Egypt. J. Agric. Res., 81 (4), 2003.

PRE HARVEST GRAY MOLD CONTROL AND FUNGICIDE RESIDUES IN THE HARVESTED STRAWBERRIES

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(Manuscript received 2 Feb. 2003)

Abstract

Protective pre harvest spray of three fungicides *i.e.* Benlate [Methyl 1-(butyl carbamoyl)-2-benzimidazol carbamate], Prochloraz [1-(N-propyl-N- (2-(2,4,6 -tri-chlorophenoxy) ethyl) carbamoyl) imidazole] and Vinchlozolin [3 - (3,5 Dichlorophenyl) 5-ethenyl-5-methyl-2,4-oxazolidinedione] were applied to Strawberry (*Fragaria chiloensis Duchesne* var *ananassa* Baily) (cv. Pajaro) at 0.5, 1, 2 g l^{-1} under either natural or artificial infection. The applied fungicides significantly reduced the level of gray mold caused by *Botrytis cinerea*. Excellent control was obtained with Vinchlozolin, at the rate of 2.0 g l^{-1} which developed 4.8% and 1.2% rot for two successive years compared with 28.5% and 16.2% in the check under artificial infection conditions.

Gas chromatographic detection of Vinchlozolin in treated strawberries revealed residues of 0.0354, 0.3871 and 0.0838 ppm after two days, one and two weeks, respectively, in strawberries treated with 2.0 g l^{-1}.

INTRODUCTION

The gray mold fungus, *Botrytis cinerea* Pres. ex Fr. is the most common cause of post harvest rot of strawberry (*Fragaria chiloensis* var. *ananassa*). This disease causes severe losses in the field before harvest and during transit of the harvested strawberries (Jarvis, 1962 a and b; Dennis *et al.*, 1979 and Jordon, 1978). Losses due to *Rhizopus stolonifer* (Ehrenb ex Fr.) Vuillemin, *Phytophthora cactorum* (Leb & Cohn) Schroet, *Pythium spp., Mucor mucedo* L. ex pries, *Fusarium spp., Alternaria spp.* and *Trichoderma spp.* were also reported (Stoddard *et al.* 1924, Harvey and Pentzer, 1960; Powelson, 1960 and Jarvis, 1962 a). Systemic fungicides present a primary means of controlling post harvest diseases (Eckert and Ogawa, 1988). However, they have come under food safety measures as posing potential risks when applied to food (National Research Council, 1987). As the tested fungicides are still in experimenting and use in countries of origin, they were used on strawberries in this study.

Public and scientific concerns about the presence of synthetic chemicals in the food chain have generated an interest for the detection of the fungicidal residues in fresh fruits and vegetables. This study was undertaken to evaluate the gray mold pre harvest spray and fungicide residues in the harvested strawberries.

MATERIALS AND METHODS

Effect of fungicides on growth rate of Botrytis cinerea:

The fungicides given in Table (1) were tested on potato dextrose agar plates (PDA) at concentrations of 0, 1, 5, 10, 25, 50, 100, 200, and 400 ppm of the active ingredients in four replicates. Plates were inoculated with a disc (5 mm) of *B. cinerea* and incubated at 18 ± 2°C. Check plates were similarly prepared and the reduction in colony diameter was determined after 5 days.

Table 1. Fungicides used in the *in vitro* tests

Common name	Active ingredient	Trade name	Producer
Benlate	50% [methyl 1- (butyl carbmoyl) - 2- benzimidazol) carbamate]	Benlate	Du Pont Ltd
Ronilan	50% [3- (3, 5 Dichlorophenyl) 5-ethenyl - 5- methyl-2, 4-oxazolidinedione]	Vinchlozolin	BASF
Prochrolaz	50% [1-(N-propyl-N- (2-(2,4,6 – tri-chlorophenoxy) ethyl) carbamoyl) imidazole]	Octave	Shiring, England

Field Trails:

Chemical control of the main pathogen causing rot of strawberry fruits was carried out on Pajaro cultivar, using 3 fungicides under either natural infection or artificial inoculation with *B. cinerea* at the rate of 10^4 spore/ml. Benlate 50% wp, Prochloraz 50% wp and Ronilan 50% wp were sprayed at 3 rates (0.5, 1.0 and 2.0 g l^{-1}) at three week- intervals commencing at flowering and green fruit development till seven days before harvest. Four replicates were used per treatment. The experiment was conducted during 2 successive seasons. All agricultural practices were carried as usual. Notes were recorded on fruit rot disease and average number of rotten fruits was calculated.

