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Control of Strawberry Fruit Rots by Fungicides and Determination of their Residues in the Harvested Fruits

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Spraying strawberry plants with any of the following fungicides, dichlofluanid (Euparen) 50%WP, iprodione (Rovral) 50%WP and procymidone (Sumisclex) 50%WP resulted in significant reduction of the natural infection with fruit-rots with significant increase to the marketable fruits compared with the control treatment. Significant variations were observed in the rate of fruit-rot infection and the fruit yield due to the effect of the tested fungicides and the growing season.

The residues of the tested fungicides (mg a.i./kg fruits) in the harvested fruits gradually decreased by increasing the interval after spraying.. Slow degradation of dichlofluanid 50%WP was observed in and on the fruits five days after application. Then fast dissipation occurred until complete disappearance of the residues after 45 days. On the other hand, the degradation of iprodione 50%WP and procymidone 50%WP was faster in the first week then turned to be slower but their residues were still detected at the 45th day. Referring to the Maximum Residue Limits of the Codex Alimentarius Commission were 10 mg/kg for each of the fungicides under experiment on strawberry fruits. In this study, the Pre-Harvest Intervals (PHI's) of dichlofluanid 50%WP, iprodione 50%WP and procymidone 50%WP were 7, 2 and 3 days, respectively. Human consumption of strawberries following the identified withholding periods would be safer and reduce the risk for consumer health.

According to the agricultural practices, the strawberry fruits are harvested every 3 days. For this reason, the use of dichlofluanid should be restricted to be applied 7 days (PHI) before start collecting strawberry fruits and should not be permitted for application during the harvesting season. Iprodione and procymidone having shorter PHI's 2 and 3 days which enable using them during the harvesting season.

Key words: fungi, fungicides, fruit rots and strawberry.

Strawberry (*Fragaria vulgaris* Ehrh.) is one of the most important favourite and delicious fruits of which the demand has been increased in Egypt for local consumption and exportation. Under Egyptian conditions and all over the world, strawberry fruits are vulnerable to infection by many destructive pathogens that cause fruit-rots (Khafagi, 1982; Bhardwaj *et al.*, 1998; Abada *et al.*, 2002; Legard *et al.*, 2002 El-Neshawy *et al.*, 2003 and Saber *et al.*, 2003) in addition to physiological disorders (Ulrich *et al.*, 1980).

The cultivated area with fresh strawberry plantation during 2001 / 2002 growing season reached about 1200 feddan and exceeded 2000 feddan during 2003 / 2004 growing season with an average of 16-24 ton / feddan during the two seasons. In addition, the exported amount ranged between 35-60% from the total production and the remained production is for local market, but with high price compared with frozen (Frigo) strawberry (Anonymous, 2004). Therefore, strawberry plantations represent the highest cash crop either for the growers or the national income.

The use of fungicides in agriculture is necessary to combat a variety of fungi that could destroy crops and to improve the quality of plant production. As a consequence of this use, the presence of residues is unavoidable. The placement, timing, and type of fungicides application affect the likelihood of terminal residues. On the other hand, because strawberry fruits are consumed and/or exported directly from the growing fields, therefore using of fungicides cause high risk in this respect. But fungicides are still the effective tool for management strawberry fruit-rots. Therefore, selecting short persistence fungicides as well as adjusting the time of application are very important to resolve this problem.

The present investigation was planned to study the role of three recommended fungicides by Ministry of Agriculture, *i.e* dichlofluanid, iprodione and procymidone in management of strawberry fruit-rots and to determine their residues in the harvested fruits.

