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The antinematicidal activity of vitamin E and its derivatives on *Sterinernema feltieae*

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Abstract. Vitamin E is found naturally in some foods, and also available as a dietary supplement. "Vitamin E" is the fat-soluble compounds with distinctive antioxidant activities. The vitamin E acts as an antioxidant, which protects cell membranes. *Sterinernema feltieae* was selected as a test organism is because this organism is a complex organism and can be used as a model for whole organisms. The objective of this study is to evaluate the activity of vitamin E and its derivatives on *Sterinernema feltieae.* The results showed that the derivative of vitamin E ($C_{37}H_{55}NO_3$) was most active against *S. feltiae* with the LD_{50} value was 207.77 µM, followed by vitamin E ($C_{29}H_{50}O_2$) with the LD_{50} value was 209.09 µM. These results indicated that derivative of vitamin E ($C_{37}H_{55}NO_3$) exerts more potent toxicity than vitamin E against *Sterinernema feltieae.*

Keywords: Vitamin E, derivatives of vitamin E, thiabendazole, hydrogen peroxide, *Steinernema feltiae*.

INTRODUCTION

Entomopathogenic nematodes (EPNs) are deadly parasitic worms for insects. EPNs are a family of *Steinernematidae* and *Heterorhabditidae*. These species are infective juveniles (IJs) that carry symbiotic bacteria of the genus *Xenorhabdus* and *Photorhabdus*, respectively. EPNs are generally used as an alternative to pest management research in the area of biological control. EPNs are widely used by researchers to determine the activity of a drug compound because these animals represent high-level organisms. (higher organisms) [1].

An antioxidant is a compound that is significantly capable of reducing or inhibiting the oxidation of a

substrate, both enzymatically and non-enzymatic. Antioxidants have the ability to terminate the chain oxidation reaction, which produces reactive oxygen species (ROS), by inhibiting free radicals, and other oxidative reactions by oxidizing itself. There are some differences in the oxidation system of an antioxidant depending on the properties, solubility, and source of the antioxidants. Antioxidants include enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT), vitamins (such as vitamins A, C, E), herbal antioxidants (such as gingo biloba, turmeric, and garlic), phytochemical antioxidant (caretonoids and flavonoids), mineral antioxidant (zinc and selenium), and hormone anti-oxidants (melatonin) [2].

Vitamin E is generally synthesized by plants and obtained from a variety of food sources. (dietary sources). Sources of vitamin E are mostly found in nuts, spinach, seeds, olive oil, and sunflower oil. There are several types of vitamin E, i.e. α-,β-,γ-, and σ-tocopherols, tocotrienol. While α-tocoferol (α-Toc) is the most abundant vitamin E derivative in human tissues and sera. Vitamin E is included in the compounds tocopherols and tocotrienols. Vitamin E, also called α-tocopferol, is biologically active in the form of Vitamin E, and its forms are very varied and widely found in nature, such as in wheat oil, and sunflower oil. Vitamin E have been extensively studied for their biological properties. Vitamin E natural isoforms have been shown to act in a preventive and therapeutic manner against several types of cancer and are still being widely investigated for their potential efficacy [3]. The structure of vitamin E and its derivatives as well as thiabendazole as a positive control can be seen in **Figure 1**.

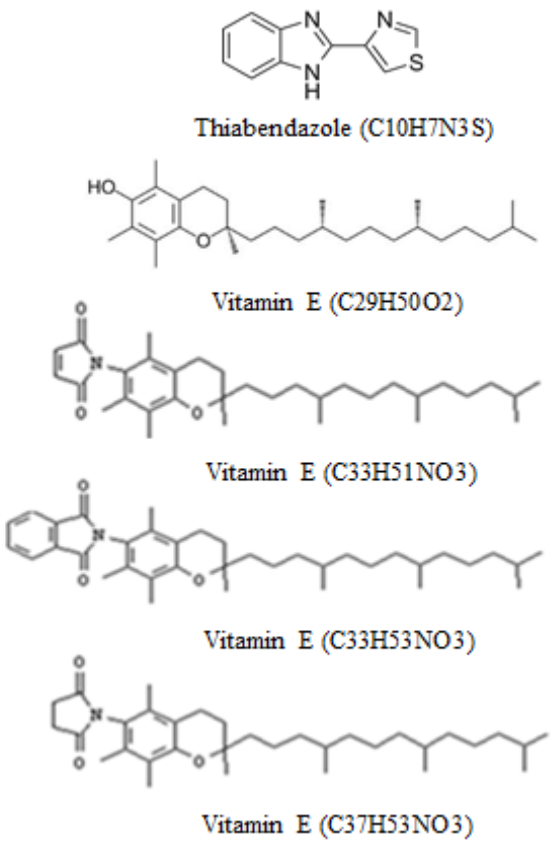

Figure 1. The structure of thiabendazole, vitamin E and derivatives of vitamin E.

