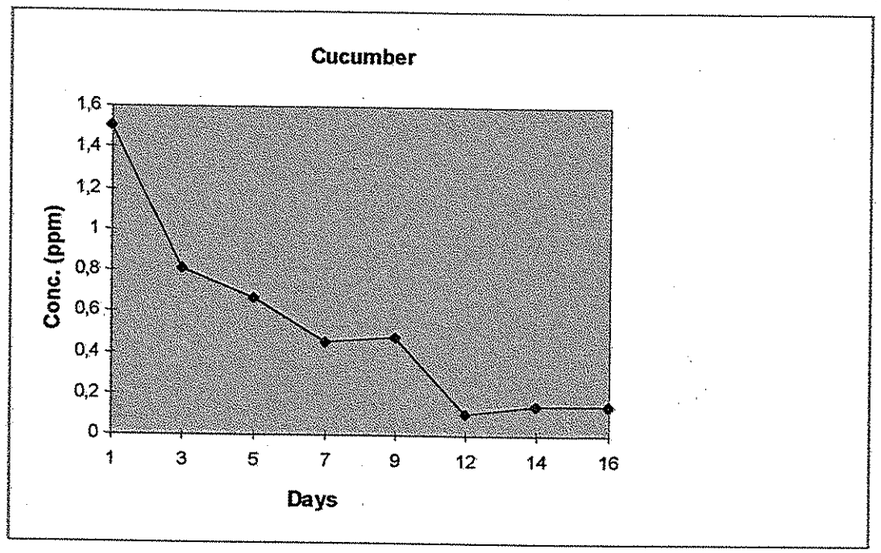

Figure 1: Degradation of mancozeb residue in cucumber after spraying

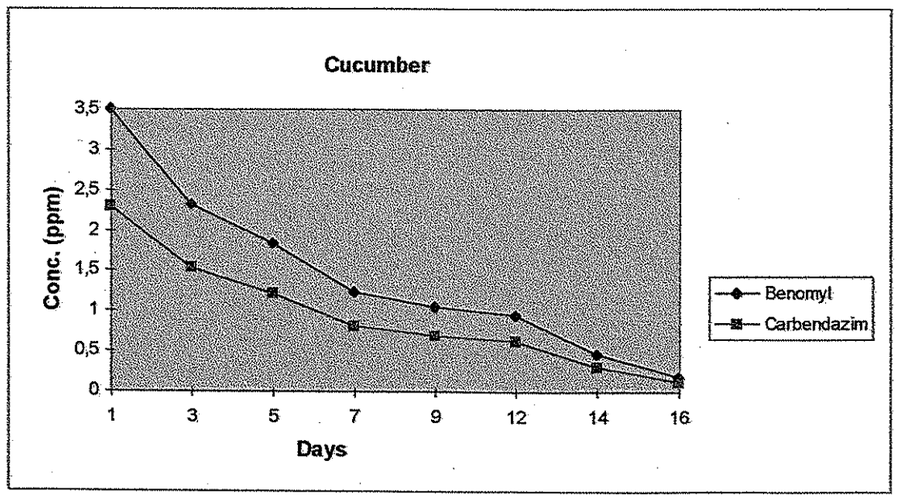

Figure 2: Degradation of benomyl and carbendazim for cucumber after spraying

Table 3: The mancozeb and benomyl residues for tomatoes (ppm)*

Date	Sampling day	Mancozeb residue (ppm)	Benomyl residue (ppm)	Temperature (°C)	% RH
11.03.1998	Spraying			19.0	52.3
12.03.1998	1. day	4.51	3.295	15.1	68.3
14.03.1998	3. day	4.50	3.231	14.1	70.0
16.03.1998	5. day	4.48	2.582	12.9	72.0
18.03.1998	7. day	3.97	2.236	9.2	73.7
20.03.1998	9. day	3.76	2.088	11.1	59.7
23.03.1998	12. day	3.02	2.085	12.1	67.7
25.03.1998	14. day	1.65	1.847	10.1	53.7
27.03.1998	16. day	0.03	1.053	14.8	61.7