Materials and Methods

1. Field preparation for fresh strawberry planting:

A field located at Berkash district, Giza governorate was prepared for planting strawberry (cv. Sweet Charlie) by fresh transplants using the recommended practices of Ministry of Agricultural. In this respect, the land was divided into bridges (beds) of 120-cm width and 42 m (about 50 m^2) long with 40 cm in between bridges, irrigated with enough water. Apparently healthy fresh strawberry transplants (previously grown in fumigated nursery and irrigated with under-ground water) were dipped in the preparation of the non-systemic fungicide iprodione 50%WP; 0.1 % for 20 minutes just before transplanting. Four rows were transplanted on each bridge (30 cm. interval) and 25 cm. were left between each two transplants. The transplants were sprayed, just after transplanting, with water using sprinkler system when it was necessary for three weeks until forming new leaflets. Also, drip irrigation was used, after the establishment of the transplants. One month after transplanting, the plastic sheets (soil mulch) were put above the beds and under the grown plants. Field experiments were carried out during 2001/2002 and repeated in 2002/2003 growing seasons and transplants were planted during mid of September in each season. Moreover, the grown plants were covered with plastic sheets (80 µm thick) as low tunnels at the end of October at night until mid of November and most of the day after that until the end of February, then when it was necessary according to environmental conditions. The grown fruits were left to the natural infection with the causal fruit rots. Also, strawberry plants received the normal agricultural practices as recommended by the Ministry of Agriculture.

2. Chemical control:

Three recommended fungicides namely: dichlofluanid 50%WP, iprodione 50%WP and procymidone 50%WP, were sprayed on strawberry plants two times at each of the three fruiting cycles at the rate of 250, 90, and 90 g/100 l water, respectively. The first spray was done at the beginning of flowering stage and the second one was two weeks later. The same periods of spraying these fungicides were done in the other two fruiting cycles. Four randomly replicate plots were used for each treatment.

2.1. Determination of fungicide residues:

2.1.1. Sampling:

In another experiment, the aforementioned three fungicides were sprayed on strawberry plants at the beginning of harvesting fruits, one spray, and samples used for determination of fungicide residues were then collected at different intervals, *i.e.* zero time (one hour following application), 1, 2, 3, 5, 7, 14, 21, 30 and 45 days after applying fungicides.

Four replicated samples, 350 g from each treatment fungicide replicate as well as the control, were put in polyethylene bags. The four replicates were collected together and mixed to form one representative sample.

2.1.2. Sub-sampling:

One kg of each fruit representative sample was homogenized. Two sub-samples each 50 g were taken, one for extraction and the second was kept in a deep freezer at -20 °C as a stock. Extraction was carried out instantly.

2.2.1. Multi residue method:

The AOAC (1995) was followed with some modification. The sample and solvent volume were only half of those in the AOAC method. A rotary evaporator and blowing down with air were used instead of Kuderna-Danish concentrator. The total volume of acetone extract was measured to make the reading calculations. However, the AOAC method uses tabulated water percentages of commodities. After drying, the aromatic phase was concentrated just to dryness. The pesticide residues were reconstituted in n-hexane/acetone for gas chromatography determination.

The method allows the determination of the three fungicides residues. Table (1) described the recoveries of the dichlofluanid 50%WP, iprodione 50%WP and procymidone 50%WP of strawberry fruit, spiking levels as well as coefficient of variation CV%.

Table 1. Recoveries of fungicides from strawberry spiked samples

Compound	Spiking level (mg/kg)	No. of Samples	Averages recovery (%)	CV (%)
Dichlofluanid	0.1	10	106	6.34
Iprodione	0.5	10	105	3.45
Procymidone	0.3	10	88	14
	0.06	9	88	17

2.2.2. Pesticides reference standard:

Dichlofluanid, iprodione and procymidone, reference materials are certified standards provided by (Dr. Ehrenstorfer Gmbh, Gogginer Str. 78D-8900 Augoburg) and prepared in n-hexane/acetone mixture.

2.2.3. Apparatus:

Gas chromatography:

Hewlett Packard (HP) 5890 equipped with double Electron Capture Detector's (ECD) with two capillary columns.

Operating conditions: Nitrogen carrier gas 2.5 ml/min, 75-90 ml/min, (carrier = makeup), column head pressure 82 Kpa. Injector temp. 225°C and detector temp. 300°C.