Vitamin E has several biological functions, and the most common is as an antioxidant [4]. As an antioxidant, vitamin E acts as a peroxyl radical scavenger, i.e. prevents the production of peroxyl radicals in cell tissues, by forming tocopheryl, which will subsequently be reduced by the presence of a donor of hydrogen atoms [5]. Vitamin E is fat-soluble, and binds into membrane cells, thus protecting cells from oxidative damage. As a radical scavanger, vitamin E plays a role in preventing the propagation of free radical reactions [6].

Steinernema feltiae (Steinernematidae) is an entomopathogenic nematode that is fatogenic to plant pests [7]. *Steinernema feltiae* (*S. feltiae*) (**Figure 3**) or also called microscopic worms because they can only be seen under a microscope and are also classified in entomopathogenic nematodes (EPNs) because their ability as a natural insecticide. The farmers abroad use these worms as predators (biological control) for pests because the worms have the ability to infect host insects so they are called endoparasite worms [8, 9]. These nematodes, symbiotically associated with enterobacteria of the species *Xenorhabdus* [10, 11]. In addition to being used as a biological control, *S. feltiae* can also be used as an organism model in many laboratory studies.

The study aims to evaluate the toxicity of vitamin E derivatives on *S. feltiae* nematodes. In this study, *S. feltiae* was incubated with vitamin E derivatives at various concentrations to determine the viability of the nematode against the test compound. The viability of the worms was further observed under a microscope and the LD_{50} value was further determined. In addition, this study also determined the viability of *S. feltiae* nematodes against hydrogen peroxide (H_2O_2).

METHODOLOGY

The materials used in this investigation were 10 mL falcon tube, 1 mL Eppendorf tube, 96-well culture flask (Greiner bio-one, Cellstar), microscope with four-fold magnification (VWR International, Belgium), volume pipette, tube heater plate and manual counter (manual counter). Meanwhile, the substances used in the study are dimethyl sulfoxide (DMSO), vitamin E derivatives, thiabendazole (TBZ), hydrogen peroxide (H_2O_2) and distilled water.

Nematisidal Assay on *Steinernema feltiae*

The *S. feltiae* nematode used in this study in the form of soft cakes came from Sautter & Stepper Inc. Before use, the nematodes were stored at temperatures below 12°C. The *S. feltiae* suspension solution is prepared by mixing 200 mg of *S. feltiae* cake powder with 50 ml of distilled water.

Sample Preparations

For nematode testing, dissolve 200 mg of *S. feltiae* soft cake in 50 ml of distilled water to form a suspension solution. Before use, the solution of this suspension is then inhaled for 10-15 minutes at room temperature for the adaptation of *S. feltiae* to its environment. For activity testing, the *S.feltiae* suspension solution, dissolved in the sample based on concentration variations. The final concentrations for testing sample activity were 50, 100, 200, and 400 µM. For negative control, 100 µL of worm suspension solution is inserted into one sterile well-plate (96 well plate flat bottom tissue cell culture) [12]. For each concentration made three repetitions, and for each repetition, made three samples, and each sample contained 100 µL. Living and dead nematodes are counted immediately after exposure to the sample using a four-fold magnification microscope. (VWR International, Belgium). They then analyzed the percentage viability of the nematode against the test sample at the time of 0 hours of incubation. As soon as the percent viability at 0 hours is calculated, the test plate is closed and incubated at room temperature in a closed room for 24 h. Then the counting is done again after 24 h of exposure. The design of the nematicidal assay design can be seen in **Figure 2**.

