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Physiological Effects of Captan Fungicide on Pepper (*Capsicum annuum* L.) Plant

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Abstract: The aim of the study was to investigate the physiological effects of the fungicide Captan on germination as well as some leaf contents of pepper plant *Capsicum annuum* L. For the germination experiments, seeds of pepper were treated with a recommended concentration of 2.5 g l^{-1}, two higher concentrations of 5.0 g l^{-1} and 7.5 g l^{-1} as well as a water-treated control. For the effect of fungicide on leaf contents, the seedlings were treated 4 times with the three different concentrations of Captan with 6-day intervals starting with 2-month old seedlings. The results showed that germination rate decreased with all concentrations of Captan, this effect being more pronounced at the higher concentration and after 144 as well as 168 hours. Analysis of the treated leaves showed that the recommended concentration resulted in higher amount of chlorophyll a as well as a/b and carotenoid contents of the leaf than the higher concentrations and control while the amount of chlorophyll b and total chlorophyll was reduced in all the treated plots. All concentrations of Captan gave higher amount of chlorophyll a/b than the control. Generally, treatment of higher fungicide concentration yielded in lower values of all the investigated leaf contents. Concentrations of 2.5 as well as 5.0 g l^{-1} showed higher values of protein, proline as well as internal ABA contents of the leaf than the higher concentration as well as control. The higher concentration gave lowest values for these three leaf contents.

Key words: Abscisic acid, *Capsicum annuum*, Captan, fungicide, proline, protein

INTRODUCTION

Fungicides are most commonly used against diseases of agricultural crops in Turkey as in many other countries of the world. Farmers believe its application secures yield and quality of the crop. Consequently, worldwide 95% of the total agricultural area is cultivated conventionally, while only 5% of the area is treated with either biological control methods or cultivated according to organic farming regulations (Öztürk, 1997).

Although fungicide application results in quick and high control of the diseases but it is associated with a number of problems too, such as environmental and food contamination, acute and chronic risk of poisoning, disruption of the natural balance, risk of teratogenic, mutagenic, and carcinogenic effects on humans and animals. Some fungicides tend to accumulate in the environment and within the food chain. The excessive and inappropriate use of fungicides has led to serious environmental concerns such as potential contamination of surface and underground water resources (Anonymous, 1993). Excessive dosages and incorrect applications of fungicides results in phytotoxic effects, negatively affect fruit quality as well as yield and lead to anatomical as well as morphological abnormalities in plants.

Several studies have shown adverse effects of fungicides on plants. Steward and Krikorion (1971) reported that carbonate derivatives of the fungicide Antrakol, used for control of tobacco mildew, not only speed up cell division, but also inhibit photosynthesis. Similarly, the fungicide Simazin had an impeding effect on the 'Hill reaction' by preventing fixation even in concentrations as small as 1 ppm (Ashton *et al*., 1960). Stone *et al*. (1987) showed that the fungicide Metalaxyl applied to *Brassica compestris* was absorbed and translocated, eventually accumulating in the lower leaves of the plant.

Many authors believe that application of such plant chemicals like fungicides causes stress in plants. The word stress includes responses of plants to detrimental factors like fungicides and extreme environmental factors (Selye, 1936; Edreva, 1998). Levitt (1980) classified stress factors in two main groups: physicochemical and biotic factors. Physicochemical factors comprise of heat, radiation, chemicals, water, salt, and electrical and magnetic fields, while biotic factors include competition with contagious microorganisms (fungi, bacteria, viruses), other organisms, and pesticides (fungicides, insecticides, etc.).

Pepper is an important vegetable crop in Turkey. It is consumed fresh or dried and used in spice, paste and sauce production. More recently, it is utilized in pharmaceutical products, which has further increased its importance. Captan is a widely used fungicide in Turkey to control diseases of fruits and vegetables. In a study conducted on apple fruits sold at various markets in Izmir, residues of Captan were found in all the samples tested (Tama, 1993). Application of Captan on pepper crop is also very common but its physiological effects on the plant were unknown.

Therefore, the aim of the study was to examine effect of different dosages of the fungicide Captan on germination rate of pepper seed and also on the various leaf contents, i.e., chlorophyll a, b, as well as a/b, carotenoid, protein, proline and internal ABA.

