by time interactions for the activities of soil enzymes (DHase, POD, and PPO) indicates that responses are not consistent over time; this suggests differently functioning or structured microbial communities (Papatheodorou et al., 2014). Overall, all enzymes activity was affected, positively or negatively, depending on the exposure time. Furthermore, the test of fumigants can provide various results, depending on the chemical structure of fumigant and concentration of fumigant (Jastrzębska, 2011).

Table 3. Effect of tested fumigants on soil polyphenol oxidase activity over 28 days

Polyphenol oxidase activity % (as control activity 100%)								
Treatment		**Concentration (mg/kg) or (mg/L)**	**Time (day)**					
			1	**4**	**7**	**14**	**21**	**28**
Soil drench method								
Bio-fumigants (Essential oils)	Garlic	2000	106.06^{a}	104.1^{a}	99.89^{b}	92.50^{b}	132.10^{b}	102.01^{ab}
	Jojoba	2000	114.18^{a}	97.42^{a}	100.75^{b}	124.44^{a}	134.90^{b}	100.76^{b}
	Citrus	2000	124.31^{a}	98.94^{a}	105.33^{a}	102.30^{b}	147.82^{a}	101.83^{ab}
	Argan	2000	121.48^{a}	89.2^{a}	103.24^{b}	111.79^{a}	102.01^{a}	104.65^{ab}
True	DMDS	2000	75.36^{b}	81.79^{b}	109.27^{a}	96.78^{a}	98.80^{cde}	89.40^{b}
Thiophanate-methyl		200	60.12^{c}	77.45^{b}	102.33^{b}	98.98^{b}	91.6^{e}	97.73^{b}
Mini-atmosphere method								
Fumigant generators	Zn_3P_2	150	79.38^{b}	88.50^{a}	104.07^{b}	102.26^{a}	98.26$_{cde}$	98.46^{b}
	AlP	150	77.36^{bc}	89.22^{a}	102.93^{b}	102.61^{a}	96.96^{cde}	104.79^{ab}
	1,4-D	500	66.99^{c}	77.62^{b}	104.19^{b}	103.40^{a}	96.18^{cde}	101.17ab
	Formaldehyde	500	75.60^{b}	79.83^{b}	104.66^{b}	95.29^{b}	96.29^{cde}	110.71^{a}

Means in the column followed by different letters indicate significant differences among treatments at $P \leq 0.05$
Concentration is (mg/kg) for essential oils and DMDS, concentration is (mg/L) for fumigant generators