Mancozeb residues for tomatoes: Turkish tolerance value: 1.0 ppm; CODEX value: 3 ppm
Benomyl residues for tomatoes: Turkish tolerance value : 0.5 ppm; CODEX value: 5 ppm

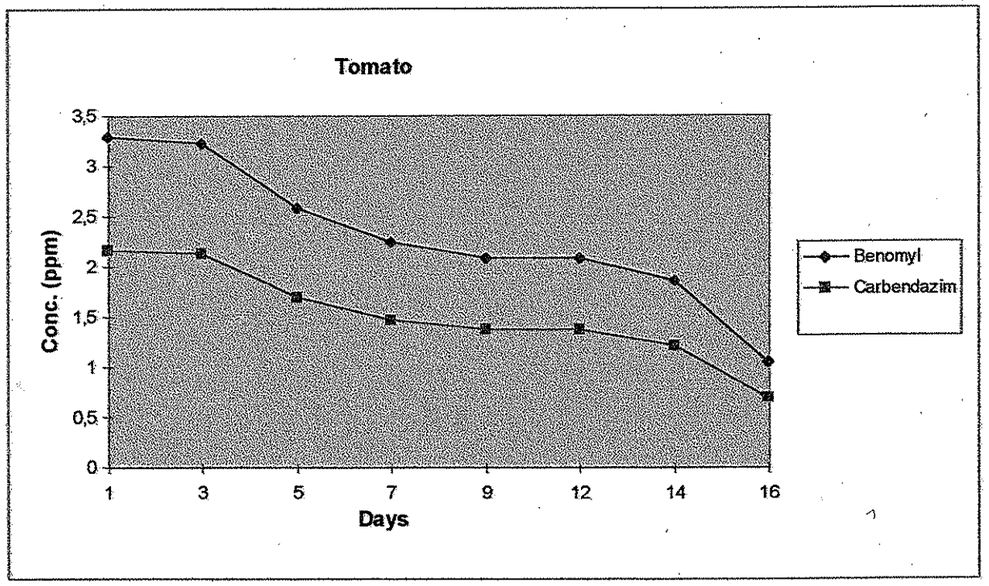

Figure 3: Degradation of benomyl and carbendazim for tomato fruits after spraying (days)

Table 4: Mancozeb and benomyl residues for green pepper (ppm)*

Date	Sampling day	Mancozeb residue (ppm)	Benomyl residue (ppm)	Temperature (°C)	% RH
09.10.1997	Spraying			26.5	54.0
10.10.1997	1. day	7.16	17.89	25.2	64.7
13.10.1997	4. day	8.43	17.63	23.2	81.3
15.10.1997	6. day	5.42	11.79	25.6	60.7
17.10.1997	8. day	4.86	4.01	25.5	65.3
20.10.1997	11. day	4.70	3.81	16.1	83.3
23.10.1997	14. day	4.55	3.11	18.2	77.0
27.10.1997	18. day	3.00	1.84	18.7	78.7

* Mancozeb residues for green pepper: Turkish tolerance value: 1.0 ppm
* Benomyl residues for green pepper: Turkish tolerance value: 1.0 ppm, CODEX value: 5 ppm