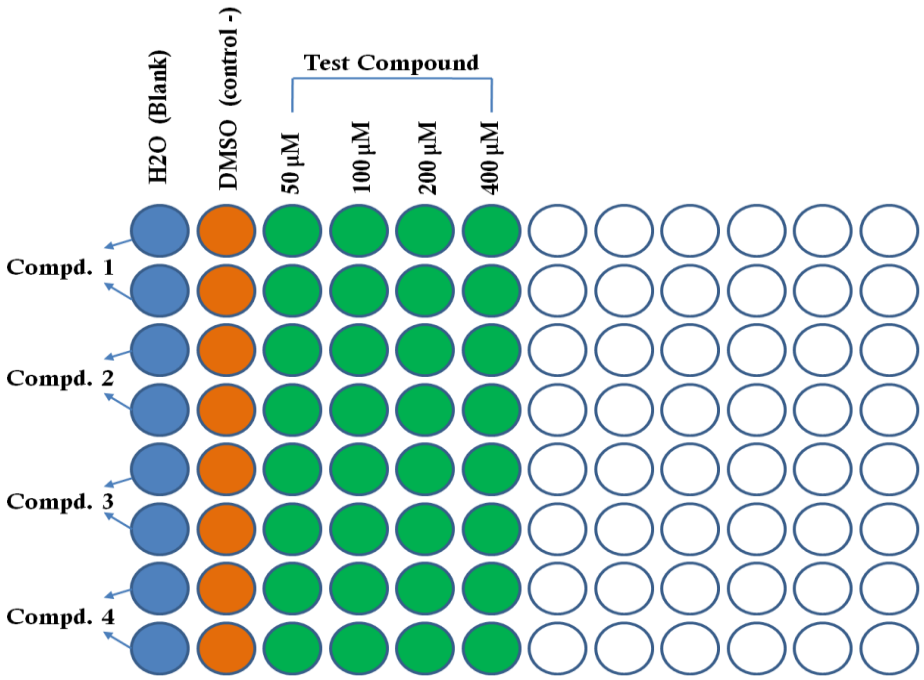

Figure 2. Nematicidal assay by using 96 well plate flat bottom tissue cell culture.

The Viability of *Steinerma feltiae*

The viability of the nematode produced at each concentration of the compound, is expressed as a percentage of viability (%). A viability value is calculated on the basis of the number of nematodes that survive after 24 h of exposure, indicated by W_{24h}, whereas the numbers of nematodes that die at initial exposure (0h), are expressed by W_{0h}.:

$$Viability\ (\%) = \frac{W_{24\,h}}{W_{0\,h}} x100$$

RESULTS AND DISCUSSION

The toxicity of the hydrogen peroxide (H_2O_2), TBZ, vitamin E and derivatives of vitamin E against *S. feltiae* was evaluated at several concentrations ranging from 50 µM to 400 µM. In this study, TBZ was used as a positive control. In this study, the LD_{50} value was also determined after 24 hours of incubation against *S. feltiae*. The viability of *S. feltiae* were observed under a microscope with four-fold magnification (**Figure 3**).

Figure 3. The viability of *S. feltiae* were observed under a microscope with four-fold magnification.

The Antinematicidal Activity of Hydrogen Peroxide and Thiabendazole

The toxicity of hydrogen peroxide and thiabendazole on *S. feltiae* was determined at 0; 4; and 24 h at room temperature. These results can provide very important information about the oxidative damage of hydrogen peroxide on the vitality of *S. feltiae*. Hydrogen peroxide is an oxidative stress compound that can inhibit the viability of nematodes. The results show that hydrogen peroxide compounds at a concentration of 80 µM with a 24 h incubation time produce highly toxic activity with a viability percentage of 12%. Whereas at a 20 µM concentration with a 24 h incubatory time produces 52% (**Figure 4**).

