

## Effects of Some Meteorological Parameters and Formulation on Phytotoxicity of the Fungicide Captan

Ali Reza Ebadollahi Natanzi<sup>1\*</sup>, Shima Mahmoudian<sup>2</sup>, Hamid Reza Rahimi<sup>2</sup>, Moslem Mohammadpour<sup>1</sup>

Received: 29.4.2011

Accepted: 21.5.2011

### ABSTRACT

**Background:** This study investigated the effects of some important meteorological parameters and filler type compounds on phytotoxicity of Captan in peach trees in the north of Iran. Captan is a phthalimide fungicide which has frequently been used in northern regions of Iran to control certain plant diseases. This fungicide has caused intense phytotoxicity in peach trees which was first observed in the northern districts of Iran in 2005. Since early research has only reported that some changes in the climate could be associated with this phytotoxic effect and little is known on phytotoxicity of filler in the formulation, therefore, we studied the role of filler in this toxic effect and also its interaction with this phytotoxicity.

**Methods:** Filler (calcium carbonate) and climatic parameters, including minimum and maximum temperatures, relative humidity, daily precipitation, and sunshine hour, were analyzed.

**Results:** The means of calcium carbonate concentrations measured by atomic absorption and EDTA analyses were  $17.41 \pm 0.4$  and  $20.93 \pm 1.25$   $\mu\text{g}/\text{ml}$ , respectively. The final ranges of calcium carbonate in formulation were found to be 42-43.5%. pH values were measured from 8.55 to 8.75. Furthermore, there were no significant differences between meteorological parameters in the year 2005 in comparison with previous year ( $P > 0.05$ ).

**Conclusion:** The high amounts of calcium carbonate in the formulation of Captan, resulting in alkaline hydrolysis followed by the increasing uptake of thiophosgen in peach trees and consequently interacting with meteorological parameters, have caused a broad spectrum of phytotoxicity in northern areas of Iran.

**Keywords:** Calcium Carbonate, Filler, Phtalimide Fungicide, Plant Toxicity.

### INTRODUCTION

Captan is a phthalimide type fungicide which is used to control some diseases such as peach leaf curl disease (*Taphrina deformans*) (1).

Captan was first registered for use in Iran in 1968 (2) and since then it has been used to help farmers to protect their crops from diseases. Captan has a wide range of applications and has become the most abundantly applied fungicide for apple and peach trees (3). It has a broad spectrum of functions and works extremely well under a wide range of growing and climatic conditions. It is also used for citrus fruits, almonds, potatoes and

ornamentals (4,5). The most currently used formulation of this fungicide in Iran is as 50% wettable powder (1,6). It is typically applied to orchards by a hand sprayer.

Captan is considered an irritating toxicant, especially for the eyes (7). Oral and dermal LD50 values of captan are 9000 and  $> 4500$  mg/kg, respectively (8). It is not classified by the Environmental Protection Agency (EPA) as a human carcinogen, however, prolonged high-level exposure to it can result in carcinogenicity (9).

Captan undergoes metabolism in the body after oral administration and is converted to tetrahydrophthalimide moieties which can be

1- Department of Medicinal Plants and Natural Resources, Imam Khomeini Higher Education Center, The Institute of Applied Scientific Education of Jihad-e- Agriculture, Karaj, Iran.

2- Department of Toxicology and Pharmacology, Faculty of Pharmacy, Tehran University of Medical sciences, Tehran, Iran.

\* Corresponding Author: Email: ebad@iheec.ir

excreted in urine (10). This fungicide is used each year in the northern districts of Iran by farmers as a general preventative procedure with an application rate of 3/1000 to control some diseases of fruit trees (5). Application time for Captan in the northern districts of Iran by farmers depends on weather conditions, but it generally begins around mid-April each year. There were several local reports from Northern provinces of Iran in 2005, regarding captan phytotoxicity, especially in peach trees (11,12). The extent of phytotoxicity was high in some places, so that the estimated loss of products in these regions was around 70 % or more. Preliminary studies for finding the agents that caused this extent of phytotoxicity showed that changes in weather conditions, such as reduced temperature and other factors such as the amount of cloud at spring time, induced this phytotoxic effect. However, the farmers believed that components of the captan formulation were responsible for this extensive phytotoxicity. In fact, they believed that all of the circumstances for spraying captan in peach trees were exactly the same as before. Hence, this study was carried out to determine whether the weather conditions alone induced this phytotoxic effect on peach trees or changes in the formulation of captan, as well as meteorologically parameters were responsible.