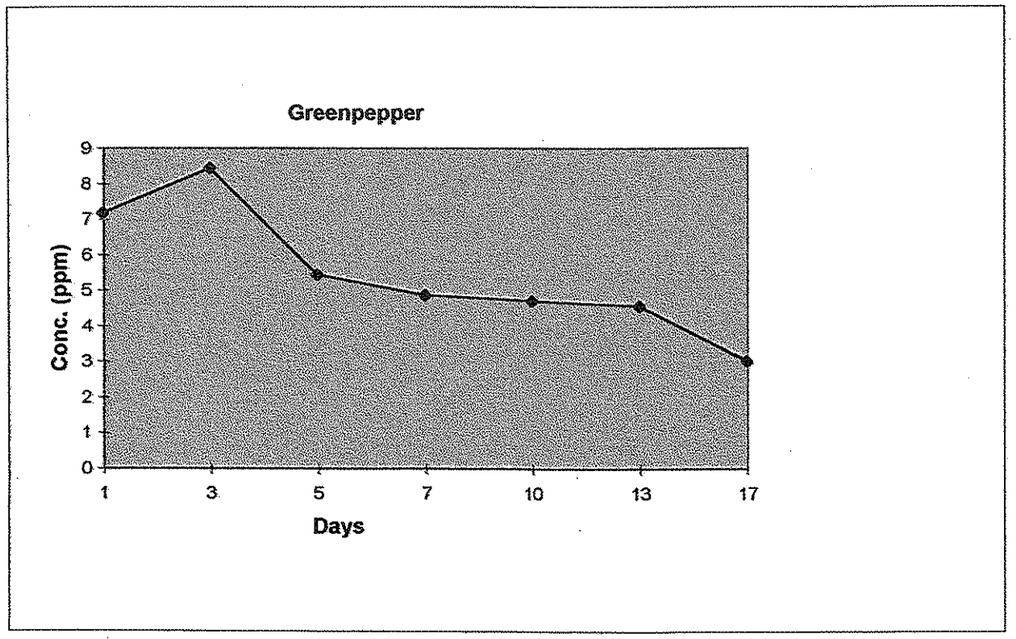

Figure 4: Degradation of mancozeb residues in green pepper after spraying (days)

Table 2: The Mancozeb and benomyl residues found in cucumber (ppm)*

Date	Sampling day	Mancozeb Residue (ppm)	Benomyl Residue (ppm)	^{0}C	% RH
11.03.1998	Spraying			19.0	52.3
12.03.1998	1. day	1.50	3.496	15.1	68.3
14.03.1998	3. day	0.80	2.316	14.1	70.0
16.03.1998	5. day	0.66	1.827	12.9	72.0
18.03.1998	7. day	0.45	1.236	9.2	73.7
20.03.1998	9. day	0.47	1.048	11.1	59.7
23.03.1998	12. day	0.10	0.943	12.1	67.7
25.03.1998	14. day	0.14	0.470	10.1	53.7
27.03.1998	16. day	0.14	0.171	14.8	61.7

Mancozeb residues for cucumber: Turkish tolerance value : 1.0 ppm, CODEX value: 0.5 ppm
Benomyl residues for cucumber: Turkish tolerance value : 0.5 ppm, CODEX value: 0.5 ppm

ingredients). Therefore, it is of great interest to know the contamination values of benomyl and mancozeb primarily used during cultivation periods. Additionally, these values were determined for carbendazim, the main metabolite of benomyl. Carbendazim was determined spectrophotometrically at 281 nm (see benomyl).

The recovery values for mancozeb and benomyl were found as 80 % and 73 %, respectively. The residue values in cucumber fruits were given in Table 2 and the degradation curve in Fig. 1.

As shown in Fig. 1 and Table 2, Mancozeb residues for cucumber decreased below 0.5 ppm, which is the Codex tolerance value, after 7th day of spraying, after third day it became 0.80 ppm, that is lower than the Turkish tolerance value 1 ppm. Büyükurvay at al. [8] found in their study that mancozeb residue decreased below the Turkish tolerance value after third day and below the Codex value after five days. Benomyl residue for cucumber is also given in Table 2 and degradation curve in Fig. 2. The benomyl residues for cucumber decreased below the Turkish and Codex tolerance limits 12 days after spraying.

The degradation values of MBC are additionally determined and shown in Fig. 2. MBC follows a similar degradation as benomyl. The values decrease from 2.3 ppm one day after spraying to 1.5 ppm after 3 days and 8.0 ppm after 7 days (CODEX value: 0.5 ppm).

Internationally, MRL values are for the total residue arising from the use of benomyl or carbendazim, often additionally including thiophanate-methyl, expressed as carbendazim. These values range from 2 (European Union) to 5 ppm (New Zealand, Canada) in the case of wine and table grapes [9].

The residue values and degradation rates for both fungicides and carbendazim obtained after spraying of tomatoes and green pepper are shown in Tables 3 and 4 / Figs. 3 to 6.
As shown in Table 3 and Fig. 3, mancozeb residues for tomatoes are below the Codex value after 12 days and after 16 days below the Turkish tolerance value from spraying.

