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SHORT COMMUNICATION

# Effect of Dose-Dependent Application of Fungicides Propineb and Mancozeb on $H_2O_2$, Lipid Peroxidation, and Photosynthetic Pigment in Tomato Leaves

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## ABSTRACT

**Objective:** The study investigated the growth, photosynthetic pigment, hydrogen peroxide ($H_2O_2$), and malondialdehyde (MDA) contents of tomato leaves under different concentrations of two modern fungicides, mancozeb and propineb.

**Materials and Methods:** Tomato plants were cultivated for 45 days and irrigated with ¼ Hoagland solution. Three different concentrations of propineb and mancozeb; half of the recommended dose (1.5 g/L and 1 g/L), recommended dose (3 g/L and 2 g/L), and two times higher (6 g/L and 4 g/L) sprayed on tomato leaves, respectively. After the fungicide treatment, tomato leaves were harvested at 1, 3, and 7 days after the treatment (DAT).

**Results:** The highest doses of propineb and mancozeb inhibited shoot growth compared with the control at 7 DAT. The chlorophyll a, b and carotenoid contents were significantly reduced with all mancozeb and propineb treatment doses at 3 and 7 DAT. The phytotoxic effects of fungicides were determined by $H_2O_2$ and MDA content 1, 3, and 7 days after treatment in leaves. The foliar application of propineb and mancozeb altered the production of $H_2O_2$ and MDA, depending on the time and concentration. The analysis of the data revealed that the application of propineb and mancozeb at higher concentrations significantly increased $H_2O_2$ and MDA levels, which caused toxicity in tomato leaves.

**Conclusion:** The findings revealed that higher doses of mancozeb and propineb fungicides exert phytotoxic effects by inhibiting growth and photosynthetic pigment production and increasing oxidative stress in tomato leaves.

**Keywords:** Fungicides, Tomato, Cholorophyll, Oxidative stress, Malondialdehyde

## INTRODUCTION

Fungal infections cause diseases in grain, fruit, and vegetables, reducing yields by 20% of food production worldwide.[1] Fungicides, which are low cost, easy to use, and have a broad spectrum range, have become an effective solution to control fungal disease in recent decades in agriculture. In addition to the protective role of fungicides against fungal disease, fungicide application is commonly used in postharvest packaging plants, parks in urban areas, and protected forest areas.[2] Global fungicide application is four hundred thousand tonnes, indicating 17.5% of global pesticide usage worldwide.[2] According to the FAO report[3], global pesticide use increased by 53 percent for herbicides, 111% for fungicides and bactericides, and 44 percent for insecticides compared with the most recent decade with the 1990s. However, the most used pesticide ratio report fungicides (38.4%), herbicides (27.4%), and insecticides (23.0%) in Turkey in 2022.[4]

A member of the Solanaceae family, tomato (*Lycopersicon esculentum* Mill.) is a popular vegetable. The tomato is consumed fresh and processed, so it has great economic value worldwide. According to an agricultural production statistics report by the FAO[3], tomatoes were the most produced vegetable, with 189 million tonnes in 2021. Moreover, annual tomato production exceeds 13 million mT in Türkiye which is the third largest tomato producer in the world.[5] Tomato is commonly grown in fields and greenhouses in the Aegean, Mediterranean, and Marmara regions of Türkiye. Greenhouse cultivation of tomatoes occurs in two seasons and is monoculture. This continued plantation causes an increase in fungal diseases that cause notable yield loss in greenhouse and

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field tomato cultivation.[5] In particular, fungal diseases, such as fusarium wilt, grey mould, early blight etc., reduce the yield of tomatoes, and tomato cultivation is dependent on fungicide use for the control of fungal disease. In the data of table tomato production in Turkiye, the insecticides, fungicides, and herbicides applied per hectare on average per hectare were nearly 1.3 g and 1.4 g, 1.8 g and 2.0 g, 1.5 g and 1.7 g respectively.[6] The results indicate that fungicides are the most used pesticide in tomato cultivation in Turkiye. In addition, Engindeniz and Öztürk Çoşar revealed that mancozeb (Manzep, Tri-miltox), copper oxychloride (Hektas), propineb (Antrocol), metalaxyl + mancozeb (Ridomil), and copper salts of fatty and rosin acids (Tenn-Cop) are commonly used fungicides in tomato production.[6] They suggested that common and high levels of pesticide use relate to farmer opinion. The farmers believed that if they did not use pesticides to control pests, productivity would lose more than half of the yield. Engindeniz and Öztürk Çoşar also showed that farmers use an excess amount of pesticides in tomato cultivation.[6] Excessive use of pesticides induces environmental contamination, exposure to side effects on non-target organisms, and pesticide residues accumulate at unacceptable levels in foods.