Chromatography columns:

(1) PAS-5 tested ultra 2 silicon, 25m x 0.32mm, and film thinness 0.52 µm.
(2) PAS-1701 tested 1701 silicon, 25m x0.32mm, and film thinness 0.25 µm.

Temperature programs of GC instrument: initial temperature 90°C for 2 min , ramp (1) 20(°C/min)to 150°C, ramp (2) (6°C/min) to 270 °C hold 15 min .

2.2.4. Reagents:

- Acetone, Dichloromethane, n-hexane, petroleum ether, (Pestiscan Chromatography grade).
- Anhydrous sodium sulphate (Riedel-de Hean).
- Sodium chloride, reagent grade.

2.3. Quality assurance procedure:

The analytical method and instruments are fully validated as a part of the laboratory quality assurance system which are audited and accredited by (FINAS Centre for Metrology and accreditation Helsinki, Finland 2004). This quality system is referred to (ISO/IEC 17025).

3-Disease assessment:

The naturally rotten fruits (caused mainly by *Botrytis cinerea, Phytophthora cactorum* and *Rhizoctonia solani)* were counted for each treatment at harvesting intervals and the average was calculated. Also, the obtained marketable fruit yield (un-rotten fruits) was weighed at each harvest and the average final weight was recorded. In addition, the residues of the sprayed fungicides were assessed at each sampling time after spraying the tested fungicides.

Data obtained were statistically analyzed using split plot design (Snedecor and Cochran, 1967). The averages were compared at 5% level of probability using L.S.D. (Fisher, 1948).

Results and Discussion

Data (Table2) demonstrate that strawberry treated with each of the three fungicides, *i.e.* dichlofluanid 50%WP, iprodione50%WP and procymidone 50%WP, showed significant reduction in the average of the rotten fruits (during both seasons), being 4.8, 6.1 and 6.4%, respectively, compared with control treatment (17.8%).

Table 2. Effect of spraying fungicides on the natural infection with fruit-rots and fruit yield (cv. Sweet Charlie) during 2001/2002 and 2002/2003 (field experiments at Giza governorate)

Fungicide	Dose/100 l water	Rotten fruits infestation (%)		Mean	Average of marketable fruit yield (kg / plot)		Mean
		2001/02	2002/03		2001/02	2002/03	
Dichlofluanid	250 g	5.0	4.5	4.8	248.0	245.8	246.9
Iprodione	90 g	6.0	6.2	6.1	244.0	243.1	243.6
Procymidone	90 g	6.8	6.0	6.4	245.0	242.4	243.7
Control	---	18.0	17.6	17.8	218.0	220.0	219.0
Mean	---	9.0	8.6	---	238.8	237.8	---
L.S.D. at 5% for:							
Treatments (T)=				1.4			2.9
Season (S) =				n.s.			n.s.
T x S =				1.8			2.1

Also, significant increase in the marketable fruit yield was recorded due to spraying these fungicides, being 246.9, 243.6 and 243.7 kg/plot (42 m^2), respectively compared with control treatment, 219.0 kg/plot (42 m^2). The data also indicated that no significant differences were noticed due to the effect of the growing season on fruit-rots and the marketable fruit yield. Concerning the efficacy of these fungicides in reducing the infection, no significant differences were recorded.

It is well known that, the use of fungicides in agriculture is necessary to combat a variety of diseases that could destroy crops and to improve the quality of plant products. In this regard, Wang and Wang (1997) reported that treatment of strawberry with 50% procymidone at a concentration of 1:1000 gave an average of 80% control of grey mould disease.

Sarrazyn *et al.* (1997) found that all used fungicides have significantly reduced the total incidence of fruit rots. Chlorothalonil, dichlofluanid, thiram and iprodione when spray increased the yield significantly of healthy fruits compared with that from untreated plants by 43-114%.

