



Effects of dodine on total protein content and peroxidase activity in *Vicia faba* L.

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ABSTRACT

This research was to evaluate the effects of dodine on the physiology of *Vicia faba* L. through total protein content and peroxidase (POX) activity. According to this, four weeks old seedlings were treated with 0.04 mL/L, 0.08 mL/L and 0.16 mL/L concentrations of dodine. Then, the leaves of plant samples were harvested with 24, 48 and 72 hours intervals. Our results have showed that while the total protein content were decreased significantly, POX activity was increased with the increased concentration of dodine and exposure time when compared with control seedlings. This research have shown that dodine can stimulate the plant defense system.

Keywords: dodecylguanidinium acetate, enzyme activity, *Vicia faba*, pesticide, total protein

Dodin fungusinin *Vicia faba* L. bitkisinde total protein miktarı ve peroksidaz aktivitesi üzerine etkisi

ÖZ

Bu araştırmada dodin fungusinin *Vicia faba* L. fizyolojisi üzerindeki etkisi, total protein miktarı ve peroksidaz (POX) aktivitesi aracılığıyla değerlendirilmiştir. Bu doğrultuda, dört haftalık *V. faba* fideleri 0.04 mL/L, 0.08 mL/L ve 0.16 mL/L'lik dodin konsantrasyonları ile muamele edilmiştir. Bu işlemlerden sonra bitki örneklerinin yaprakları 24, 48 ve 72 saat ara ile hasat edilmiştir. Yapılan analizler sonucunda, artan dodin konsantrasyonu ve muamele süresiyle total protein miktarı kontrole göre önemli ölçüde azalırken, peroksidaz aktivitesi yükselmiştir. Elde edilen sonuçlar dodinin bitki savunma sistemini uyardığını göstermiştir.

Anahtar Kelimeler: 1-dodesil guanidium asetat, enzim aktivitesi, *Vicia faba*, pestisit, total protein

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1. INTRODUCTION

A wide variety of synthetically produced pesticides including fungicide, herbicide and insecticides are used to protect plants and plant products all over the world. Especially fungicides are most commonly used to improve crop yields against diseases of crops in many countries [1] [2] [3]. Fungicides are chemical compounds used to inhibit or kill fungal spores or fungi which can cause severe damage in agriculture resulting in serious losses of yield, profit and quality [4]. The extensive use of fungicides in plant protection against fungal disease generate long term residue in food and in the environment [5]. Although fungicides have positive effects, these chemicals or derivatives can accumulate in organisms and sometimes cause risk of mutagenicity, carcinogenicity or teratogenicity [6] [7] [8]. Furthermore, recent studies indicate that toxic action of pesticides can lead to oxidative stress and accumulation of free radicals including superoxide anion, hydroxyl radical as well as non radical molecules like hydrogen peroxide, singlet oxygen in the cell [9] [10]. Under normal conditions, these reactive oxygen species (ROS) are generated as products of a variety of metabolic pathways and are detoxified by cellular scavenging mechanism [11]. If plants are exposed to stressful conditions, ROS are prone to increase in cells [12] and the unhindered accumulation of ROS is toxic to plants and may result in DNA damage also loss of other cellular activities. The production of ROS must be carefully regulated to avoid unwanted cellular cytotoxicity and oxidative damage. Therefore, plants have developed antioxidant defense mechanism including POX, glutathione peroxidase (GPx), ascorbate peroxidase (APX), superoxide dismutase (SOD), Catalase (CAT), glutathione-S-transferase (GST) and low molecular weight antioxidants (phenolics, glutathione, ascorbate and tocopherols) to protect themselves against ROS [13] [14].