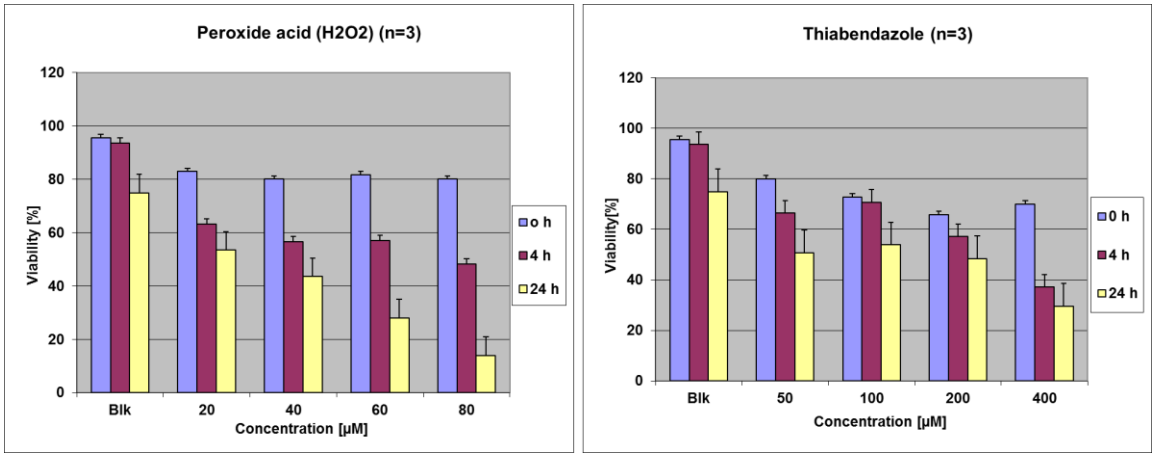

Figure 4. The antinematicidal activity of hydrogen peroxide and thiabendazole compounds against *S. feltiae*. DMSO was used as a blank (blk).

In this study, thiabendazole (TBZ) was used as a positive control. TBZ is an anti-parasitic compound and has nematisidal properties against roundworms worms (causes strongyloidiasis), hookworm worms, and other species of worms [12, 13]. Similarly with hydrogen peroxide (H_2O_2), TBZ also showed effects on *S. feltiae* and the percentage viability of *S. feltiae* was tends to decrease with increasing TBZ concentrations. The results showed that TBZ at concentration of 400 µM has a highest toxicity value against *S.feltiae* with the percentage viability of 30%. The percentage of viability of the compound hydrogen peroxide (H_2O_2) and positive control TBZ can be seen in **Figure 4**.

The Antinematicidal Activity of Vitamin E and Derivatives of Vitamin E

Studies have been conducted on the antinematicidal activity of vitamins E and its derivatives against *S. feltiae*. In this study, before analysis the nematodes (4 and 24 h), 50 µL of warm water was added to each well to make sure the worms were still alive or dead, and then the number of live and dead nematodes were counted. The results showed that vitamin E and all of derivatives of vitamin E tends to have the same antinematicidal activity against *S. feltiae* after 24 h of time incubation. However, the results revealed that vitamin E ($C_{29}H_{50}O_2$) and derivative of vitamin E ($C_{37}H_{55}NO_3$) have better antinematicidal activity compared to other derivatives vitamin E with the percentages viability of 42% and 45% respectively at concentration of 400 µM after 24 h of time incubation. At this concentration, the movement and the activity of *S. feltiae* suffered many paralysis and even death.

