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Chemical stability and biological toxicity of some fungicides sprayed on citrus fruits against *Spodoptera littoralis* (Lepidoptera: Noctuidae) under storage conditions

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Abstract

The study investigates the effect of a storage stability test on the degradation of various fungicides. The fungicides tested in the study include Imacid (7.5% LS), which contains Imazalil; Citrocil Plus (27.4% EC), which contains a combination of Imazalil (5% EC) and Ortho-phenylphenol (22.4% EC); Ortosol (20% EC), which contains Ortho-phenylphenol; Scholar (23% SC), which contains Fludioxonil; Fruitgard (45% flow SC), which contains Thiabendazole; and Decco (36% SC), which contains Pyrimethanil as an active ingredient. The tested fungicides were stored at 54±2°C for 14 days. During the different storage periods, samples were taken after 7 and 14 days to determine the physical properties of the emulsion concentrate EC**,** suspension concentrate SC, and solution for seed treatment LS, and evaluate the chemical stability of the active ingredient by HPLC and GC/MS. The results show that the Imazalil, Ortho-phenylphenol, Fludioxonil, Thiabendazole, and Pyrimethanil active ingredients were 7.46, 19.92, 22.62, 44.99, and 35.96%, respectively, and reached 7.24, 19.70, 21.65, 44.76, and 35.88% after 14 days of storage at 54±2^{o}C for Imacid 7.5% LS, Ortosol 20% EC, Scholar 23% SC, Fruitgard 45% flow SC, and Decco 36% SC, respectively. Also, Imazalil 5% active ingredients were 4.99% and reached 4.91% after 14 days of storage at 54 ± 2^{o}C, and Ortho-phenylphenol 22.45 active ingredients were 22.43% and reached 22.05% after 14 days of storage at 54±2^{o}C for Citrocil Plus 27.4% EC. Additionally, GC/MS was used for the degradation of Imazalil, Ortho-phenylphenol, Fludioxonil, Thiabendazole, and Pyrimethanil fungicides. From this, it was found that the major degradation products were 1-(2-ethoxy-2-phenylethyl)-1H-imidazole; [(3E)-penta-1,3-dien-3-yl] benzene; 2,2-difluoro-2H-1,3-benzodioxole; (E)-2-(1H-benzimidazol-2-yl) ethene-1-thiol; and 4,6-dimethylpyrimidin-2-amine, respectively. Furthermore, the biological data of the fungicide toxicity test proved its ability to kill larvae at small concentrations, and statistically no significant differences were found between treatments for the 4th instar larvae of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) that all revealed high toxicity levels, and Imazalil was the most effective one.

Introduction

Fungicides are known as pre- and post-harvest treatments of fruit and vegetables because they reduce mold, rot, and other pests like insects (Zanuncioa, 2018), act as anthelmintic drugs, and are commonly found in food product additives (World Health Organization, 2021). Imazalil (IMZ) [(RS)-1-(b-allyloxy-2,4-dichlorophenethyl) imidazole] is a systemic fungicide. The structure of Imazalil is shown in Table (1). Its primary use is for postharvest treatment of citrus (Laville *et al*., 1978, and Farm Chemicals Handbook, 1993). Thiabendazole (TBZ) (4-1(1H-benzimidazol-2-yl)-1,3-thiazole (TBZ)) is a benzimidazole pesticide. It is also known as the food additive E233 from the class of preservatives (World Health Organization, 2021). TBZ is a fungicide and anthelmintic drug commonly found in food products. Due to its toxicity and potential carcinogenicity, its determination in various samples is important for public health (Budetić *et al*., 2023). Imazalil (IMZ) and thiabendazole (TBZ) are systemic fungicides employed to control a wide range of fungal diseases on fruits, vegetables, and ornamentals (Tomlin, 1994a). Both fungicides are most widely used in packinghouse treatments to control postharvest decay in citrus fruit (Eckert and Eaks, 1988).