## MATERIALS AND METHODS

### Materials

Calcium carbonate, hydrochloric acid, ethylenediaminetetraacetic acid (EDTA) and eriochrome black T were obtained from Merck (Darmstadt, Germany). A one kilogram whole sample of the fungicide captan (WP 50 %) with good conditions of storage and the same batch number was obtained from Mazandaran province.

### Assay of Calcium Carbonate

The calcium content of sub-samples was assayed using a Shimadzu atomic absorption/flame emission spectrophotometer (model-AA680, Shimadzu Corporation, Kyoto, Japan) in accordance with the method described by Fausett *et al* (13).

To reduce the interfering agents in absorption, lanthanum chloride (0.2%) was

added to both the standard and unknown samples.

### Sample Preparation

The fungicide samples taken from the whole sample were reduced to the 50g size, and then dried by placing them in a 105-110 °C oven for at least 6 hours. The samples were then allowed to cool for 2-3 hours in a dessicator. After drying, a 0.5g sub sample was taken and the following procedures were carried out on it.

### Digestion Step

This process was completed by adding 5 ml of hydrochloric acid to the sample prepared from the previous step.

### Preparing the Standard Solution of Calcium Carbonate

Here, 0.25 g of the dried calcium carbonate was dissolved in 1 ml hydrochloric acid and then diluted with distilled water to a total volume of 100 ml (1.00mg ca/ml). The standard calcium carbonate solutions were then made through serial dilution to obtain 40, 30, 20 and 10 ppm concentrations.

### Instrument Analysis

The instrument conditions were set at the wavelength of 422.7nm. Flame fuel, air-acetylene, and a hollow cathode lamp were selected. The calcium concentrations in the unknown diluted solution were read from the calibration curve and the concentration of calcium in the original unknown solution was calculated with a factor dilution of 50.

### Calcium Carbonate Analysis by EDTA Titration

Briefly, 0.05 g of the prepared sample of captan was added to 50 ml of the distilled water. 5ml of the pH 10 buffer and 2 drops of indicator, 200 mg of the mixture of indicator, were then added and titrated by 0.01 N of EDTA to blue endpoint.

### PH survey

The pH of 0.05 % solution of captan was analyzed at room temperature using an electronic pH meter (HANNA Model 211, accuracy 0.01 pH).

### Meteorological analysis

Analysis of the meteorological parameters was carried out by the hourly amount of cloud, hourly relative humidity (RH), daily precipitation, and hourly bulb and wet bulb temperature data (minimum and maximum temperature). Data on meteorological parameters was obtained from a station located in Sari city with these specifications: Latitude 36 33 N; longitude 53 0 E; elevation 23.0 meters (14).

### Statistical Analysis

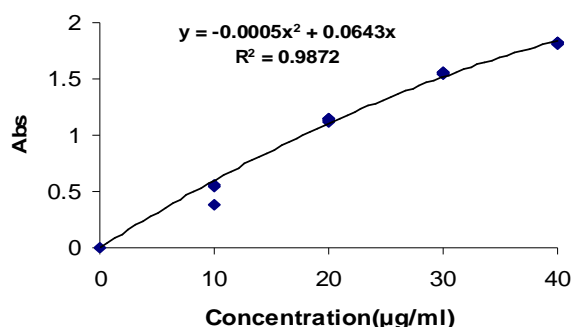
All comparisons were performed by unpaired t-test. P-values of less than 0.05 were considered significant.

## RESULTS

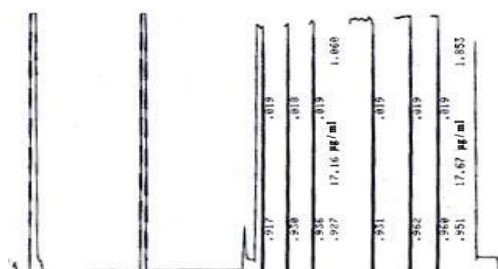
### Calcium Carbonate Quantification

Calcium carbonate concentrations in the formulation of captan are shown in Figure 1(a), (b). As indicated in Figure 1(a), there was a significant correlation ( $r^2 = 0.9872$ )

between Ca concentration and absorbance which was determined by atomic absorption method. Quantification of  $\text{CaCO}_3$  in samples from the formulation of captan which were plotted against a standard solution of  $\text{CaCO}_3$  is shown in Figure 1(b) and Table 1. As seen in Figure 1(b), the mean concentration of  $\text{CaCO}_3$  in the first and the second three replications were 17.16 and 17.67 ( $\mu\text{g}/\text{ml}$ ), respectively. The mean concentrations of  $\text{CaCO}_3$  in captan formulation determined by atomic absorption and EDTA titration methods are shown in Table 1. Mean concentrations of  $\text{CaCO}_3$  using atomic absorption and EDTA analysis were 17.41 and 20.93 ( $\mu\text{g}/\text{ml}$ ), respectively Table 1. As indicated in this table, the final range of  $\text{CaCO}_3$  in captan formulation was determined to be from 42 to 43.5%. The range of pH measured in a 0.5 % solution from captan was from 8.55 to 8.77 (Table 1).