Among these ROS-detoxifying enzymes, peroxidases can scavenge H₂O₂. Peroxidases are oxidoreductases that can use several organic and inorganic substrates as hydrogen donors in the presence of hydrogen peroxide. Peroxidases have a great number of functions in plant metabolism [15]. Peroxidases play important roles in auxin catabolism, being responsible for the oxidative carboxylation of indole-3-acetic-acid [15] [16]. Therefore, they are critical in plant development, cell growth and elongation, ethylene formation, apical dominance and fruit development [15] [17]. Peroxidases also have an important role in lignification by catalyzing the polymeric oxidation of phenolic units during lignin

synthesis and cell wall formation [15] [17] [18]. Peroxidases (POXs) are the most important part of the multi-component defense system. Because they are the first enzymes to increase their levels considerably after a stress stimulation [15]. They are involved in defense reactions of plants against every kind of stress factors [9], such as copper, zinc and iron [19], mercury and lead [20], pesticides [21] [22] [23] [24], salt and drought stress [24], osmotic stress [25], pathogens [26] and many other stress factors [9] [25] [27]. The changes in peroxidase activity are important for plant defense under stress conditions and there are many studies on increase and decrease peroxidase activity [9] [25] [27].

Another important factor for plant defense under stress conditions is variation in total protein content. When plants are exposed to stressful conditions, the resulting hydroxyl radicals cause protein modifications, including specific amino acid alterations, polypeptide fragmentation, denaturation and tend to proteolysis [28]. The increasing number of references reported that in certain conditions, proteolysis is also associated to oxidative stress promoted by reactive oxygen species [29].

The physiological changes caused by chemicals in plants must be observed. Plant based bioassays are widely employed to detect and evaluate the toxicity of many chemicals, to people, animals and plants. Among higher plants, *V. faba* is routinely used for cytological, physiological, radiobiological studies and toxicity evaluation [30]. After exposure to the agents, the effects of agents are to be identified by monitoring changes in defense mechanisms in plants. Peroxidase, superoxide dismutase, catalase activity can be used as a monitor connected with total protein contents.

Dodine (1-dodecylguanidinium acetate) is an aliphatic fungicide and bactericide used to control scab on apples, pears and pecans, brown rot on peaches and several foliar diseases of cherries, strawberries, peaches, sycamore trees and black walnuts. In industry, dodine is used as a preservative and biocide [31]. There is no study available about the effect of dodine on the peroxidase level and protein content in the plant system. The goal of this study is to evaluate the effect of commonly used dodine fungicide on peroxidase activity and protein content in *Vicia faba*.

2. MATERIAL AND METHODS

2.1. Plant Material

Vicia faba seeds were planted in plastic pots containing mixture of torf and perlite (2:1). Seedlings were grown under 16/8 hrs photoperiod at 25±2°C, 60-70% relative humidity with 350 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density in a growth chamber.

2.2. Fungicide Treatments

EC₅₀ value (0.08 mL/L) which is the concentration where root growth is reduced by 50% compared with the control, was determined according to Çördük et al [32]. Four weeks old seedlings were treated with 0.04 mL/L (EC₅₀/2), 0.08 mL/L (EC₅₀) and 0.16 mL/L (2xEC₅₀) concentrations of dodine by spraying to the leaf. Leaf samples were harvested with 24, 48 and 72 hours intervals after dodine treatments. One group was selected as a control group in each treatment period. The experiment was repeated five times, for each treatment.

2.3. Preparation of Leaf Extracts

Healthy leaf of *V. faba* seedlings were harvested and leaf tissues (0,5 g) were grinded in liquid nitrogen with an eppendorf micro-pestle and homogenized in cold 0.05 M sodium acetate buffer (pH 6.5). Then homogenates were centrifuged at 13000 rpm at 4°C for 15 min. The supernatants were collected after centrifugation, for analysis.

2.4. Determination of Protein Content

Protein concentrations were determined according to Bradford (1976) using bovine serum albumin (BSA) as a standard [33]. 100 μL sample was taken in a glass tube and 5mL Protein Reagent Blue G-250 was added in each test tube. Between 15-60 minutes, absorbances were measured in spectrophotometer at 595nm. Absorbance values were placed in the standard graphic to determinate protein content of plant extracts.