Fungicides are classified into a broad range of compounds by their mode of action and chemical composition. Contact fungicides prevent the growth and development of fungi by killing their spore germination in plant tissue. Among the contact fungicides, dithiocarbamates were developed in the 1940s for fungal diseases, leading to improved anti-fungal formulations. After the first dithiocarbamate fungicide, thiram, ferbam and ziram, was commercialised, mancozeb (manganese ethylene bisdithiocarbamate) and propineb (zinc propylene bisdithiocarbamate) have become widely used fungicides in plant protection since 1962.[7] The mode of action of these fungicides inactivates the thiol group of enzymes and metabolites in fungus cells.[1] For a long time, the use of mancozeb and propineb on a variety of vegetable, fruit, and grain crops has caused environmental problems. In addition, these fungicides have deleterious effects on humans, fish, birds, and plants. Contact fungicides remain on the plant surface to prevent the germination of fungal spores and the penetration of spore germ tubes into the host epidermis.[7] Contact fungicides affect $CO_2$ assimilation and stomatal activity due to leaf surface action. Thus, fungicide treatments primarily have a deleterious effect on the photosynthesis. Many physiological studies have revealed that fungicide treatment decreases chlorophyll a fluorescence[8], $CO_2$ assimilation[9], intercellular $CO_2$ concentration[10], Rubisco content, ribulose 1.5 bisphosphate regeneration[1], and pigment content in plants.[11] Dias et al.[12] reported that the commercially recommended dose of mancozeb in *Lactuca sativa L.* leaves reduced the efficiency of photosystem II, decreased photosynthetic pigments, and induced lipid peroxidation. Different concentrations of benzimidazole (Carbendazim) and dithiocarbamate (Mancozeb) fungicides decrease root/shoot length and germination, and they affect chlorophyll (Chla, Chlb, total chlorophyll) content in chickpea seedlings.[13]

Reactive oxygen species (ROS) are rapidly produced due to breakdown of the cellular balance in plants. Fungicide-induced toxicity triggers the accumulation of ROS species such as hydrogen peroxide ($H_2O_2$), superoxide ($O_2^-$), and hydroxyl radicals (OH•). ROS, highly reactive molecules, induce oxidative stress that damages the fatty acids of the cell membrane. This phenomenon is known as lipid peroxidation and is a marker of disturbed redox status in plants.[14] Lipid peroxidation determined by the product of polyunsaturated precursors that include small hydrocarbon fragments and MDA (malondialdehyde). Many studies have shown that fungicide-induced toxicity triggered accumulation MDA in lettuce[12], wheat[15], and pea[16].

Recently, the over-application of pesticides has become a potential risk due to their biomagnification and long life span in the environment. Moreover, excessive pesticide use results in ecosystem pollution that causes serious problems for non-target organisms such as humans, fish, bees, and plants. The present study investigated the effects of different doses of a broad range of fungicides (propineb and mancozeb) on the growth, photosynthetic pigment, $H_2O_2$ and MDA contents in tomato leaves. The findings of this study revealed the effects of the dose-dependent phytotoxicity of propineb and mancozeb in non-target plants. Also, the results can provide a new perspective on the formulation and concentration of fungicides for minimising the adverse effects on non-target plants.