**Figure 1 (a).** Correlation of Ca concentrations (X) versus absorbance (Y) determined by atomic absorption method



**Table 1.** Measurement of calcium carbonate in captan formulation based on two different methods

Sample name & formulation	Mean of $\text{CaCO}_3$ concentration ( $\mu\text{g/ml}$ ) by atomic absorption	Mean of EDTA used (ml) in titration	Final range of $\text{CaCO}_3$ (%) in formulation of fungicide	pH range (0.5%)
Captan, WP50%	17.41 $\pm$ 0.4	20.93 $\pm$ 1.25	42-43.5	8.57-8.77

## Results of Meteorological Analyses

### Comparisons of Temperatures

Results of minimum and maximum temperatures which were compared in a one-month period are indicated in Table 2. No significant differences between the minimum and maximum in the years 2004 and 2005 were

observed. Daily mean of minimum temperatures during one month for the years 2004 and 2005 were 8.48 and 8.86 ( $^{\circ}\text{C}$ ), respectively. On the other hand, daily means of maximum temperatures for these two years were 20.14 and 18.73 ( $^{\circ}\text{C}$ ), respectively (Table 2).

**Table 2.** Comparison of minimum and maximum temperatures in the years 2004 and 2005 from 21 March to 20 April

Meteorological Parameters	Daily mean of Min. Temperature ( $^{\circ}\text{C}$ ) in 2004 and 2005 during one month		Daily mean of Max. Temperature ( $^{\circ}\text{C}$ ) in 2004 and 2005 during one month	
	2004	2005*	2004	2005*
Mean	8.48	8.86 <sup>ns</sup>	20.14	18.73 <sup>ns</sup>
Number of point	31	31	31	31
STD deviation	2.75	4.05	5.92	7.96
Minimum	3.60	1.40	8.20	8.60
Maximum	13.60	19.20	33.40	38.80

\* P-value ( $P>0.05$ ) and ns means not significant.

### Comparisons of Relative Humidity and Daily Precipitation

Percentages of relative humidity compared between two years within one month are shown in Table 3. As it can be seen, no significant differences in RH were observed. The percentage of relative humidity for the years 2004 and 2005 were 75.64 and 72.58,

respectively. The results obtained from daily precipitation in these two years specified that there were no significant differences between the amounts of precipitation during the time of phytotoxicity (Table 3). These results also showed that daily precipitation values for the years 2004 and 2005, within one month, were 4.31 and 2.77mm, respectively (Table 3).

**Table 3.** Comparison of the percentage of relative humidity and daily precipitation in the years 2004 and 2005 from 21 March to 20 April

Meteorological Parameters	Relative humidity(%) in 2004 and 2005 during one month		Daily precipitation(mm) in 2004 and 2005 during one month	
	2004	2005*	2004	2005*
Mean	75.64	72.58 <sup>ns</sup>	4.31	2.77 <sup>ns</sup>
Number of point	31	31	31	31
STD deviation	11.27	16.84	10.86	6.62
Minimum	43.60	35.40	0.000	0.000
Maximum	95.30	94.00	49.00	29.00

\* P-value ( $P>0.05$ ) - ns means not significant.

### Comparison of Cloudiness

Comparison of the amount of cloud and bright sunshine hours showed no significant differences between these meteorological parameters during one month in two successive

years ( $P = 0.06$ ). The percentage of cloud during one month in 2004 and 2005 were 46.71 and 62.33, respectively. Bright sunshine hours for these two years were 7.00 and 5.19 hours, respectively (Table 4).