MATERIALS AND METHODS

For the experiments, pepper plants *Capsicum annuum* L. were grown from seeds (Demre™ Long Green Pepper, Simge Incorporation, Turkey). The seeds were sterilized before growing by immersing in 2% sodium hypochlorite for 5 min and then repeatedly rinsed with distilled water. Germination experiments were conducted in 5 cm diameter Petri dishes containing a sheet of blotting paper, moistened with either 1 ml distilled water for control or 1 ml of one of the three different Captan (Agro-Captan 50 WP™, AGRO-SAN, Istanbul, Turkey) concentrations: 2.5 g l^{-1} (recommended concentration), 5.0 g l^{-1} or 7.5 g l^{-1}. Each Petri dish contained 25 seeds, and each treatment was replicated four times. Germination tests were carried out at 25 ±1°C under dark conditions. Germination rates were estimated 24 hours after the first germination occurred and then every 24 hours for seven consecutive days using radicle protrusions as criterion.

To study the effect of Captan on the various leaf contents in the seedlings, seeds were grown in 20 plastic bags filled with standard planting substrate, each containing five seeds. The seedlings were kept in a screen house at the botanical garden and were regularly watered in the morning and evening. Two-month old seedlings were thinned out leaving three seedlings in each bag.

The first Captan application was carried out on two-month old seedlings using a hand sprayer until all the leaves became drop-wet. Three more applications were done with 6-day intervals using the same method. Treatments included three different Captan concentrations of 2.5, 5.0 as well as 7.5 g l^{-1} and a control. In the control treatment, the seedlings were sprayed with distilled water as per Captan application. Leaf samples were collected and processed following a waiting period of 48 hours required for protein analysis. Photosynthetic pigment substances were determined by a method after Witham *et al.* (1971), total protein after Bradford (1976), proline after Bates *et al.* (1973) and internal abscisic acid (ABA) after Scott and Jacobs (1964). There were three replications of all the experiments.

The data were analyzed with Tukey's Multiple Range Test (Tukey, 1953).

RESULTS

The results of present studies showed that germination rate of pepper decreased markedly with all the three concentrations of Captan as compared to control (Table 1).

Germination rate was similar by seed treatment with concentrations of 2.5 and 5.0 g l^{-1} except at 144h after application where it was 5% lower at the higher concentration. Germination rate at the higher concentration was higher than the two lower concentrations after 24 as well as 48h, lower after 72h, similar after 96 as well as 120h and then remained lower on the last two sampling dates. Overall, the germination rate was quite low, even in the control.

Application of different concentrations of Captan gave mixed responses regarding quantities of leaf pigment substances (Table 2).

The recommended concentration resulted in higher chlorophyll a value with 1.793 mg ml^{-1} than the control with 1.744. The middle concentration gave 1.568 while the higher concentration the lowest value of 1.465 mg ml^{-1}.

Table 1: Effect of three different concentrations of Captan on germination rate of Pepper plants

Treatments Captan (g l^{-1})	Germination rate in % (hours after first germination) 24	48	72	96	120	144	168
2.5	15	20	35	40	50	55	65
5.0	15	20	35	40	50	50	65
7.5	25	30	30	40	50	50	55
Control	30	30	40	50	60	70	75

Table 2: Effect of three different concentrations of Captan on Pepper leaf contents

Treatments Captan (g l^{-1})	Chlorophyll a mg ml^{-1} Mean±SE	Chlorophyll b mg ml^{-1} Mean± SE	Total Chlorophyll mg ml^{-1} Mean±SE	Chlorophyll a/b mg ml^{-1} Mean	Carotenoid mg ml^{-1} Mean±SE
2.5	1.793±0.10	0.792±0.19	2.584±0.11	2.260	7.180±0.27b
5.0	1.568±0.09	0.733±0.03	2.301±0.12	2.140	6.260±0.41b
7.5	1.465±0.05	0.688±0.02	2.153±0.05	2.130	5.800±0.22b
Control	1.744±0.24	0.896±0.13	2.639±0.54	1.950	6.710±0.80a

Table 3: Effect of three different concentrations of Captan on protein, proline and internal ABA quantities in pepper leaves

Treatments Captan (g l^{-1})	Protein (mg ml^{-1}) Mean±SE	Proline (µmol g^{-1}) Mean±SE	Internal ABA (ppm) Mean±SE
2.5	0.1916±0.0008a	5.90±0.007b	19.5±0.01b
5.0	0.1910±0.0010a	5.65±0.007b	17.0±0.07b
7.5	0.1902±0.0026a	4.10±0.005b	15.7±0.05b
Control	0.1904±0.0012a	5.50±0.007a	16.0±0.01a

Means in columns followed by different letters are significantly different at P<0.05 (Tukey's HSD test)