## MATERIALS AND METHODS

### Experimental Design

The tomato (*Lycopersicon esculentum* Mill. Narcan-8) seeds were purchased from Seed Corporation (Balıkesir Küçükçiftlik) in Turkey. Before the imbibition, the seeds were sterilised in a 5% NaOCI solution for 10 min. Three seeds were planted in plastic pots containing perlite irrigated by ¼ Hoagland solution (Caisson Labs, USA). The tomato seedlings were grown for 45 days in a plant growth chamber. The chamber conditions were as follows: 16–8 h photoperiod, 25°C/20°C, and 60% relative humidity. Fungicides were purchased commercially as mancozeb (MAYCEB M-45) and propineb (Antracol® WP 70). MAYCEB M-45 included 80% mancozeb, which was recommended at a dose of 2 g/L (3 mM). Antracol® WP 70 included %70 propineb, which was recommended at a dose of 3 g/l (7,25 mM).

45-day-old tomato seedlings were sprayed with mancozeb at 1 g/L (half of the recommended dose), 2 g/L (recommended dose), and 4 g/L (two times higher) in tomato laves. Propineb was sprayed at 1.5 g/L (half of the recommended dose), 3 g/L (recommended dose), and 6 g/L (two times higher) in tomato laves. The control tomato plants were sprayed the deionised water. Fungicide treatment was performed in 18 pots, and 3

pots were used for each fungicide treatment. Each pots were consisted the three plants. The control group also had three replicated pots. The tomato leaves were harvested from three independent plants and randomly pooled at 1, 3, and 7 days after treatment.

### Growth Parameter

The shoot lengths of the plants were recorded in cm for all experimental groups.

### Photosynthetic Pigment Content

Photosynthetic pigment contents were determined using the method of Lichtenthaler and Buschmann.[17] Fresh weights of leaf samples were collected and extracted in 100% acetone. After the samples were kept at 4 °C for 24 h and centrifuged at 3.000 x g for 15 min, the absorption values of the supernatants were measured using a spectrophotometer (Epoch 2 Microplate Spectrophotometer) at 661.6, 644.8, and 470 nm.

### Hydrogen Peroxide Content

The amount of $H_2O_2$ was determined according to the method of Velikova et al.[18] The fresh leaf samples (0.5 g) were extracted in 5 mL of 0.1% trichloroacetic acid in an ice bath. After the extract was centrifuged at 12.000 x g for 15 min at 4 ºC, 0.5 mL of 10 mM potassium phosphate buffer (pH: 7.0) and 1 mL of 1 M potassium iodide were added to 0.5 mL of the supernatant. The absorbance of the mixture was determined using a spectrophotometer (Epoch 2 Microplate Spectrophotometer) at 390 nm. The amount of $H_2O_2$ was calculated in µmol/mL from the standard curve.

### MDA Content

Lipid peroxidation was analysed for MDA content using the method of Jiang and Zhang.[19] Fresh leaf samples (0.5 g) were extracted using 10 mL of 0.25% thiobarbituric acid in 10% trichloroacetic acid in a cold mortar. The mixture was boiled at 95 °C for 30 min and quickly cooled in an ice bath. The absorbance of the supernatants obtained from the samples centrifuged at 5.000 x g for 10 min was measured using a spectrophotometer (Epoch 2 Microplate Spectrophotometer) at 532 nm and 600 nm. The MDA level was calculated in µmol/g fresh weight using an extinction coefficient (EC) value of 155 $mM^{-1}cm^{-1}$.

### Statistical Analysis

The standard error values (±) were calculated for at least five replicates. All data sets statistically evaluated by GraphPad Prism version 10.1.2 software (GraphPad Software, San Diego, CA, USA). A one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test was performed to compare the significance between the results.

## RESULTS AND DISCUSSION

Successful tomato production requires fungicides and insecticides to prevent diseases, minimise production loss and meet quality standards. However, the widespread use of fungicides causes numerous environmental concerns, including soil and water contamination, and side effects on non-target organisms. The mode of action of pesticides affects both pests and non-target organisms such as humans and plants because of similar targeting systems or enzymes. The beneficial effects of pesticides have become a risk due to their phytotoxicity when applied at higher concentrations in plants. A small percentage of the pesticides reach the sites of action for pest control (approximately <0.1%), and larger amounts are degraded via light and heat and non-target accumulation in the environment. Pesticide toxicity is related to many factors, such as environmental conditions (temperature, moisture, pH), application dose, and technique. Pesticide toxicity shows several morphological symptoms, such as necrosis, chlorosis, stunting, burns twisting of leaves, and it also negatively affects plant growth and development.[20] Pesticides inhibit biological processes such as reactive oxygen balance, synthesis of photosynthetic pigments, cell division, enzyme function, and photosynthesis.[21]