Analysis of Vinchlozolin (Ronilan) Residues In Treated Strawberry Fruits:

a . Sample treatment

Strawberry plants were treated twice at two week- intervals, with Vinchlozolin. Three concentrations (0.5 g l^{-1}, 1 g l^{-1} and 2 g l^{-1}) were experimented. After two, seven and fifteen days, fruits from about 30 plants, per each treatment were taken and kept in a closed plastic container. A 50 g sub sample from each treatment was crushed then mixed with 10 g of anhydrous sulfate and filtered through 10 g of anhydrous sodium sulfate in a funnel. Acetone was filtered through the sample until total volume was 50 ml for each sample. The samples were kept frozen until analysis.

b. Analytical Procedure:

Anhydrous sodium sulfate (10 g) was added to reduce moisture in the sample. The homogenate was filtered and the extract was quantitatively transferred to a 100 ml round bottom flask. The acetone was removed by a rotary vacuum evaporator at 40°C and the sample was dissolved in 100ml of hexane. The extract was then processed through the Florisil clean-up procedure as described in the following:

A 10 X 200 mm chromatographic column was prepared by inserting a plug of glass wool and 5 g of activated Florisil topped with 2 g of anhydrous sodium sulfate. The column was rinsed with 100 ml of hexane and the wash was discarded. Three 5 ml rinses with hexane were added to the extract and transferred to the column. When the last rinse reached the top, the column was eluted with 50 ml of 10% ethyl ether in hexane (v/v). The eluate was completely evaporated and the residue was taken up in an appropriate amount of hexane for analysis (Fig.1).

c. Standards and Reagents:

An analytical standard was needed to inject in the gas chromatograph. Stock solution was prepared by dissolving 0.10 g Vinchlozolin in 100.0 ml hexane; then dilutions of 10 mg/ml, 1 mg/ml, and 0.1 mg/ml were prepared.