Many investigators used fungicides in controlling strawberry fruit-rots and obtained adequate control (Washington *et al.*, 1999; Blacharski *et al.*, 2001; Legard *et al.*, 2002; El-Neshawy *et al.*, 2003 and Saber *et al.*, 2003).

Degradation of fungicides residues:

Dichlofluanid 50%WP residue determined one hour after application on strawberry (the initial deposit), was 16.43 mg/kg fruits (Table 3). The residues slowly decreased by the laps of time after treatment. Faster dissipation of residues occurred between the 5th and 14th day following application showing concentrations of *11.20* and *0.63* mg/kg, *respectively*.

Table 3. Dichlofluanid 50%WP residues and percentage of loss in treated strawberry fruits at different intervals after treatments (2003/2004 growing season)

Time after treatment (days)	Residue in mg/kg fruits	Loss (%)
Initial	16.43	0.0
1	15.95	2.9
2	14.74	10.2
3	13.13	20.0
5	11.20	31.8
7	5.99	63.5
14	0.63	96.1
21	0.56	96.6
30	0.13	99.2
45	ND	100.0

The rates of loss in fungicide residues at the days 5, 7 and 14 reached 31.8%, 63.5% and 96.1%, respectively. At the end of the experimental period; 45 days, no residues could be detected on strawberry fruits.

The Maximum Residue Limit (MRL) of the Codex Alimentarius Commission for dichlofluanid is 10 mg/kg on strawberry. Based on that, 7 days could be considered the Pre-Harvest Interval (PHI) that should pass after the last application of the fungicide and before setting the strawberries on the market for safe consumption.

Similar data was obtained by Eades and Gardiner (1967) and Brewerton and Gibbs (1968).

Data (Table 4) indicate that the amount of iprodione 50%WP residue, determined one hour following application on strawberry, was 11.9 mg/kg. The initial residues gradually decreased by increasing the time after treatment,

Two days following application iprodione residues reached 7.1 mg/kg and continued degradation to reach 2.1 mg/kg after 30 days of application. However, after 45 days 0.18 mg/kg iprodione were detected in and on strawberry fruits.

At the second day after application, strawberry applied with iprodione 50% WP could be safely consumed based on the Maximum Residue Limit of the Codex Alimentarius Commission of 10 mg/kg. The Pre-Harvest Interval (PHI) of two days should be appropriate.

Data (Table5) indicate that the initial deposit of procymidone 50%WP applied on strawberry and determined one hour after application reached 12.13 mg/kg.

Table 4. Iprodione 50% WP residues and percentage of loss in treated strawberry fruits at different intervals after treatments (2003/2004 growing season)

Time after treatment (days)	Residue in mg/kg fruits	Loss (%)
Initial	11.9	0.0
1	10.9	8.4
2	7.1	40.5
3	7.1	40.5
5	6.5	45.6
7	6.1	45.3
14	4.9	58.7
21	3.1	74.3
30	2.1	82.0
45	0.18	98.5

Table 5. Procymidone 50% WP residues and percentage of loss in treated strawberry fruits at different intervals after treatments (2003/2004 growing season)

Time after treatment (days)	Residue in mg/kg fruits	Loss (%)
Initial	12.13	0.0
1	11.25	7.3
2	9.2	24.2
3	7.92	34.7
5	4.8	60.4
7	3.9	67.9
14	3.1	74.4
21	1.3	89.3
30	1.1	90.9
45	0.47	96.1

The residue of procymidone gradually decreased by increasing time after application, The initial deposit decreased to 11.25, 9.2, 7.92, 4.8, 3.9, 3.1 1.3, 1.1 and 0.47 at the intervals of 1, 2, 3, 5, 7, 14, 21, 30 and 45 days following application, respectively. The Codex maximum residue limit for procymidone is 10 mg/kg which indicate that the Pre-Harvest Interval (PHI) of 2 days is appropriate when the residues reached 9.2 mg/kg. For precautionary reasons, 3 days PHI would be safer for human consumption of strawberry fruits treated with procymidone 50% WP.