One of the most essential reasons for non-target pesticide toxicity is the use of doses higher than the recommended dose by farmers. Although higher doses help temporarily the pest struggle, they can cause stress to non-target plants, resistance to pesticides, and pesticide residue in soil and water, and finally, it results in environmental pollution in the long term.[2] For this reason, it is important to understand how the dose-dependent application of pesticides affects non-target plants. This study aimed to investigate the effects of different doses of the fungicides mancozeb and propineb on the growth, oxidative stress, and photosynthetic pigments of tomato leaves.

Plant growth is an indicator of changes in plant performance and monitoring responses to environmental stress factors. Three different concentrations of propineb and mancozeb; a half of the recommended dose (1,5 g/L and 1 g/L), recommended dose (3 g/L and 2 g/L), and two times higher (6 g/L and 4 g/L) were sprayed on 45-day-old tomato plants. Plant growth was recorded 3 and 7 days after treatment with propineb and mancozeb (Figure 1). Shoot growth was affected by different doses of propineb and mancozeb application. At 3 and 7 days after treatment, shoot growth did not differ significantly between the recommended and half-dose fungicide application and control. However, the highest dose of mancozeb and propineb (two times higher dose) inhibited shoot growth compared with the control. Shoot growth was negatively affected at 7 days after fungicide application (Figure 1). All concentrations of mancozeb inhibited shoot growth in 45-day-old tomato plants. Pereira et al.[22] determined showed that amino acid metabolism was disturbed in the later growth stages of lettuce under mancozeb treatment, as well as decreased levels of Ala, Asn, GABA, Ile,

Leu, and Val. They also reported that the expanded growth stage of lettuce leaves was negatively affected by exposure to mancozeb. Based on the results of this study, mancozeb inhibited the growth of lettuce leaves because of decreased amino acid metabolism. In contrast, Shakir et al.[23] reported that four commonly used pesticides (emamectin benzoate, alpha-cypermethrin, lambda-cyhalothrin and imidacloprid) reduced tomato growth at higher concentrations. Growth inhibition can be associated with oxidative stress in tomato leaves following pesticide application.

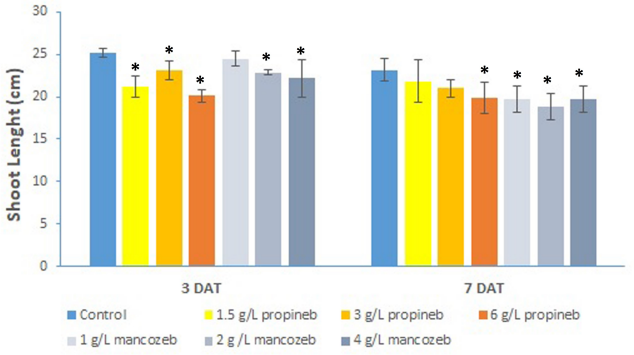


**Figure 1.** Effect of half of the recommended dose (1.5 g/L and 1 g/L), recommended dose (3 g/L and 2 g/L), and double of the recommended dose (6 g/L and 4 g/L) of propineb and mancozeb on shoot growth in 45-day-old tomato seedlings. Astericks showed adjusted p <0.05 for statistically significant differences from the control as determined by one-way analysis of ANOVA followed by Dunnett's Multiple Comparison Test. Bars represent standard deviations (SD). DAT: Days after treatment.