2.5. Peroxidase (POX; EC 1.11.1.7) Activity Analysis

200 μL of 0.1 M pyrogallol, 680 μL sodium acetate buffer (pH 6.5, 0,05M) and 20 μL raw leaf homogenates were mixed, then 100 μL of 90 mM H₂O₂ were added to the mixture. Immediately after adding hydrogen peroxide to the mixture, the reaction mixture (1 ml) was measured by spectrophotometer (Spectroquantpharo 300). The peroxidase enzyme activity was calculated at 300 nm [34]. The kinetic enzyme reaction was monitored over 90 s and peroxidase measurements were taken in every 10 s. Peroxidase enzyme activity was expressed as

$\Delta\text{OD}_{300\text{nm}}/\text{min}/\text{mg protein}$.

3. RESULTS AND DISCUSSION

In this research, we have evaluated the effect of dodine on the physiology of *Vicia faba* through protein content and peroxidase activity. Different concentrations of dodine (0.04 mL/L (EC₅₀/2), 0.08 mL/L (EC₅₀) and 0.16 mL/L (2xEC₅₀) were applied to *Vicia faba* seedlings. Protein content and peroxidase activity were determined and compared in each concentration of dodine and exposure time. Our data have showed that the total protein content significantly decreased with the increase of concentration of dodine in each exposure time as compared to their controls (Fig 1). The most significant decline was found in the plant samples which were treated with 0.16 mL/L (2xEC₅₀) dodine for 72 h.

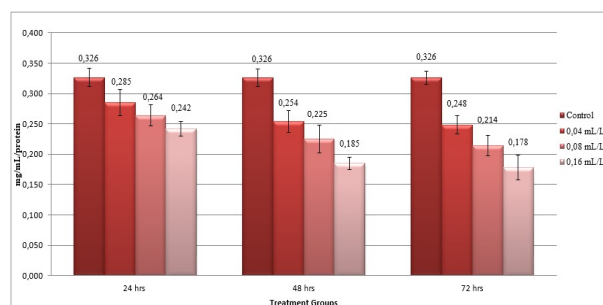


Figure 1. Effects of Dodine treatments on total protein content in *Vicia faba*

The change in the total protein content may be associated with inhibition of protein synthesis and protein degradation. According to our results, dodine may cause oxidative stress in plants resulting in inhibition of the protein synthesis or protein degradation. It is known that protein degradation can occur under conditions induced oxidative stress. Cells also may exhibit increased rates of proteolysis following exposure to oxidative stress inducing agents [35] [36]. According to some reports, the toxicant produced by the application of systemic fungicide may block protein synthesis by binding the larger ribosomal subunits inducing change in the enzyme system [37], ceasing NADP and ATP formation [38] [39].

Apart from protein degradation resulting from oxidative stress, it is determined that dodine fungicide leads to denaturation of proteins. In a study, dodine was determined to be a better chemical denaturant than guanidine denaturation for phosphoglycerate kinase. In addition to this, it was reported that dodine denaturation is less reversible than guanidine denaturation. Dodine can be used to destabilize proteins for thermal or pressure denaturation at concentrations over 1000-fold lower than the widely used nitrogen-based denaturants [40]. In another study, researchers have showed that dodine

unfolds some small proteins at milimolar concentrations. They also suggest that dodine allows fine tuning of the protein's unfolded state, unlike traditional "all-or-none" denaturants. But researchers have noted that the effectiveness of dodine is protein-dependent such as the five-helix bundle $\lambda 6-85$, and the triple stranded β -sheet Fip35 WW domain [41].

In parallel with our work, the use of calixin and benlate systemic fungicides induce a significant decrease on total protein content of resistant (MexiPak) and susceptible (Povan) *Triticum aestivum* L. [39] and same researchers also found that combine effects of four pesticides significantly decreased total protein content with the increase of concentration on soybean [42]. As can be seen almost all of the researches about pesticides effects on plant defence system, pesticides lead to decrease the protein content. On the other hand, in some studies no influence on protein content between pesticide treated plants and controls has been reported [43].