It was concluded from data obtained that, dichlofluanid 50%WP, iprodione 50%WP and procymidone 50%WP, are effective in controlling fruit rots disease and increased the strawberry fruits yield and quality. However, dichlofluanid 75% WP, iprodione 50%WP and procymidone 50%WP could be safely applied on strawberries provisioned to follow the good agriculture practices and the PHI's of 7, 2 and 3 days, respectively. According to the agricultural practices, the strawberry fruits are harvested every 3 days. For this reason, the use of dichlofluanid should be restricted to be applied 7 days (PHI) before start collecting the strawberry fruits and not permitted for application during the harvesting season. Iprodione and procymidone having shorter PHI's 2 and 3 days which enable using them during the harvesting season.

The recommended pre-harvest interval of these fungicides on the manufacture pamphlet for strawberry fruits is 7 and 14 days for both dichlofluanid and iprodione, respectively. But, in case of procymidone this interval is not determined for strawberry, but was mentioned for grapevine as 21 days. Therefore, obtained data of the PHI's is in full harmony for dichlofluanid and is less than that mentioned for both iprodione and procymidone, being 2 and 3 days, respectively. Hence, the obtained data are of great importance for the health of the strawberry consumers, as it could recommend both iprodione and procymidone not dichlofluanid for spraying strawberry for controlling fruit-rots, even during harvesting, without any hazard on the human health. The fluctuation in the number of days before harvesting strawberry fruits between the recommended days by the formulated company of the tested fungicides and the obtained data may be due the effect of the environmental conditions.

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مكافحة أعفان ثمار الفراولة بالمبيدات الفطرية وتقدير متبقياتها في الثمار عند جمعها

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** المعمل المركزي لتحليل متبقيات المبيدات والعناصر الثقيلة في الأغذية- مركز البحوث الزراع،

أدى رش نباتات الفراولة بأي من المبيدات الفطرية ايوبارين ، روفرال ، سوميسكلكس إلى حدوث انخفاض معنوى فى الإصابة الطبيعية بأعفان الثمار مع حدوث زيادة معنوية في محصول الثمار القابل للتسويق مقارنة بالنباتات التي لم ترش بأي مبيد. لم يكن هناك فروق معنوية في قيم الإصابة بالمرض وكذلك محصول الثمار نتيجة لتأثير المبيدات المستخدمة وكذلك موسم الزراعة .

انخفضت متبقيات المبيدات المرشوشة في الثمار (مجم مبيد / كجم ثمار) تدريجيا بزيادة الفترة بعد رش هذه المبيدات وحتى جمعها. وكان سلوك متبقيات مبيد اليوبارين في ثمار الفراولة مختلفا حيث أظهر بطئا فى هدم متبقياته حتى ٥ أيام من رش الثمار ثم زاد معدل إختفاء المتبقيات بعد ذلك خاصة بين اليوم الخامس والرابع عشر ولم يوجد أي متبقى له في الثمار بعد ٤٥ يوم من الرش. بينما كان معدل تحلل المبيدين روفرال ، سوميسكلكس أسرع في المدة الأولى (٥ أيام) ثم انخفض هذا المعدل بعد ذلك (١٤ يوم) بدون حدوث اختفاء تام للأثار المبيدين بعد ٤٥ يوم من الرش .

ونظرا لأن الحدود المسموح بها من كل من المبيدات تحت الدراسة والمنشورة من لجنة الكودكس الدولية تبلغ ١٠ مجماكجم فإن فترة ما قبل الحصاد الواجب مرورها بعد آخر معاملة بالمبيد وقبل جمعه للإستهلاك تبلغ ٧ و٢ و٣ أيام لكل من اليوبارين والروفرال والسوميسكلكس على التوالى. وهذة الفترات عند إتباعها كعنصر من عناصر الممارسة الزراعية الجيدة فى معاملة نباتات الفراولة بالمبيدات المذكورة فإن ثمارها تصبح آمنة للإستهلاك الأدمى.