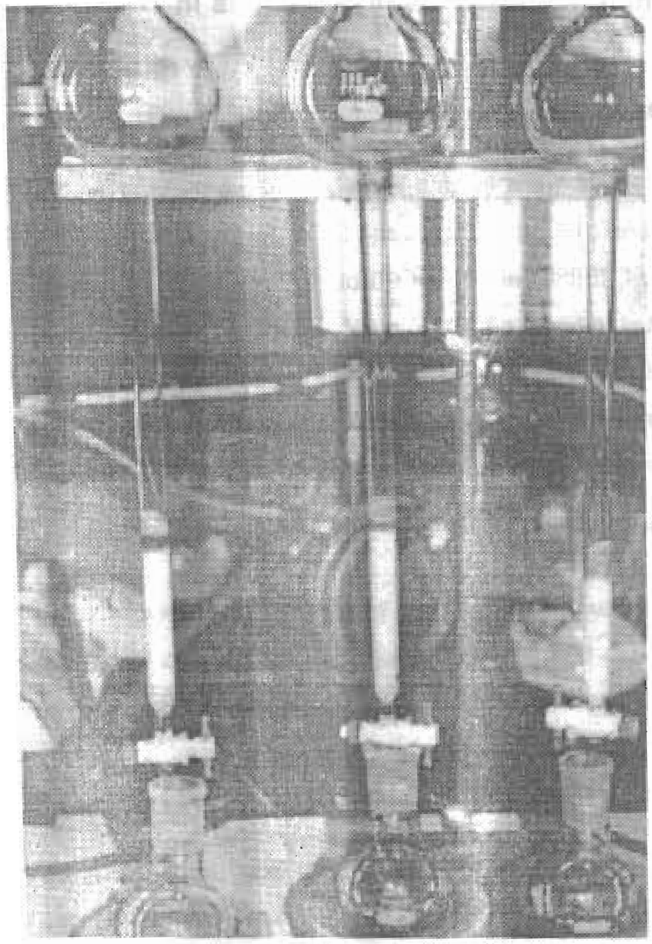

Fig 1. Clean-up step of Florosil / anhyd. Na_2SO_4
Chromatography column.

Standards of 0.01 mg/ml, 0.02 mg/ml, 0.04 mg/ml and 0.08mg/ml were prepared from 0.10 mg/ml. All solvents were of analytical grade. Sodium sulfate was of reagent grade (anhydrous). Florisil (PR grade), 80/100 mesh, was activated for 6 h. at 130°C and cooled in a dessicator before use. Vinchlozolin reference standard (97%) was kindly supplied by BASF.

d. Gas Chromatography Analysis :

A Tracor 222 gas chromatograph, equipped with a Ni^{63} electroncapture detector (ECD) and a Helwett-Packard 3390 A integrator were employed. The gas chromatographic column was a 1.6 m x 2 mm i.d. glass column packed with 10% ov-1 on 80/100 mesh Gas-chrom Q. The following conditions were used for gas chromatography analysis. Temperature: inlet, 230°C; oven 200 C detector. 280°C; gases: nitrogen (carrier), 40 ml/min. nitrogen (detector), 50 ml/min. With these chromatographic conditions Vinchlozolin was eluted for approximately 4-5 min. A 5-µl aliquot from a total volume of each concentration was injected into the gas chromatograph. Peak areas determined by a computing intergrator were compared to standard curves of Vinchlozolin in order to calculate the amount of residues present. The detection limit was 0.05 ng for Vinchlozolin (Zanini, 1980). Two injections of each sample and standard were made. Standards of 0.00, 0.01 mg/ml and 0.02 µg/ml gave a linear response and were used to calculate the sample concentrations.

The concentrations were calculated using the mathematical method of interpolation according to this equation:

$$\frac{Ac_2 - Ac_1}{As - Ac_1} = \frac{C_2 - C_1}{C_s - C_1}$$

$$C_s = \frac{(As - Ac_1)\,(C_2 - C_1) + C_1\,(Ac_2 - Ac_1)}{Ac_2 - Ac_1}$$

where :

C_s = Sample concentration.

C_2, C_1 = Two consequent standard concentrations: sample in between.

A_s = Sample Area.

Ac_2, Ac_1 = C_2 and C_1 Area

RESULTS

The extent of inhibition of mycelial growth of *B. cinerea* caused by the tested fungicides is given in table (2). Ronilan and Benlate were the most effective fungicides against *B. cinerea* as shown by the total inhibition at 5 ppm compared to Prochloraz which resulted in similar inhibition at 10 ppm. The effect of Ronilan and Benlate, at all tested concentrations, showed no significant differences.

Table 2. Mycelium linear growth of *Botrytis cinerea* after 5 days incubation at 18 ± 2 ºC

Fungicide	Linear growth (mm)								
	0	1	5	10	25	50	100	200	400
	(ppm)								
Benlate	9.0	8.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Prochloraz	9.0	12.0	7.2	0.0	0.0	0.0	0.0	0.0	0.0
Ronilan	9.0	6.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0

L S D at 0.05

Fungicides 0.24

Concentration 0.27

Pre-harvest Fungicidal application Under Natural Infection Conditions

The experiment was carried out in two successive seasons with Ronilan, Benlate, and Prochloraz fungicides at three tested doses (0.5, 1.0, and 2.0 gl^{-1}) at Kafr Hakiem farm, in Berquash/ Giza. The percentage of infection was calculated as the number of rotten fruits to the whole number of fruits in each replicate.