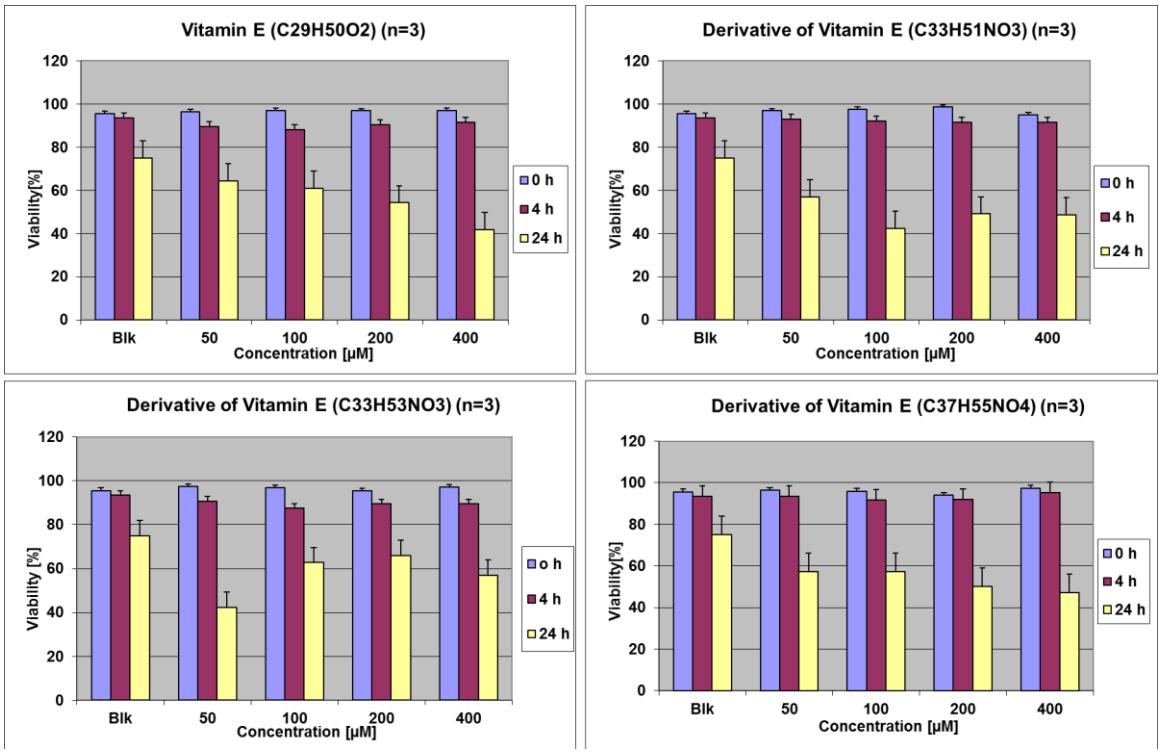

Figure 5. The antinematicidal activity of vitamin E ($C_{29}H_{50}O_2$) and derivatives of vitamin E against *S. feltiae* after incubation at 0, 4, and 24 h of time incubation. DMSO was used as a blank (blk).

Based on the **Figure 5**, the degree of toxicity of vitamin E and the derivatives of vitamin E on *S. feltiae* after 24 h of incubation is vitamin E ($C_{29}H_{50}O_2$) > derivative of vitamin E ($C_{37}H_{53}NO_3$) > derivative of vitamin E ($C_{33}H_{51}NO_3$) > derivative of vitamin E ($C_{33}H_{53}NO_3$). Zuckerman and Geist have shown that vitamin E at a concentration of 200 µg/mL can significantly not able to length of the reproductive at prereproductive stage of *Caenorhabditis elegans* [14], *Caenorhabditis briggase* [15], *Turbatrix aceti* [16, 17]. Meanwhile, Epstein and Gershon mentioned that vitamin E (α-tocopherol and α-tocoferol quinone) at a concentration of 400 µg/ml was able to inhibit the growth of *Caenorhabditis briggase* [15]. In this study, the LD_{50} value was also determined. Table 1 shows the LD_{50} value of thiabendazole (TBZ), vitamin E and some derivatives of vitamin E. The LD_{50} values are determined in micromolars (µM). In this study, the LD_{50} value of hydrogen peroxide (H_2O_2) was not determined.

Table 1. The LD_{50} values of thiabendazole (TBZ), vitamin E and some derivatives of vitamin E *on S. feltiae* after 24 h of time incubation.

Compounds	**LD_{50} value [µM] after 24 h of time incubation**
Thiabendazole ($C_{10}H_7N_3S$)	238.57
Vitamin E ($C_{29}H_{50}O_2$)	209.09
Derivative of Vitamin E ($C_{33}H_{51}NO_3$)	207.77
Derivative of Vitamin E ($C_{33}H_{53}NO_3$)	>400
Derivative of Vitamin E ($C_{37}H_{53}NO_3$)	>400

Table 1 shows that the derivatives of vitamin E ($C_{33}H_{51}NO_3$) was the most active compounds against *S. feltiae* with LD_{50} values of 207.77 µM. The LD_{50} value of the compound vitamin E ($C_{29}H_{50}O_2$) was 209.09 µM, whereas the value of LD_{50} of the derivative of vitamin ($C_{33}H_{53}NO_3$) and ($C_{37}H_{53}NO_3$) have the same LD_{50} values of > 400µM. In this assay, DMSO is used as a negative control. This result are a preliminary study to evaluate the antinematicidal activity of vitamin E and its derivatives.

CONCLUSION

Vitamin E derivatives tend to have the same antinematicidal activity against *S. feltiae*, however, vitamin E derivative compounds ($C_{33}H_{51}NO_3$) have better antinematicidal activity compared to vitamin E $C_{29}H_{50}O_2$). Meanwhile, the derivatives of vitamin E ($C_{33}H_{53}NO_3$) and ($C_{37}H_{53}NO_3$) have moderate antinematicidal activity against *S. feltiae*

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