In this study, peroxidase activity was calculated in all treatment groups to evaluate the effects of dodine. In all exposure time (except 24 h, 0,16mL/L), it was demonstrated an increase of the peroxidase activity compared to the control by the increase of dodine concentration (Fig 2).

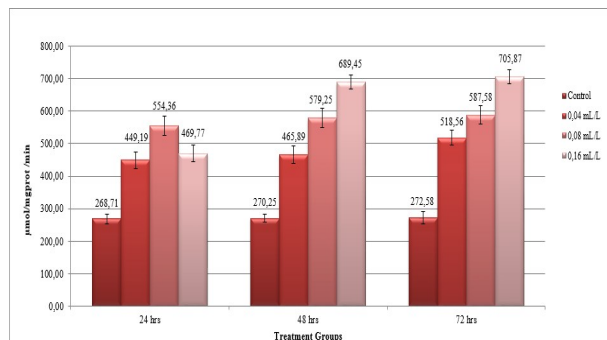


Figure 2. Effects of Dodine treatments on peroxidase activities in *Vicia faba*

This increase in POX activity may indicate that the *V. faba* seedlings likely began to scavenge the ROS occurring as a result of oxidative stress caused by dodine. Oxidative stress occurs as a result of the deterioration of compliance of the speed of the repair and injury and ROS can not be cleaned. Plants produce more antioxidant enzymes to cope with damages caused by ROS [44]. Antioxidant enzymes play an important role in removing ROS, and their activation is directly related to defense against abiotic stress [45]. Several studies have been demonstrated that once the plants are exposed to chemicals, physiological changes occur. Several pesticides are lead to highly effective defense reactions; peroxidase activity [20], guaiacol-peroxidase activity

[22], catalase and pyrogallol peroxidase [21]; SOD, APX and CAT activities [24]. It was indicated that these associated with increasing levels of H_2O_2 and decreasing level of O_2^- in the fungicide treated plant and these results indicated that the fungicide-induced delay of senescence was due to an enhanced antioxidant enzyme activity protecting the plants from harmful ROS. Furthermore, it was indicated that activities of the antioxidative enzymes SOD, CAT, POX in leaves of the fungicide treated plant were increased [46].

Similar to our results, it was reported that peroxidase activity increased with treating plants with different fungicides such as acrobat MY 90/60 fungicide [47], copper based fungicides [48], azoxystrobin and epoxiconazole [22], carbendazim, JS399-19 and tebuconazole [46].

Peroxidase, superoxide dismutase, catalase activity can be used as a monitor connected with total protein contents. Effects of three commercial products (Gusation 35PH®, Paration CE720®, and Tamaron 600 LM®) and an active ingredient (metamidofos) on *Capsicum annuum* L. were investigated in a study and similar to our results, it was found that total protein content was decreased [49] and peroxidase activity was increased with the increase of concentration [50]. Likewise, in another study, researchers found that industrial pesticide effluents decreased total protein content and increased peroxidase activity with the increase of concentration on *Nicaotiana tabacum* and *Vigna radiate* [51].

As a result of our other research, dodine increased the mitotic aberrations in root tip cells of *A. cepa* and it can be accepted as a genotoxic agent [32]. On the other hand, in this study, we have demonstrated that dodine has potentially stimulate the plant defense systems by the meaning of POX activity even used as 0.04 mL/L half of the maximal effective concentration ($EC_{50}/2$).

4. CONCLUSION

In spite of the fact that the use of fungicides is considered to be healthy in order to increase yield of crops but fungicides have adverse effect on plants if it was used in excessive doses. Dodine, widely used in agricultural areas, is known to cause genotoxic, cytotoxic effects and protein degradation in plants. Our results have shown that dodine treatment increases peroxidase activity and decreases total protein content in *V. faba*. These results indicated that dodine may cause oxidative stress in plants so dodine stimulate the plant defense systems. Since dodine, even at low doses, cause many effects in plants, use of dodine should be applied under control in the agricultural fields or less effective pesticides than dodine

may be chosen. So, negative effects of dodine on human health may be reduced.

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