Benomyl residues in tomatoes were found to be above the Turkish tolerance limits until the 16th day after spraying but below the Codex limit from the first day (Table 3, Fig. 4).

Mancozeb residue values for green pepper (Fig. 5, Table 4) vary between 8.4 and 3.0 ppm. The values were still above the Turkish tolerance limit even after 18 days of spraying (temperature 16.1 to 26.5 °C). The waiting period for vegetables after use of mancozeb in Turkey normally is 14 days [10]. Zeren and Deger [11] formerly indicated that harvesting of tomatoes and green pepper is already possible 3 and 4 days after the last mancozeb spraying. These experiments were done at temperatures of 25.1 to 38.6 °C. Temperature seems to play an important role for degradation of both fungicides. The higher the temperature, the faster the degradation.

Also benomyl and carbendazim values in the greenhouse experiments with green pepper are extremely high after the 18th day of spraying (Table 4, Fig. 6). Therefore, it must be concluded according to this study that during relatively cold cultivation periods the time of harvest must be at least 18 days after the last spraying.

in polyethlene bags until working them up. Benomyl (DuPont, purity 95%) and mancozeb (Elf Atochem, purity 87.2%) were used as standards to quantify the fungicides in residue analysis.

The tested fungicides and their rates of application during the greenhouse experiments are as given in Table 1.

A 100 m^2 area was used as test-field during the whole study. In October,1997 the green pepper plants were treated with pulverized benomyl and mancozeb. Almost 1 kg aliquots of green pepper were harvested 1, 4, 6, 8, 11, 14 and 18 days after pulverisation and kept at -18 °C until analysis. Tomatoes and cucumber were similarly treated with benomyl and mancozeb during first half of March 98. About 1 kg of ripe fruits were harvested after 1, 3, 5, 7, 9, 12, 14 and 16 days of fungicide treatment and also kept at -18 °C. Mancozeb residue was analysed according to the methods of Hennet *et al.* [5] and Lo and Ho [6]. Benomyl residues were analyzed according to Anonymous [7].

Mancozeb residue analysis

50 g aliquots of samples were homogenized in a blender. The homogenate was filled into a round-bottom three-necked flask and 150 ml 2N H_2SO_4 and some boiling stones were added. Nitrogen gas at a flow speed of 8-10 ml min^{-1} was passed through a washing bottle filled with „Viles reactive" solution (0.05 g copper acetate, 20 ml triethanolamine and 1 ml diethylamine dissolved in Na_2SO_4-treated ethanol). This washing bottle was connected with one neck of the reaction flask by means of a tube. In the neck on top of the flask a reflux condenser was connected with three further washing bottles, the first filled with lead acetate solution and the two others again filled with „Viles reactive" solution. Now the samples have been boiled for 90 min without vacuum and after boiling the two washing bottles at the end of the reaction plant have been filled up to 24 ml with „Viles reactive" solution. 15 min after addition the uniform solutions have been measured against „Viles reactive" as blank at 430 nm with a Shimadzu Mark 160-A model UV-VIS spectrophotometer. The values corresponding to fungicide concentration have been found by multiplication of the measured values with the constants of 1.74 (maneb), 1.75 (mancozeb), 1.90 (propineb) and 1.58 (thiram).

The samples of tomatoes which have not been sprayed, have also been homogenized and spiked with a certain amount of standard pesticide. These control mixtures are also boiled as described above to determine the percentage residues of pesticides.

Benomyl and carbendazim residue analysis

100 g of samples were crushed into small particulates and extracted with 300 ml ethyl acetate and 12 g sodium bicarbonate for 30 min. The diluted phase was filtered through 50-75 g sodium sulphate. These ethylacetate extracts were extracted two times with 25 ml of 1 N H_2SO_4 and one time with 25-35 ml of distilled water. The combined H_2SO_4 phases were washed with 25 ml of chloroform and then neutralized with 100 ml of $NaHCO_3$. The phases were collected in a 500 ml extraction funnel and extracted three times with 25 ml of chloroform. The combined extracts were filtered through sodium sulphate and evaporated until dryness. The residue was dissolved in 25 ml 1 N H_2SO_4 and measured against 1 N H_2SO_4 at 281 nm.