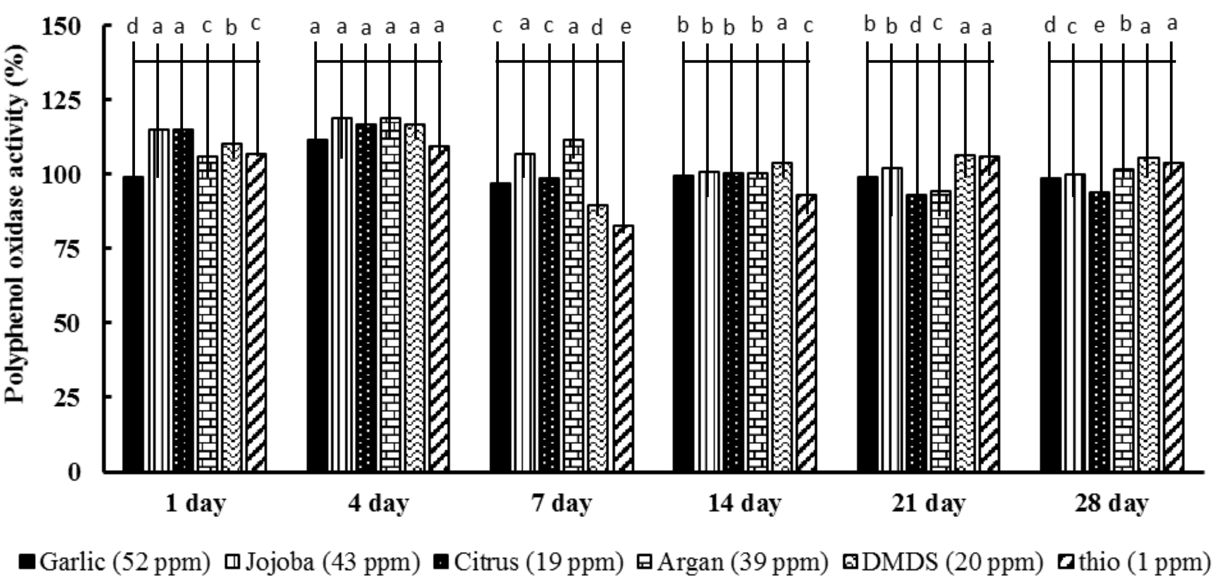

Fig. 5. Effect of essential oils and DMDS at their EC_{50} concentrations on polyphenol oxidase activity using soil drench method

The columns in each time interval followed by different letters indicate significant differences among treatments at $P \leq 0.05$

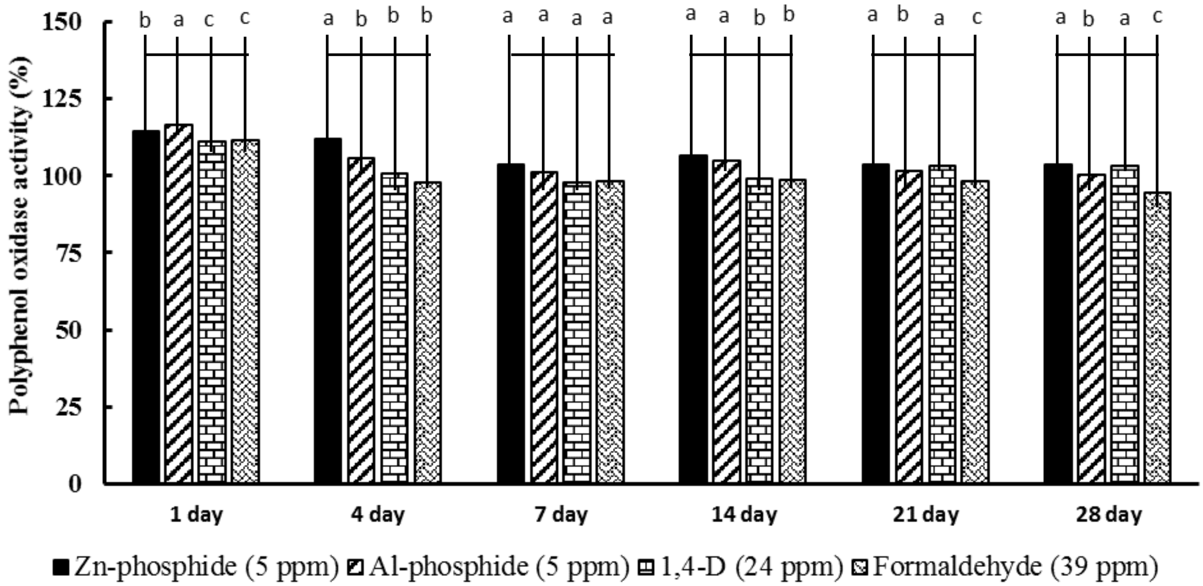

Fig. 6. Effect of fumigant generators at their EC_{50} on polyphenol oxidase activity using soil fumigation method
The columns in each time interval followed by different letters indicate significant differences among treatments at $P \leq 0.05$

CONCLUSION

It must never be that the effects on soil enzymes activities can be positive or negative and had different patterns. Consequently, the effect of fumigants could be due to the fumigant type, chemical structure, concentration as well as exposure time. The activities of DHase, POD, and PPO found to be sensetive to fumigants, proved to be a good indicator of the degree of soil contamination with tested fumigants. Various essential oils proved to be harmless or very low effect for the soil enzymes. Overall, this observation provides general bases for the development of novel biopesticides for sustainable crop protection. Also, the inhibitory effect by 1,4-D was negligible. Almost the same trend of the effect of phosphine was obtained from either aluminum phosphide or zinc phosphide. Phosphine at its EC_{50} (5 mg/L) caused an increase in the DHase, POD and PPO activities to the maximum level, which was then maintained at a steady level while, at 150 mg/L, it caused inhibition approximately of 15%. DMDS in 2000 and 20 mg/kg soil inhibited soil enzymes approximately at all time intervals, then reach the level of the enzymatic activity of a control soil sample after 28 days. In general, the activity of soil enzymes has been recovered from the inhibitory or stimulative effect and reach the level of the enzymatic activity of a control soil sample after 28 days. Although the important use of alternative that safe remedies, the effect of tested fumigants on DHase, POD, and PPO never must be great concern, where the effects are transient. The further study must be carried out about the effect of pesticides and fumigants on spectrum of soil enzymes particularly on the long-term as well as on the specific microorganisms in soil.