Data in table (3) indicated that spraying the fungicides under natural infection condition at the concentration used decreased the gray rot. The disease incidence was completely checked during the second season with Benlate and Ronilan at a concentration of 2g/l. Significant differences were obtained among concentrations 0.0, (0.5 and 1.0), and 2.0 $g\ l^{-1}$

Table 3. Efficacy of pre-harvest fungicidal application on gray mold rot of strawberry 'Pajaro' under natural infection conditions:

	% Infection							
	Season 1				Season 2			
Dose ($g\ l^{-1}$)	0.0	0.5	1.0	2.0	0.0	0.5	1.0	2.0
Benlate	16.2	7.6	6.6	0.3	10.2	5.4	3.9	0.0
Prochloraz	16.2	1.4	1.5	1.4	10.2	4.1	2.5	1.6
Ronilan	16.2	2.2	2.2	1.6	10.2	2.5	1.7	0.0

LSD at 0.05 for fungicides: 1.499 1.5

Significance of fungicides concentrations

Conc. g/l	mean	Grouping
0.0	13.2	A
0.5	4.3	B
1.0	3.0	B
2.0	0.8	C

LSD at 0.05 for concentrations: 1.7309 1.7

Pre-harvest Fungicidal Treatment and strawberry Gray Fruit Rot Under Artificial Infection Conditions:

Benlate, Ronilan, and Prochloraz at the rates 0.0, 0.5, 1.0 and 2.0 g l^{-1} were sprayed two weeks before harvest in two successive seasons.

Fungicidal application was made 48 hours after artificial inoculation with *B. cinerea*. It was observed that all tested fungicides gave sufficient control of fruit rot (Table 4). A negative relation between fungicide concentrations and percentage of infection was recognized and a significant interaction between the tested fungicides (Ronilan with Benlate or Prochloraz with Benlate) and their concentrations was found in both seasons. The concentration 2 g l^{-1} of the tested fungicides achieved the lowest percentage of infection in both seasons with greatest effect of Prochloraz in season 1 (1.8 %) and of Ronilan in season 2 (1.2%).

Table 4. Efficacy of pre-harvest fungicidal application on gray mold rot of strawberry 'Pajaro' under artificial infection conditions:

	% Infection							
	Season 1				Season 2			
Dose ($g\ l^{-1}$)	0.0	0.5	1.0	2.0	0.0	0.5	1.0	2.0
Benlate	28.5	13.4	13.3	9.8	16.2	7.4	5.2	3.4
Prochloraz	28.5	3.8	3.3	1.8	16.2	7.2	6.7	5.9
Ronilan	28.5	5.8	5.0	4.8	16.2	6.0	4.3	1.2

LSD at 0.05 for fungicides: 2.1521

Significance of fungicides concentrations

Conc. g/L	mean	Grouping
0.0	22.8	A
0.5	7.3	B
1.0	6.8	BC
2.0	4.5	C

LSD at 0.05 for concentrations: 2.485

Vinchlozolin (Ronilan) Residues in Treated Strawberry fruits:

Data in table (5) present the results of analysis of the summer samples after two applications at the beginning and at the end of fruit ripening. The detection limit was 0.05 ng of Vinchlozolin. Low residue values were found in the samples analyzed. It was determined that residues after two weeks were 0.008 and 0.084 ppm respectively for the concentrations 1.0 $g\ l^{-1}$ and 2.0 $g\ l^{-1}$. Arrows identify the Vinchlozolin peaks at different concentrations after three tested intervals. No run peaks were sorted for the non- treated fruits (Fig.2).