Chlorophyll b as well as total chlorophyll quantities were reduced in all the fungicide treatments as compared to control. They were lowest in higher concentration treatments with 0.688 as well as 2.153 mg ml^{-1} and highest in recommended concentration treatment with 0.792 as well as 2.584 mg ml^{-1}, respectively. These quantities were 0.896 as well as 2.639 mg ml^{-1} in control, respectively. All the Captan treatments increased Chlorophyll a/b value as compared to control. It was 2.260 mg ml^{-1} with recommended dosage, 2.140 with the middle dosage, 2.130 with the higher dosage, while 1.950 mg ml^{-1} in the control. Carotenoid content was increased only with the recommended dosage of Captan with 7.180 mg ml^{-1}. It was 6.260 with the middle dosage and 5.800 with the higher dosage, while 6.710 mg ml^{-1} in control.

Analysis of the data showed that the fungicide had no influence on the protein contents of the leaves, as there was no significant difference in the treated and control treatments (Table 3).

But, higher dosage of the fungicide resulted in significantly lower value of proline with 4.10 μmol g^{-1} as compared to control with 5.50. There was a significant difference among proline values with recommended as well as middle dosages and control. On the other hand, Internal ABA content was significantly higher with the recommended dosage of the fungicide with 19.5 ppm as compared to control with 16.0 ppm. There were significant differences in Internal ABA values among the middle as well as higher dosages of Captan and control.

DISCUSSION

The present studies indicated that Captan application reduced germination rate of pepper plant. Furthermore, different dosages of the fungicide had different effects on germination. Germination rate was lower with the application of lower dosages till 48 hours, it was similar with all the dosages from 48 till 120 hours but was lower with the higher dosages from 144 till 168 hours. Hopkins (1995) reported that Enzymes play an important role in the process of germination. Certain ions or molecules of the pesticide molecule can combine to enzymes so as to obstruct their catalytic activities and thus, may negatively affect germination. Prado *et al.* (2000) demonstrated that under saline conditions germination decreased markedly in *Chenopodium quinoa* wild seeds. It is however premature to conclude that this effect is entirely due to inhibition of enzymes during germination, as other factors such as osmotic effects cannot be excluded.

Captan application resulted in reduction of chlorophyll a, b, as well as total chlorophyll and carotenoid contents in pepper leaves but the recommended dosage resulted in increase of chlorophyll a and carotenoid contents as compared to higher dosages and control. Reduction of these contents was higher at the higher dosages of the fungicide. Ashton *et al.* (1960) found that the fungicide Simazin impede effect of the ‘Hill reaction’. Steward and Krikorion (1971) determined that the fungicide Antrakol, inhibited photosynthesis in *Nicotiana tabacum.* The present as well as the earlier results indicated that fungicides might decrease the amount of chlorophyll in leaves. This decline in photosynthetic pigment substances may also cause the nutritious value of the pepper plant to diminish, considering the fact that the vitamin synthesis takes place in chloroplasts.

Carotenoid is an important component of defense mechanism of plants (Çakmak and Marschner, 1992). It was observed in the presented study that the application of Captan in the recommended dosage of 2.5 g l^{-1} increased the amount of the carotenoid, which may be attributed to stress induced by the fungicide. In contrast, higher dosages slightly decreased the amount of carotenoids in leaves.

Upadhyaya *et al.* (1989) stated that various kinds of stress cause accumulation of proline in plant tissues. They further said that many plants synthesize proline from glutamine under stress conditions. Flores *et al.* (1988) reported that fungicide applications increased proline accumulation, which helped the plants to tolerate stress of frost. Leul and Zhou (1999) found that flooding increased free proline content in winter rape treated with Uniconazole. Hamilton III *et al.* (2001) discovered that Na applications in Sporobutus species induced stress, increasing the quantity of heat shock proteins as well as that of proline. The results of this study indicated increase of proline content in leaf tissue if treated with 2.5 and 5.0 g l^{-1} Captan. Higher dosage however resulted in decrease of proline amount.

It was found in the present study that Captan application resulted in increase of internal ABA concentration of the leaves except at the higher dosage. Sivakumaran and Hall (1978) stated that in cases where environmental conditions negatively affect plants, the amount of abscisic acid (ABA), a hormone hindering the metabolic activity in the plant, increases, for example, in *Euphorbia lathyrus* water stress increased the ABA concentration in developing leaves by a factor ten and in mature leaves by a factor five. Alfredo and Setter (2000) also found accumulation of higher amounts of ABA in Manioc leaves in the event of water deficiency. The increase in the internal ABA levels in pepper plant with the application of recommended dose of Captan displays similar reactions of pepper plant to fungicide induced stress as reported in the literature.

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