ROS production is the balance between the antioxidative defence system under optimal growth conditions in plants.[14] The balance between ROS production and scavenging may be disrupted in plants exposed to abiotic and biotic stress factors. This imbalance of the ROS system causes an increase in ROS levels, which causes injury to nucleic acid and oxidising proteins and membrane lipids in plant cells.[14] $H_2O_2$ is produced from the univalent reduction of $O_2$ by superoxide dismutase $H_2O_2$ is the most stable ROS because of its long half-life, but it can inactive enzymes by oxidating their thiol groups.[14] Excessive accumulation of $H_2O_2$ triggers oxidative stress, and it has become an important indicator of toxicity against different stress factors in plant cells.[24] Fungicide spraying (propineb and mancozeb) remarkably induced the $H_2O_2$ level, proving the occurrence of oxidative stress in tomato leaves (Figure 2). Different concentrations of propineb and mancozeb changed the $H_2O_2$ levels 1, 3 and 7 days after treatment. Propineb at 1.5 and 3 mg/L significantly decreased the $H_2O_2$ level 1 day after treatment, whereas it remarkably increased the $H_2O_2$ level 7 days after treatment. However, 6 mg/L propineb caused the highest increase in $H_2O_2$ level 1 day after treatment. In the following days (3 and 7 days after treatment), the $H_2O_2$ level decreased according to 1 days after treatment, even if it was higher in the control groups. In propineb administration, half (1.5 g/L) of the recommended dose and recommended dose (3 g/L) showed a significant increase in the $H_2O_2$ level at 7 day after treatment, yet two doses (6 mg/L) suddenly increased in the $H_2O_2$ level at 1 day after treatment (Figure 2). The results of the $H_2O_2$ level indicated that a low dose of propineb slowly caused oxidative stress, whereas a high dose of propineb quickly triggered oxidative stress after treatment in tomato leaves. The application of 1, 2, and 4 g/L of mancozeb gave rise increment of the $H_2O_2$ level 1, 3, and 7 days after treatment compared with the control. Moreover, the highest level of $H_2O_2$ was observed in 4 mg/L mancozeb spraying (two times higher doses) 3 days after treatment in tomato leaves.

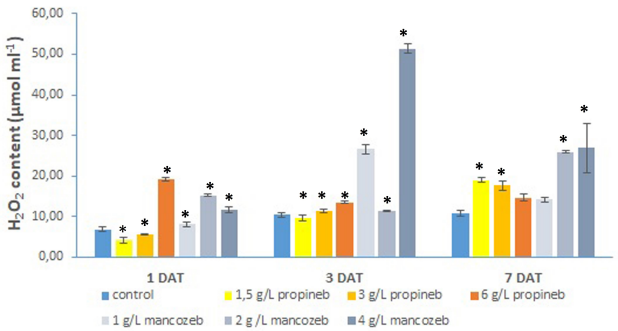


**Figure 2.** Effect of half of the recommended dose (1.5 g/L and 1 g/L), recommended dose (3 g/L and 2 g/L), and double of the recommended dose (6 g/L and 4 g/L) of propineb and mancozeb on $H_2O_2$ content in 45-day-old tomato seedlings. Astericks showed adjusted p<0.05 for statistically significant differences from the control as determined by one-way analysis of ANOVA followed by Dunnett's Multiple Comparison Test. Bars represent standard deviations (SD). DAT: days after treatment.

Excessive ROS accumulation can damage many cellular functions by disrupting nucleic acids, proteins, carbohydrates, and lipids in plants. Lipid peroxidation is an indicator of oxidative injury caused by ROS in cells. The harmful effects of lipid peroxidation are associated with fluidity, specific ion channels, proteins, receptors, and enzymes in membranes.[14] Lipid peroxidation determined by MDA, a polyunsaturated precursor that includes small hydrocarbon fragments. The analysis of MDA content proved that the application of the two fungicides significantly induced membrane damage in the leaves of tomatoes compared with the control plants (Figure 3). The half of recommended (1.5 g/L) and recommended dose (3 g/L) of propineb showed trends similar to control or decreased MDA content at 1 and 3 days after treatment, yet these two concentrations of propineb triggered the MDA content at 7 days after treatment. In addition, two-fold higher doses of propineb (6 g/L) remarkably increased MDA content compared with the control in tomato leaves at 1, 3, and 7 days after treatment. On the first day after treatment, there were similar trends in MDA content at all concentrations of mancozeb. Besides, two times higher doses of mancozeb (4 g/L) significantly increased MDA content compared with the control at 3 and 7 days after treatment (Figure 3). The experimental results revealed that different doses