Pyrimethanil (PYRI) fungicide, N-(4,6-dimethylpyrimidin-2-yl) aniline (Table 1), a novel anilino-pyrimidine fungicide (AgrEvo), is used to control grey mold and leaf scab on grapes, strawberries, tomatoes, other fruits, vegetables, and ornamental plants (Tomlin, 1994b). Pyrimenthanil (PYRI) is a fungicide used in agriculture to control *Botrytis cinerea*, *Venturia inaequalis*, and *V. pirina*. (Couderchet, 2003 and YongBing *et al*., 2004). Cabras and Angioni (2000) observed that PYR was the most resistant to degradation of the anilinopyrimidines tested on grapevines.

Ortho-phenylphenol (OPP) is used as an antibacterial and disinfectant agent and fungicide in a variety of different agricultural, industrial, and domestic uses (Lambert and Eastmond, 1994; WHO, 2003 and Balakrishan and Eastmond, 2006).

Fludioxonil (FLUDI) is a phenylpyrrole fungicide that is registered in Europe for post-harvest uses (European Food Safety Authority, 2007). Fludioxonil acts on the transport-associated phosphorylation of glucose, inhibiting mycelial growth (Baroffio *et al*., 2003 and Brandhorst and Klein, 2019). Application of post-harvest fungicides by fruit dipping or spraying in drench chambers (Russouw *et al*., 2021).

Due to its toxicity and potential carcinogenicity, chemical stability, and deterioration determination in various samples, especially during storage because it is an important argument for public health. Different analytical methods can be used to determine the presence and concentration in samples, as liquid chromatography (LC) and high-performance liquid chromatography (HPLC) are the most commonly used methods Budetić *et al.* (2023).

Cotton is the most important economic fiber crop in Egypt, the growth season infected by many pest species like beet armyworm *Spodoptera exigua* (Hübner) and the fall armyworm *S. frugipedra* (J.E.Smith) and the cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), that can defoliate about 40 economically important plant families and considered the most serious polyphagous pest distributed at different crops around the world causing economic losses. Insecticide applications are the most prominent effective way to control this pest (Hosny *et al.,* 1986). Pest management practices (IPM) in modern agriculture have increasingly depended on practice-based insecticides such as seed treatments, foliar applications during plant growth stages, and relations with pest density

or crop damage. Several strategies and tactics for management were suggested and tested in fields in subjects of mitigation of insecticide problems. Thus, there is an urgent need to use different chemicals or alternative structures that have the ability to maximize the efficacy of control during the crisis of failure to control, (Abdel-Rahim *et al*., 2009 and Yones *et al.,* 2012). The desirable investigation of any new chemical products for availability to control field pests is initially considered a hazard category for toxicity to pollinators, beneficial arthropods, and environmental risk limits. The most practical insecticide synthetic chemicals class novelew last decades against *S. littoralis* and gave curative results were the conventional insecticide classes such as Organophosphates, carbamates, pyrethroids, and biopesticides, followed by the novel compounds with unique mode of actions (Khan *et al*., 2011; Saeed *et al*., 2012; Dahi *et al*., 2016, and Abd El-Kareem, 2016). However, those classes have become ineffective.

Various fungicides are permitted for use on citrus fruits to control citrus pests, including *S. littoralis* in Japan. The Food Sanitation Law regulates the levels of fungicides used in these fruits. The common fungicide uses, including: FLUDI, was newly permitted in 2011, and AZO and PYRI were also approved in 2013 by the Ministry of Health, Labour and Welfare of Japan. The antifungal agents AZO, DP, FLUDI, IMZ, OPP, PYRI, and TBZ1 (Inoue *et al.,* 2012 and Vorstermans and Creemers, 2007) belong to different chemical classes and are contained mainly in imported citrus fruits.