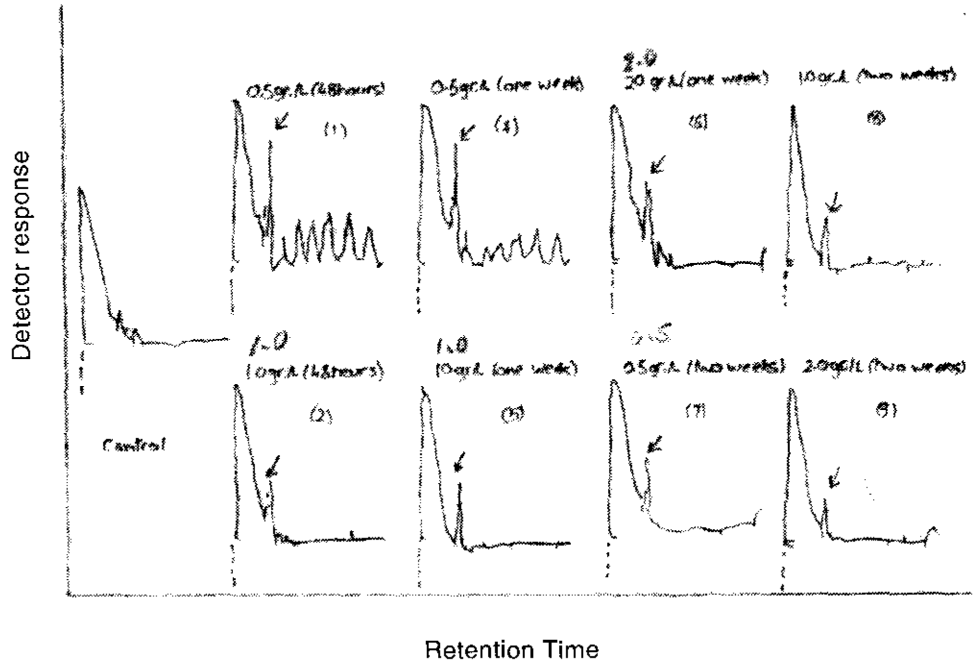

Fig 2. Typical Chromatograms of Vinchlozolin-sprayed Strawberry fruit extract.

Table 5. Gas chromatographic determination of Vinchlozolin residues in treated strawberry fruits.

Concentrations g l^{-1}	Residues of Vinchlozolin in treated strawberry fruits (ppm)		
	Two days	One week	Two weeks
0.0	0.00	0.00	0.00
0.5	0.0172	0.1616	——*
1.0	0.0142	0.3183	0.0082
2.0	0.0354	0.3871	0.0838

* missed sample

DISCUSSION

The fungicides used in this study varied in their effect on *Botrytls cinerea* linear - growth. It was found that Ronilan and Benlate was more effective than Prochloraz. These fungicides completely inhibited the fungal growth at a range of 5 - 10 ppm, respectively. Cohen and Dennis (1975) tested *in vitro,* the activity of a range of fungicides on strains of *B. cinerea, Mucor mucedo, Rhizopus stolonifer* and *Rhizopus exualis.* Dichlofluanid and Dichloran were more active than the other fungicides against all strains of *B. cinerea.*

Under natural infection of strawberry fruits in the field, spraying the fungicides Ronilan, Benlate, and Prochloraz at three rates, 0.5, 1.0, and 2.0 g l^{-1} minimized the incidence of gray fruit rot to different extents according to the concentrations. Significant differences were obtained among concentrations used, being high at greater concentration.

Benlate, Ronilan, and Prochloraz at concentrations of 0, 0.5, 1.0, and 2.0 g l^{-1}, were sprayed two weeks before harvest. The application was made 48 hours after the artificial inoculation with *B. cinerea.* Results indicated that all tested fungicides gave sufficient control against fruit rot disease. The lowest percentage of infection was obtained with 2.0 g/liter for Ronilan as compared with the other fungicides. These findings confirmed results by many investigators. Borecka *et al.* (1973) reported that gray mold in strawberries caused by *B. cinerea* can be adequately controlled by three to four applications of selected chemicals beginning at the white bud stage and continuing at 7 days intervals until the first fruits begin to ripen. Benomyl provide excellent control accompanied by marked increase in yield.