**Table 1.** Effect of half of the recommended dose (1.5 g/L and 1 g/L), recommended dose (3 g/L and 2 g/L), and double of the recommended dose (6 g/L and 4 g/L) of propineb and mancozeb on chlorophyll a, chlorophyll b, carotenoid, and total chlorophyll content in 45-day-old tomato seedlings. Astericks showed adjusted p values<0.05 for statistically significant differences from the control as determined by one-way analysis of ANOVA followed by Dunnett's Multiple Comparison Test. Bars represent standard deviations (SD). DAT: days after treatment.

| Time | Treatment | Chl a (µg/mL) | Chl b (µg/mL) | Carotenoid (µg/mL) | Total Chlorophyll |
|---|---|---|---|---|---|
| **1 DAT** | Control | 380.38 ± 5.41 | 147.30 ± 5.44 | 179.68 ± 3.01 | 527.67 ± 8.86 |
| | 1.5 g/L propineb | 380.76 ± 17.42 | 156.84 ± 8.46 | 196.28 ± 10.31* | 537.60 ± 25.75 |
| | 3 g/L propineb | 313.28 ± 6.02* | 126.30 ± 9.01* | 154.93 ± 5.44* | 439.59 ± 15.00* |
| | 6 g/L propineb | 227.26 ±3.69* | 97.36 ± 5.11* | 114.67 ± 2.78* | 324.62 ± 7.20* |
| | 1 g/L mancozeb | 364.46 ±10.70 | 141.61 ± 15.35 | 174.88 ± 9.61 | 506.07 ± 26.03 |
| | 2 g/L mancozeb | 284.41 ±2.20* | 112.37 ± 5.22* | 139.86 ± 3.65* | 396.78 ± 6.85* |
| | 4 g/L mancozeb | 280.73 ±2.76* | 111.32 ± 3.77* | 138.41 ± 3.15* | 392.05 ± 6.15* |
| **3 DAT** | control | 356.11 ± 1.94 | 151.98 ± 8.47 | 178.01 ± 4.62 | 508.09 ± 6.53 |
| | 1.5 g/L propineb | 316.63 ± 2.66* | 122.63 ± 5.39* | 148.91 ± 3.44* | 439.26 ± 7.66* |
| | 3 g/L propineb | 255.42 ± 2.68* | 102.36 ± 1.92* | 121.86 ± 2.26* | 357.77 ± 3.42* |
| | 6 g/L propineb | 242.90 ± 7.38* | 97.82 ± 5.99* | 124.84 ± 5.70* | 340.72 ± 12.01* |
| | 1 g/L mancozeb | 300.54 ± 2.25* | 121.37 ±5.98* | 148.80 ± 3.86* | 421.91 ± 8.20* |
| | 2 g/L mancozeb | 280.78 ± 3.70* | 105.49 ± 1.47* | 129.68 ± 1.63* | 386.27 ± 3.88* |
| | 4 g/L mancozeb | 298.01 ± 9.20* | 116.99 ± 3.04* | 144.37 ± 2.22* | 414.99 ± 6.84* |
| **7 DAT** | control | 303.27 ± 1.41 | 107.76 ± 1.56 | 136.70 ± 0.68 | 411.03 ± 1.96 |
| | 1.5 g/L propineb | 257.76 ± 1.36* | 95.19 ± 1.81* | 121.82 ± 1.09* | 352.95 ± 2.20* |
| | 3 g/L propineb | 258.89 ± 5.90* | 100.09 ± 9.08 | 122.68 ± 3.85* | 358.98 ± 14.90* |
| | 6 g/L propineb | 284.03 ± 1.93* | 108.15 ± 1.26 | 133.36 ± 1.56 | 392.18 ± 3.12* |
| | 1 g/L mancozeb | 201.51 ± 0.92* | 90.01 ± 4.52* | 105.96 ± 2.70* | 291.52 ± 5.39* |
| | 2 g/ mancozeb | 196.19 ± 1.94* | 83.76 ± 8.47* | 100.07 ± 4.62* | 279.95 ± 6.53* |
| | 4 g/L mancozeb | 243.84 ± 0.60* | 96.50 ± 0.89* | 120.58 ± 0.93* | 340.34 ± 1.19* |

of mancozeb and propineb enhanced the $H_2O_2$ and MDA levels within 7 days of treatment in tomato leaves. The increase in $H_2O_2$ and MDA content indicates oxidative stress caused by the application of propineb and mancozeb in tomato leaves. Shahid et al.[16] reported that three different fungicides enhanced the accumulation of $H_2O_2$ and MDA content with increasing dosages of the fungicides.