**Table 4.** Comparing the amount of cloud (percentage) and bright sunshine hours in the years 2004 and 2005 from 21 March to 20 April

Meteorological Parameters	Amount of cloud(%) in 2004 and 2005 during one month		Bright Sunshine (hr) in 2004 and 2005 during one month	
	2004	2005*	2004	2005*
Mean	46.71	62.33 <sup>ns</sup>	7.00	5.19 <sup>ns</sup>
Number of point	31	31	31	31
STD deviation	37.01	34.55	4.51	4.58
Minimum	0.00	0.00	0.00	0.00
Maximum	100.00	100.00	11.20	11.30

\* P-value ( $P > 0.05$ ) - ns means not significant

### DISCUSSION

Measurement of calcium carbonate by atomic absorption and titration methods in our research showed that large amounts (42- 43.5 %) of this compound were present in the formulation of captan (Table 1). Generally, the appropriate amount of components considered in the formulation of captan consist of 50%  $\pm$  2 of active ingredient, the wetting and dispersant agents (<10%), and the filler compounds such as kaolin (>40 %) (15, 16). The company that produces and distributes captan has declared that there is an equal ratio of kaolin and calcium carbonate in the formulation of captan. The presence of this high amount of calcium carbonate has been previously reported by another research group (17). Considering the high quantity of measured calcium carbonate and the permissible amount of other components in this formulation, it is obvious that the ratio of kaolin and calcium carbonate are not equal.

Kaolin is an important filler which is mostly used in the formulation of phthalimide fungicides (18,19). Since the interactions between kaolin particles and other components, especially lignosulfonate type surfactants are so low or sometimes lacking, this good compatibility is reason enough for placing

kaolin in most pesticide formulations (16). In addition, the properties of kaolin will not be changed in broad pH ranges. In agriculture, Kaolin is used as a carrier of wettable powder formulations to control certain fruit tree diseases. Recent advances in formulations, such as the adoption of particle film technology in the formulation of pesticides and its use as adsorbent in medical therapy has made kaolin receive more attention than before in medicine and agriculture (19,20). Compared to kaolin, calcium carbonate has a pH range of 7-9 which is completely dependent on its concentration in the formulation. Our data showed that the pH measurement in captan formulation was close to 9.0 (Table 1). It seems that this level of pH qualifies the fungicide for phytotoxic effects because captan undergoes hydrolysis and breakdown at a rapid rate in alkaline pH ranges (21). Such alkaline pH can result in the release of thiophosgen, a compound that is effective in controlling the plant pathogen or phytotoxicity (3). Compatibility of  $\text{CaCO}_3$  with the other components in fungicide formulation is very important and by choosing the most suitable wetting and dispersant agents,  $\text{CaCO}_3$  as a filler enables captan to be formulated in accordance with FAO specifications (22).

The analysis of meteorological parameters in this study showed that there are no significant

differences between metrological parameters such as the amount of cloud, hours of bright sunshine, relative humidity, daily precipitation, the means of maximum and minimum temperatures between 2004 and 2005 (Table 2-4). We previously showed that this wide range of toxicity in fruit bearing trees could not just be linked to little changes in weather conditions (23). Farmers and gardeners from districts in which phytotoxicity occur are used to applying captan on fruit bearing trees yearly around mid April. We did not observe any complete significant differences between meteorological parameters in 2004 and 2005. However, the risk of captan for fruit trees will be greatest when it is used in cloudy, cool, and damp weather conditions (24). Interestingly, in Yazd province which is located in the south of Iran and has completely different weather conditions from the north of Iran, no phytotoxicity resulting from the use of captan was reported (25). The reasons for this lack of phytotoxicity in Yazd province compared to the high amount of phytotoxicity that occurred in northern areas of Iran in peach trees should be sought in the presence of  $\text{CaCO}_3$  and its amount in formulation of captan. It seems possible that  $\text{CaCO}_3$  in warm weather where relative humidity is low can protect captan from hydrolysis while in the Northern provinces of Iran and places where humidity is high; there are higher rates of thiophosgen release in plants following alkaline hydrolysis. Furthermore, thiophosgene uptake in plants such as peach trees which have a thin layer of cuticle will be enhanced. Consequently, thiophosgene enters into cells and kills them, resulting in plant tissue damage. On the other hand, some enzymes such as glutathione S-transferase which are involved in detoxification of plants will be compromised and cannot properly play their role in defense mechanisms (26). We can conclude that the increased rate of thiophosgen due to alkaline pH and also high relative humidity in northern districts of Iran are the prominent reasons which account for this wide phytotoxicity in peach trees. For these reasons we obviously found that  $\text{CaCO}_3$  is not an appropriate substitute for kaolin in formulation of the fungicide captan and should not be used in northern districts of Iran. We presume that the presence of cloudy, cool, and

damp weather can collectively affect the severity of phytotoxicity in peach trees.

## ACKNOWLEDGMENTS

The authors are grateful to Iranian Meteorological Organization, Plant Protection Organization, and Council of Iranian Official Experts for their cooperation.