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الملخص العربي

الاثر الجانبى لمدخنات التربة ومعاملة التربة ببعض بدائل بروميد الميثيل على ثلاث من انزيمات التربة

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توجد حاجة ملحة لإيجاد بدائل للمبيدات تكون أكثر أمانًا وصديقة للبيئة فضلاً عن كونها تكون ذات فعالية عالية على الآفات. تم دراسة تأثير بعض المدخنات على نشاط إنزيم ديهيدروجينيز والبيروكسيديز والبولى فينول أوكسيديز في التربة. تم اختبار ثلاثة أنواع من مواد التدخين هى: المدخن الحقيقي ثنائي ميثيل ثاني كبريتيد (DMDS)، ومولدات المدخنات ومنها غاز الفوسفين (PH_3) الناتج من فوسفيد الألومنيوم وفوسفيد الزنك، ثنائي كلورو بنزين (1,4-D)، والفورمالديهايد (CH_2O)؛ والمخنات الحيوية وهي الزيوت الطيارة لكل من الثوم والجوجوبا والموالح والأرجان. تم استخدام طريقة مصغرة لتدخين التربة لمولدات المدخنات وطريقة الخلط مع التربة للمدخنات الحقيقية والحيوية. أظهرت النتائج أن DMDS بتركيز ٢٠٠٠ و ٢٠ مجم/كيلو جرام ثبط تقريبًا إنزيمات التربة في جميع الفترات الزمنية، وبعد ذلك ارتفع النشاط ليصل إلى مستوى النشاط الأنزيمي للكنترول خلال أول أربعة أيام. وأوضحت النتائج أن تأثير الفوسفين كان له نفس التأثير تقريبًا بغض النظر عن مصدره فوسفيد الألومنيوم أو فوسفيد الزنك. وقد سبب الفوسفين عند ٥ مجم/لتر فراغ في زيادة نشاط انزيم ديهيدروجينيز والبيروكسيديز والبولى فينول أوكسيديز بينما تسبب عند تركيز ١٥٠ مجم/لتر فراغ في تثبيط هذه الانزيمات بنسبة حوالى ٢٥٪. أثبتت النتائج المتحصل عليها أن الزيوت الطيارة المختبرة غير ضارة أو ذات تأثير ضئيل للغاية على إنزيمات التربة. وبالنظر الى التاثير العام لكل مواد التدخين يتضح أن أقصى نسبة تحفيز لانزيم ديهيدروجينيز كانت 37.5٪ وأقصى نسبة تثبيط كانت 25٪. وأيضًا كان الحد الأقصى للزيادة فى نشاط انزيم البيروكسيديز 30٪ بينما كان الحد الأقصى للانخفاض في نشاط البيروكسيديز 42٪. بالإضافة إلى ذلك، كانت أعلى نسبة تحفيز ونسبة تثبيط فى انزيم بولى فينول أوكسيديز هى 48٪ و40٪ بالترتيب. ان نشاط انزيمات ديهيدروجينيز والبيروكسيديز والبولى فينول أوكسيديز والتى كانت حساسة لمواد التدخين تعتبر مؤشر جيد على درجة تلوث التربة بمواد التدخين المختبرة. ويمكن القول أن انخفاض نشاط هذه الانزيمات بعد المعاملة يجب ان لا يمثل قلق كبير حيث يحدث اعادة لمستوى النشاط الطبيعى مع الوقت الا أنه تظل هناك ضرورة للبحث عن بدائل آمنة على البيئة بكل مكوناتها.