The photosynthesis apparatus is strongly influenced by environmental conditions that change the function and structure of the photosynthesis machinery in plants.[25] Physiological studies have revealed that fungicide toxicity primarily affects the photosynthetic process in plants.[1] Fungicide treatments have been shown to decrease photosynthetic activity and chlorophyll fluorescence, reduction of net $CO_2$ assimilation, transpiration rate, stomatal conductance, and intercellular $CO_2$ concentration. Pigment biosynthesis, chlorophyll a, chlorophyll b, and carotenoids are inhibited by fungicide application in grapevine[9], maize[26], and chickpea[13]. Petit et al.[9] reported that all concentrations of the fungicide fludioxonil (fdx) inhibited photosynthesis in non-target grapevines. In addition, lower fdx concentrations decreased photosynthesis from the first day after treatment, whereas higher concentrations decreased photosynthesis after 7 days. Dias et al.[12] the effect of a commercial dose of mancozeb on the photosynthetic pigment in *Lactuca sativa* leaves. They found that mancozeb had a neg-

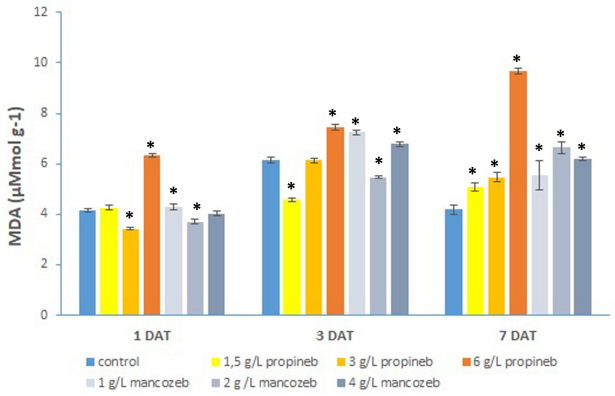


**Figure 3.** Effect of half of the recommended dose (1.5 g/L and 1 g/L), recommended dose (3 g/L and 2 g/L), and double of the recommended dose (6 g/L and 4 g/L) of propineb and mancozeb on malondialdehyde (MDA) content in 45-day-old tomato seedlings. Astericks showed adjusted p<0.05 for statistically significant differences from the control as determined by one-way analysis of ANOVA followed by Dunnett's Multiple Comparison Test. Bars represent standard deviations (SD).

ative effect on chlorophyll and carotenoids due to sensitivity of pigment-protein complex of the photosynthesis apparatus. Similarly, commonly used pesticides (emamectin, benzoate, alpha-cypermethrin, lambda-cyhalothrin and imidacloprid) decreased pigment content at higher doses in 21-day-old tomato leaves.[23] In this study, the effects of propineb and mancozeb on the pigment content of tomato leaves (Table 1). On the first day after treatment, chlorophyll a, b, and carotenoid content exhibited similar trends as the control at half the recommended dose of propineb (1.5 g/L) and mancozeb (1 g/L). However, recommended and twice-dosed propineb and mancozeb significantly decreased pigment content in tomato leaves. At 3 and 7 days after treatment, chlorophyll and carotenoid pigment degradation was remarkably induced by all concentrations of propineb and mancozeb. Interestingly, the highest pigment increment was observed in 6 g/L propineb and 4 g/L mancozeb on the first day of treatment compared with the control. Morover, after treatment (3 and 7) the pigment degradation rate slowed down as compared with the control (Table 1). The results show that propineb and mancozeb have acute harmful effects on photosynthetic pigments at two doses in tomato leaves.

## CONCLUSION

The results of this study show that the dose-dependent application of propineb and mancozeb fungicides negatively affects the growth and photosynthetic pigments of tomato leaves by triggering oxidative stress. The overdose of fungicides can be harmful and reduce the vegetative growth of tomato seedlings. These findings can help understand the toxicity of fungicides in non-target plants. The results of the study indicate the risk of excessive fungicide use in plant growth, development, and yield.

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