Dommer and Mangold (1977) reported that Ronilan (Vinchlozolin) effectively controlled *B. cinerea* on grapes and strawberries. Paulus *et al.* (1978) mentioned that for the control of *B. cinerea* on Tioga and Tufts strawberries cvs. spraying with Ronilan (Vinchlozolin) resulted in lower number of rot-infected fruits. The highest yield was obtained with BASF 352 (Vinchlozolin) and Captan + Benlate (Benomyl). Also, Duchon-Doris (1980) reported that Ronilan (Vinchlozolin) 50% at 0.2 kg/ha applied 3 times between the beginning of strawberry flowering and the appearance of the first green fruits gave satisfactory (70%) control of *B. cinerea.* Accordingly, from the obtained data, Ronilan (Vinchlozolin) 50% gave a complete inhibition of fungal development, therefore, the fungicide Ronilan was the candidate for the residue analyses in treated strawberries.

Residues of Vinchlozolin were determined as μg/kg weight. The residues after two weeks were 0.008 and 0.084 ppm for 1.0 and 2.0 gl^{-1}, respectively. For the check, no residue was detected for the non-treated fruits. These results conform with those of Duchon-Doris (1980) who reported that the permissible residue in strawberry fruit treated with Ronilan 50% is 5 ppm. The metabolism of Vinchlozolin in plants has been studied in strawberries. The metabolic breakdown begins with the opening of the ring in the heterocyclic part of the molecule and proceeds to form metabolites which are derivatives of 3, 5-dichloranilin and very polar metabolites (Anon, 1984).

As the recovered residues of Ronilan (ppm) in treated strawberries at tested concentrations is greatly lower than the permissible level (5 ppm) in strawberries, the pre harvest application of Ronilan at tested concentrations on strawberry is recommended.

AKNOWLEDGEMENT

The technical work was carried out in Strawberry experimental farm at Kafr Hakim/Berqûash in Giza/Egypt and the analytical work was carried out at the Biochemistry/Biotechnology Department, Residue research laboratory in NDSU, Fargo, ND 58105, USA.

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مقاومة العفن الرمادي قبل الحصاد وبقايا المبيدات في ثمار الفراولة

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نتج عن معاملات الرش الوقائي بالمبيدات الفطرية الثلاث وهي:

البنليت [Methyl 1-(butyl carbamoyl)-2-benzimidazol carbamate]

والبروكلوراز [1-(N-propyl-N- (2-(2,4,6-tri-chlorophenoxy) ethyl) carbamoyl) imidazole]

والفينكلوزولين [3 - (3,5 Dichlorophenyl) 5-ethenyl-5-methyl-2,4-oxazolidinedione] علي الفراولة عند معدلات ٠.٥ و ١ و ٢ جم/لتر سواء تحت ظروف العدوي الطبيعية أو الصناعية بالفطر إلي نقص معنوي لمستوي الإصابة بالعفن الرمادي علي ثمار الفراولة المتسبب عن الفطر Botrytis cinerea وأعطت المعاملة بالمبيد الفطري فينكلوزولين (رونيلان) نتائج ممتازة مقارنة بالمبيدات الأخري حيث وجد أن الثمار التي استقبلت المعاملة عند معدل ٢جم/ لتر اختزلت بها نسبة العفن الرمادي إلي ٤.٨ ٪ و ١.٢ ٪ علي مدي عامين متواليين مقارنة بنسب العفن بالثمار الغير معاملة (المقارنة) والتي وصلت الإصابة بها إلي ٢٨.٥ ٪ و ١٦.٢ ٪ علي التوالي تحت ظروف العدوي الصناعية.

أثبتت نتائج التحليل الغازي الكروماتوجرافي أن بقايا المبيد الفطري رونيلان (فينكلوزولين) بثمار الفراولة كانت ٠.٣٥٤ و٠.٣٨٧١ و٠.٠٨٣٨ جزء في المليون بعد يومين وأسبوع وأسبوعين علي التوالي من المعاملة بمعدل ٢جم/لتر.