Table 1: Fungicide properties and spraying practises

Effective Material	Effective Material (%)	Formula	Dose	Time between last spraying and harvest (days)
Benomyl	50	White solid powder	100g/100 L water	14-21
Mancozeb	80	White solid powder	200g/100 L water	14

RESULTS AND DISCUSSION

Along with squash, melons, and pumkins, cucumbers are member of the family Cucurbitaceae and collectively with these crops belong to the group of vegetables known as cucurbits or vine crops. Green peppercorns are the immature, fresh and green berries of the pepper vine - *Piper nigrum*. These berries are plucked fresh from the vines and processed into various speciality products while retaining their natural green colour and flavour. Frozen, dehydrated, brined and frozen green pepper products are mainly exported by India. Together with tomatoes, green pepper and cucumber they are also cultivated in Içel provinces and consumed immediately after harvest in a lot of different products of the Turkish cuisine (for example Shish Kebap with 2 green peppers and 4 tomatoes as

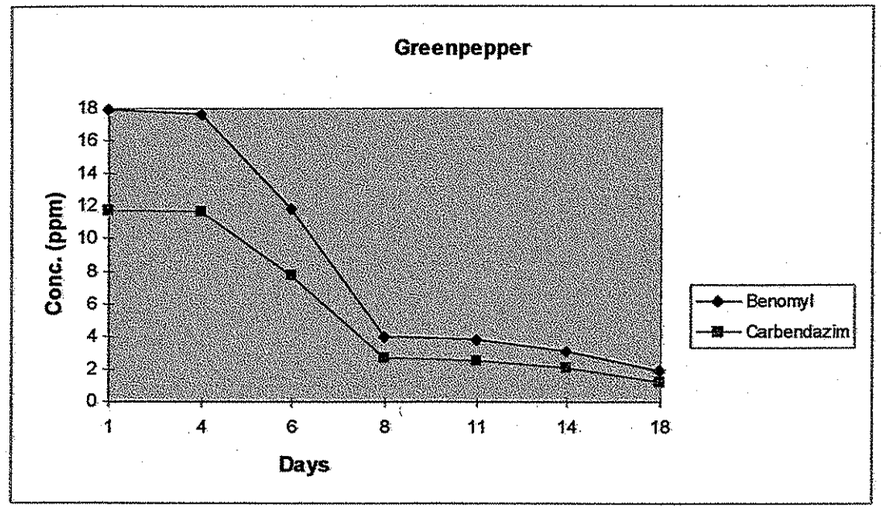

Figure 5: Degradation of benomyl and carbendazim in green pepper after spraying (days)

REFERENCES

[1] World Health Organization, Environmental Health Criteria 148 - benomyl, Geneva, Switzerland (1993)
[2] Forest Servive, Pesticide Background Statements, vol. II: Fungicides and Fumigants, U-S. Department of Agriculture, Agriculture Handbook No. 661 (1986)
[3] Food and Agriculture Organization of the United Nations, Pesticide Residues in Food - 1983: Evaluations, FAO Plant Production and ProtectionPaper 61 (1983)
[4] National Research Council, Regulating Pesticides in Food: The Delaney Paradox, National Academy Press, Washington, D.C. (1987)
[5] Henriet, J., Dejonckheere, W., Galoux, M., Gorsts, L., Rogirst, W., Wambeke, E. V. and Zenon-Roland, L., Methodes Normalises d'Analyse des Residues de Pesticides Dans Les Fruits et Dans Les Legumes, Revue de l'Agriculture **4** (33), 797-812 (1980)
[6] Lo, C. C. and Ho, M. M., Determination of Ethylenethiourea in Ethylenebisdithiocarbamate Formulations, Pestic. Sci. **37**, 247-251 (1993)
[7] Anonymous, BMC Estimation with Spectrophotometer: Apples, Pineapples, Strawberries, Cereals, Cucumbers, Potatoes, Lettuce, Stonefruit, Cirapes, Sugarbeet, BASF Akt. Agr. Res. Sta., Germany, (1985)
[8] Büyükurvay, S., Karaca, C. and Kocatürk, S., Domates ve hiyarlarda Dithiokarbamat ve ETU Kalintilarinin Arastirilmasi, Zir. Müc. Ar. Ens. Md., Ankara (1996)
[9] The Australian Wine Research Institute, MLR Database ACN 007 558 296 (1999)
[10] Anonymous, Zirai mücadele ilâglari uygulamalarinda son ilaclama ile Hasat Arasinda Gecmesi Gereken Asgari sürelerle Ilgili Teblig (91/12), Tarim ve Köyisleri Bak., KorKont. Gen. Md. Yay. 16 pp. (1991)
[11] Zeren, O. and Deger, A., Investigation on Degradation Time of Mancozeb and Ethylenethiourea on Tomatoes and Peppers, International Conference Forging Regional Cooperation in the Mediterranean Basin (May 27-28 1999, Arles, France), p. 124 (1999)