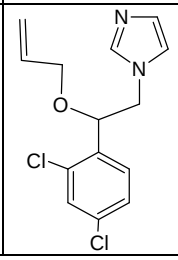
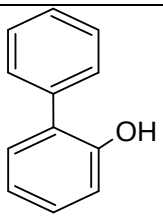
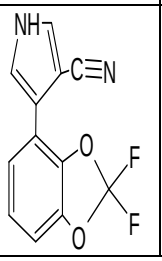
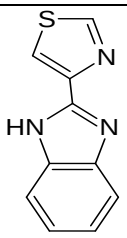
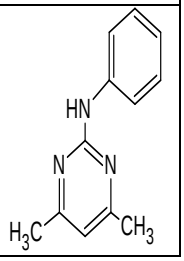
The present study aimed to evaluate the effect of storage at 54 ± 2 ºC for 14 days on fungicides (Imacid 7.5% LS, Citrocil Plus 27.4% EC, Ortosol 20% EC, Fruitgard 45% flow SC, and Decco 36% SC) and determine their physical properties. The active ingredients of Imazalil, Fludioxonil, Thiabendazole, and Pyrimethanil by HPLC and orthophenylphenol by gas chromatography, fingerprint GC/MS, as well as the evaluation of their efficacy by testing them against *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae).

Materials and methods

1. Fungicides used:

The structure of the tested fungicides used is found in Table (1).

Table (1): The structure of tested the fungicides used.

Trade name	Imacid 7.5% LS	Citrocil Plus 27.4% EC		Ortosol 20% EC	Scholar 23% SC	Fruitgard 45% flow SC	Decco 36% SC
		Citrocil Plus 5%	Citrocil Plus 22.4%				
Common name	Imazalil		Ortho-phenylphenol		Fludioxonil	Thiabendazole	Pyrimethanil
Structure							

LS: Solution for seed treatment; EC: Emulsion Concentrate; SC: Suspension Concentrates

2. Experimental procedures:

2.1. Accelerated storage procedures:

Formulations of (Imazalil) Imacid 7.5% LS (Imazalil and orthophenylphenol) Citrocil Plus 27.4% EC (Orthophenylphenol) Ortosol 20% EC (Fludioxonil) Scholar 23% SC (Thiabendazole) Fruitgard 45% flow SC and (Pyrimethanil) Decco 36% SC were placed in bottles (About 50 ml). This bottle was exposed to storage at 54 ±2ºC for 14 days.

During the storage period, samples were taken at 0, 7, and 14 days to determine the chemical and physical properties and fingerprint by GC/MS.

2.2. Standard preparation:

Standard preparation of the Imazalil, orthophenylphenol, Fludioxonil, Thiabendazole and Pyrimethanil.

Weigh 10 mg of Imazalil, Ortho-phenylphenol, Fludioxonil, Thiabendazole, and Pyrimethanil of known purity analytical content in 25 ml grade (A) flask measurement dissolved and finished methanol.

2.2.1. Sample preparation for tested fungicides:

Accurately weigh sufficient sample formulation equivalent to 10 mg of standard in a different 25 ml volumetric flask for each sample and slowly mixed with methanol.

3. HPLC Instrument Determination of Imazalil, Fludioxonil, Thiabendazole and Pyrimethanil:

The active ingredient percentages for Imazalil, Fludioxonil, Thiabendazole, and Pyrimethanil were determined before and after storage, according to the method of Yoshioka *et al.* (2015) with some modifications. An Agilent series 1100 quarterly pump with the ultraviolet (U.V.) detector and a C_{18} stainless steel column 2.6 x mm 25 cm was used. was methanol-acetonitrile (10:90) v/v was used as a mobile phase at the rate of 0.8 ml/min, 40°C column temperature, and wavelength 210, 197, 234, and 210 nm. At this condition, the retention times (Rt) of Imazalil, Fludioxonil, Thiabendazole, and Pyrimethanil were 1.335, 3.187, 1.962, and 2.18, respectively.

4. Gas Chromatography Determination for Orthophenylphenol:

A Hewlett – Packard G. C. Model 6890 instrument, equipped with a flame ionization detector (FID), capillary column 15 m × 0.53 mm. nitrogen was used as a carrier gas at a flow rate of 7 ml/min. oven temperature 200°C. Under these conditions, the retention time of Ortho-phenylphenol was 2.038 min. The quantitative determination by comparison with the standard of known purity under the identical GLC condition.