## REFERENCES

1. Iranian Plant Protection Organization (IRPPO); Ministry of Agriculture, The list of important pests, diseases and weeds of agricultural products in Iran and the recommended poisons against them based on the recommendations of the Committees determining the poisons for pests control and their applications, Tehran: Agriculture Ministry; 1991.
2. Iranian Plant Protection Organization (IRPPO) Ministry of Agriculture, Registration for eligible pesticides; Captan. Tehran: Plant protection Organization. 1968.
3. United States Drug Administration (USDA). Agricultural Chemical Usage, 2003 Fruit Summary. Washington (DC): National Agricultural Statistics Service; 2004; 14:146.
4. British Crop Protection Council (BCPC). A world compendium: The pesticide manual. 11 ed. C.D.S.Tomlin, editor. Farnham 1997:177-179.
5. Joint Meeting of the FAO/WHO Panel of Experts on Pesticide Residue (JMPPR). Captan; Pesticide residues in food, 1995. Geneva (Swiss): World Health Organization. 1996.
6. Iranian Plant Protection Organization (IRPPO). The complete list of lawful poisons in Iran. Tehran: Center for Deeds and Scientific Documents of Ministry of Jihad-e-Agriculture; 2008:296.
7. United States Environmental Agency (USEPA). Registration Eligibility Decision, Captan, EPA. Washington (DC) 1999. Report No: 738-R-99-015.
8. Stevens JT, Breckenridge C. Crop protection chemical. In: Hayes A, editor. Principle and methods of toxicology. Philadelphia: Taylor & Francis. 2001; 565- 684.
9. Arce GT, Gordon EB, Cohen SM, Singh P. Genetic toxicology of folpet and captan. Crit Rev Toxicol. 2010; 40:546-74.
10. Krieger R, Dinoff T. Captan fungicide exposures of strawberry harvesters using THPI as a urinary biomarker. Archives of environmental contamination and toxicology. 2000; 38(3):398-403.

11. Organization of Jihad-e-Agriculture of Golestan (OJAG). Plant Protection Office. Captan Phytotoxicity, Gorgan (Iran): Plant Protection Management. 2005.
12. Organization of Jihad-e-Agriculture of Mazandaran (OJAM), Plant Protection Office. Captan phytotoxicity. Sari (Iran): Plant Protection Management. 2005.
13. Fausett H, Gayser C, Dash AK. Evaluation of quick disintegrating calcium carbonate tablets. *AAPS PharmSciTech*. 2000; 1(3):37-43.
14. Iranian Meteorological Organization (IRMO), Department of Publications. Meteorological data from Sari Station, 2004-2005. Tehran (Iran): Iranian Meteorological Organization; 2008.
15. Food Agriculture Organization (FAO). FAO Specifications for plant protection products, Captan. Rome1990. Report No: AGP: CP/57.
16. Knowles DA. Chemistry and technology of agrochemical formulations: Kluwer Academic Pub; 1998.
17. Industrial Research Center for Plant Pesticides (IRCP). Analysis results of captan. Part No: 273501. Karaj (Iran): Industrial Research Center for Pesticides. 2005.
18. Virta RL. Clay and Shale. *US Geological Survey Minerals Yearbook-2005*. 2005:18.1-22.
19. World Health Organization (WHO). Bentonite, kaolin, and selected clay minerals. 2005:175.
20. Glenn DM, Puterka GJ. Particle films: a new technology for agriculture. *Horticultural reviews*. 2005:1-44.
21. Seaman A, Riedl H. Preventing decomposition of agricultural chemicals by alkaline hydrolysis in the spray tank. 1986.
22. Ajinomoto-omnichem.com [homepage on the Internet] Belgium: Captan 50 %. Omni Chem Division; 2005. Available from: <http://www.ajinomoto-omnichem.com/index.html>.
23. Ebadollahi AR. Study of some important factors affecting the phytotoxicity of captan fungicide. 10th Iranian congress of toxicology and poisoning. Tehran, Iranian Society of Toxicology. 2009:104.
24. Rosenberger D. Captan caution. *Apple Pest Report*. 2003; 11: 5. Available from: <http://pmo.umext.maine.edu/apple/AppPestReport/OldApplePestReports/AppPestRept-2003-06-01.htm>.
25. Organization of Jihad-e-Agriculture of Yazd (OJAY), Plant Protection Office. Report of performance evaluation of captan in 2005, Yazd (Iran): Plant Protection Management. 2005.
26. Teisseire H, Vernet G. Effects of the fungicide folpet on the activities of antioxidative enzymes in duckweed (*Lemna minor*). *Pesticide Biochemistry and Physiology*. 2001; 69(2):112-7.