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Investigation of degradation period of mancozeb and benomyl used as fungicides in the vegetation of tomatoes, cucumber and green pepper in Içel Provinces, Turkey.

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Key words: Degradation period, benomyl, mancozeb, carbendazim, tomatoes, cucumber, green pepper

SUMMARY: The degradation periods of the fungicides, benomyl, its degradation product carbendazim and mancozeb, were investigated after application on cucumber, pepper and tomatoes grown under agricultural conditions of Mediterranean region in Turkey. The fruits grown under greenhouse conditions were also sprayed by these fungicides. Approximately 1 kg samples were taken before and after 1, 3, 5, 7, 9, 12, 14, 16, and 18 days of spraying and kept deep-frozen at - 18 °C until residue analysis. The changes in residue amounts were determined up to the time of their falling below the tolerance levels.

INTRODUCTION

In the Mediterranean agricultural region of Turkey greenhouse and open cultivation of tomatoes, cucumber and green pepper has been mainly practised, especially in Içel provinces. Four times of the year the crops were harvested, and the farmers used extreme amounts of pesticides. Due to this extreme spraying, especially of fungicides, the problem of a possible high contamination of these freshly marketed fruits arises, because spraying is used often without taking into consideration the periods of time between the last spraying and the harvest and marketing. Although degradation periods in plants for mancozeb and benomyl usually are at least 14 days, all the tomatoes are harvested after a short time of spraying, especially in this region.

The farmers are forced to use high amounts of fungicides, because the climate and the ecological conditions are suitable for the fungal diseases. The greenhouse plants are sprayed every week. A residual problem arises because it is well-known that the residues are hazardeous for human health and environment. Benomyl (methyl 1-[(butylamino)carbonyl]-H-benzimidazole-2-yl-carbamate) which is the mostly used fungicide in this region, is not directly toxic but has a systemic effect. It is stable in soil and, furthermore, not recommended because of its mutagenic and teratogenic effects. Benomyl completely degrades to carbendazim within several hours in acidic or neutral water. The half-life of carbendazim is two months. In strongly alkaline water, benomyl breaks down to another compound [1]. Benomyl is absorbed by plants through the roots or the above-ground tissue. It accumulates in veins and at the leaf margins [2]. The metabolite carbendazim (methyl-2-benzimidazolecarbamate - MBC) seems to be the fungicidally active reagent. However, benomyl residues are quite stable, with 48 to 97% remaining as the parent compound 21 to 23 days after application [2]. The residues are easily extracted from the plant in hot water [3]. Because of results of conflicting studies concerning its metabolite MBC, the U.S. Environmental Protection Agency classified benomyl as a possible human carcinogen [4]. Mancozeb is a coordination product of zinc ion and manganese ethylene bisdithiocarbamate. ETU may be present as a breakdown product or contaminant. The EPA considers ETU to be of toxicological concern due to a potential hazard posed to the human thyroid based on animal studies. ETU is also listed as a Class B (probable human) carcinogen. Because of the cancerogenic, mutagenic and teratogenic properties of the afore-mentioned fungicides and their metabolites, these studies focus on their degradation periods in cultivation of tomatoes, cucumber and green pepper, the commonly consumed food crops in Içel region.

MATERIALS AND METHODS

Tomatoes, green pepper and cucumber were cultivated in a greenhouse experiment. They were harvested and kept