5. Gas-Chromatography-Mass spectrometry analysis of Imazalil, orthophenylphenol, Fludioxonil, Thiabendazole and Pyrimethanil:

Apparatus Agilent 7890 B, 5977 A MSD gas chromatography equipped with an Agilent mass spectrometric detector with a direct capillary interface and a fused silica capillary column (30 m × 0.025 mm HP-5-0.25 micron -60 to 325/325ºC) was used. Samples were injected under the following conditions: Helium was used as a carrier gas at approximately 1 ml/min in pulsed split mode, with a split ratio 10:1, and a split flow of 10 mL/min. The solvent delay was 4 min, and the injection size was 1 μL. oven temperature program, 50°C for 0.5 min, then 10°C/min ramp to 190°C followed by a 10°C/min ramp to 210°C for 1 min followed by a 10°C/min ramp to 300°C and held for 2 min (total run time: 34 min). The injector temperature was set at 280°C. W9N11 Mass Spectral databases were used in the identification of the separated peaks.

6. Determination of the main physical properties as emulsion stability:

6.1. Preparation and determination of the physical properties of standard water used:

According to CIPAC (1995) MT 18.1 Non-CIPAC Standard Waters, 18.3.1 WHO Standard Hard Water, (342 ppm hardness) was used in all tests of physical properties. Calcium chloride $CaCl_2$ (0.304 g) and magnesium chloride $MgCl_2$. 6 H_2O (0.139 g) were dissolved in distilled water and made up to 1000 ml.

6.2. Emulsion stability evaluation for test formulation EC:

The test was carried out by adding 5 ml of the formulation to 95 ml of standard water (In a cylinder of 100 ml). The cylinder was

invested 30 times in one min and then placed in a water bath at 30±2°C for 30 min. At the end of this time, the amount of free oil cream separated at the top of the emulsion was observed after the emulsion had been allowed to stand undisturbed for 30 min.

6.3. Suspensibility test of Scholar 23%, Fruitgard 45% flow and Decco 36% (SC) formulations:

The test was run according to CIPAC (2003). Weigh an accurately sufficient sample of SC (2.5 × 100 / conc. of the sample) after shaking of the container. Samples were transferred quantitatively into a 250 ml Stoppard measuring cylinder containing the standard water; the volume was completed to 250 ml with the water, the cylinder was inverted 30 times in one min, and then placed in a water bath maintained at 30±2°C for 30 min. At the end of this time, the separated materials or precipitation, if any, were measured.

7. Biological test:

7.1. Source and rearing of *Spodoptera littoralis* culture:

A reared strain of *S. littoralis* for many generations in the Egyptian central agricultural laboratory by methods described by Perkins (1979) was used in laboratory bioassay. When egg masses were placed in big glass jars, then larvae emerged and fed on the Castor Bean leaves, *Ricinus communis* completed their life cycle with leaves that changed daily until the pupation started. Pupae are kept individually in separate and cleaned jars until adult emergence. Newly emerged adults were placed in cylindrical transparent plastic jars. Rearing conditions in the lab were 25±2ºC, 60±5% relative humidity (RH), with a photoperiod of 16:8 (L:D) h. Adults were fed at 10% a honey solution and allowed to oviposit on the Defla (*Nerium oleander*) leaves.

7.2. Bioassay of insecticides:

Bioassays were conducted on 4th instar larvae of *S. littoralis* using a leaf-dip technique, according to Khan *et al.* (2011). Where stock solutions of each tested fungicide formulation were prepared, the subsequent dilution of five concentrations by water was done. Castor bean leaves collected from fields were washed and dipped into the insecticide solutions of each concentration for 10 seconds, gently, and left to dry at room temperature. Then placed in the Petri dishes provided with ten of the 4th instar larvae. Three replicates for each concentration were prepared, and the control was dipped in water only. Bioassay dishes were maintained in the lab room conditions described previously. Mortality was recorded 24hrs. after treatments against lab-reared *S. littoralis* larvae.

9. Data analysis:

Mortality data were used to attain toxicity lines, slope, LC_{50}, LC_{90}, and their confidence limits using Polo software from LeOra Software (1989) according to Finney (1971) included in Probit analysis and Abbot mortality correction. A one-way ANOVA was performed to show the significant differences between stored fungicides and unsorted groups of treatment using SPSS software v.20, while the main effects were insecticides, and the response was the percentage of mortality. Mean was separated by Fisher's LSD test at P=0.05 (Sokal and Rohlf, 1995).

Results and discussion

1.Effect of storage on the stability of Imazalil, Ortho-phenylphenol, Fludioxonil, Thiabendazole and Pyrimethanil:

Table (2) shows the effect of storage at 54 ± 2ºC for 14 days on the stability of the commercial (Imazalil) Imacid 7.5% LS (Imazalil 5% and Ortho-phenylphenol 22.45), Citrocil Plus 27.4% EC (Ortho-phenylphenol) Ortosol 20% EC (Fludioxonil) Scholar 23% SC (Thiabendazole) Fruitgard 45% flow SC and (Pyrimethanil) Decco 36% SC.

Evaluate the result and show that Imazalil, Ortho-phenylphenol, Fludioxonil, Thiabendazole, and Pyrimethanil active ingredients were 7.46, 19.92, 22.62, 44.99, and 35.96%, respectively, and reached 7.24, 19,70, 21.65, 44.76, and 35.88% after 14 days of storage at 54 ± 2°C for Imacid 7.5% LS, Ortosol 20% EC, Scholar 23% SC, Fruitgard 45% flow SC, and Decco 36% SC, respectively. Also, Imazalil 5% active ingredients were 4.99% and reached 4.91% after 14 days of storage at 54 ± 2°C, and Ortho-phenylphenol 22.45 active ingredients were 22.43% and reached 22.05% after 14 days of storage at 54 ± 2°C for Citrocil Plus 27.4% EC. On the other hand, the percentage losses of Imazalil, Ortho-phenylphenol, Fludioxonil, Thiabendazole, and Pyrimethanil reached 1.86, 0.9, 2.04, 0.13, and 0.08 after 14 days of storage for Imacid 7.5% LS, Ortosol 20% EC, Scholar 23% SC, Fruitgard 45% flow SC, and Decco 36% SC, respectively. However, the percentage loss of Imazalil reached 0.6 and Ortho-phenylphenol reached 1.6 after 14 days of storage at 54 ± 2°C for Citrocil Plus (27.4% EC). After storage at 54 ± 2°C for 14 days, the determined average active ingredient content must not be lower than 95% relative to the determined average content found before storage, according to FAO (2001) for Imazalil and FAO (2014) for Fludioxonil.

Aguera *et al*. (2000) found that PYR in aqueous solution degraded in about 230 min in the presence of TiO_2 if it was exposed to light, while it was persistent in the dark. The degradation of the postharvest fungicides Imazalil, Ortho-phenylphenol, and Pyrimethanil was studied on Clementine mandarins during packinghouse storage for a 28-day period at 4 °C by Natalia *et al.* (2016).

Table (2): Effect of storage on the stability of Imazalil, Ortho-phenylphenol, Fludioxonil, Thiabendazole and Pyrimethanil.

Storage Periods (Days)	Imacid 7.5% LS		Citrocil Plus 27.4% EC				Ortosol 20% EC		Scholar 23% SC		Fruitgard 45% flow SC		Decco 36% SC	
	Imazalil		Imazalil 5%		Ortho-phenylphenol 22.4%		Ortho-phenylphenol		Fludioxonil		Thiabendazole		Pyrimethanil	
	a.i	loss%	a.i	loss%	a.i	loss%	a.i	loss%	a.i	loss%	a.i	loss%	a.i	loss%
0	7.46	0.00	4.99	0.00	22.43	0.00	19.92	0.00	22.62	0.00	44.99	0.00	35.96	0.00
7	7.38	1.06	4.94	1.00	22.40	1.42	19.88	0.2	22.12	2.17	44.82	0.38	35.91	0.13
14	7.24	1.86	4.91	0.6	22.05	1.6	19.70	0.9	21.65	2.04	44.76	0.13	35.88	0.08

(a.i) active ingredient; (0) before one-hour storage; LS: solution for seed treatment; EC: Emulsion Concentrate; SC: Suspension Concentrates

2. Imazalil soluble concentration:

The formulation, after the stability test at 54°C and following dilution with CIPAC standard water D and standing at 30 ± 2°C for 30 minutes, shall give a clear or opalescent solution free from more than a trace of sediment and visible solid particles.

3. Effect of storage at 54 ± 2°C on the suspensibility of Scholar 23%, Fruitgard 45% flow and Decco 36% (SC):

The data presented in Table (3) showed suspensibility test for Scholar 23%, Fruitgard 45% flow, and Decco 36% (SC) stored at 54 ± 2°C for 14 days. Data revealed that no sediment was formed during the storage period in initial, 7 and 14 for Scholar 23%, Fruitgard 45% flow and Decco 36% (SC). According to FAO specifications, data showed that the samples conform to this specification.

Table (3): Effect of storage at 54 ± 2 ^{O}C on suspensibility of Scholar 23%, Fruitgard 45% flow and Decco 36% (SC).

Storage Periods (Days)	Scholar 23% (SC)	Fruitgard 45% flow (SC)	Decco 36% (SC)
0	-	-	-
7	-	-	-
14	-	-	-

(-) means No Sediment

4. The effect of storage on the emulsion stability of Ortho-phenylphenol:

The result showed the emulsion stability of the commercial formulation under the trade name Ortho-phenyl-phenol EC before and after 14 days of storage at 54± 2^{o}C. Results indicated that the Ortho-phenylphenol formulation passed successfully through the emulsion before and after 14 days of storage. According to the JMPS FAO/WHO Joint Meeting on Pesticide Specifications (2010)

5. Larvae responses to tested fungicide toxicities:

Toxicity response (Slope, LC_{50}, LC_{90}) values of *S. littoralis* larvae bioassay (Susceptible strain reared in the laboratory) to different new fungicides placed under storage of heat conditions (54±2^{o} C) or toxicity to unstored formulations (Data found in Tables 4 and 5). LC_{50} values appeared to arise after storage under heat conditions for all fungicides tested, except treatment by formulation of Ortho-phenylphenol and Imazalil+ Ortho-phenylphenol mixture. However, LC_{90} values of all fungicides placed under storage conditions (54 ± 2^{o}C.) appeared to have much higher toxicity than those not exposed to heat. Toxicity evaluations revealed a consistent profile, whereas the most effective compound was Imazalil, followed by Ortho-phenilphenol, at the two stages (exposed to heat or unexposed) and at LC_{50} or LC_{90} values. Moreover, the least toxic was Fludioxonil, Imazalil + Ortho-phenylphenol mixture, pyrimethanil, and thiabendazole. Fortunately, all tested fungicides reveal the ability to kill *S. littoralis* larvae at the defined concentrations (LC_{50} in Tables 4 and 5). Statistical analysis of slopes and lethal concentration lines values achieved there is not significantly different from each other at 0.5 of significance level between groups where (F=0.208, DF= 17 and P= 0.814), equality of variance p=0.752 and this statistic is delivered using Kruskal-Wallis ANOVA non-parametric test. The qui-square values refer to the good fit of toxicity lines, also the slopes and the intercept values refer to the homogeneity of the reared insect used in the study.

Similar fungicide toxicity results sustained the idea of fungicide toxicity to target insect pests. The effect of different fungicides toxicity on *S. littoralis* growth of developmental stages was discussed by some researchers like Srivastava *et al.* (2017), where two fungicides, mancozeb and ridomil, were evaluated against *S. litura.* Results showed influences on growth, and larval development duration was significantly shortened. Where he said that fungicides can serve as a practical tool to reduce population numbers and can play a greater role in IPM to manage insect pests and pathogens. Another example was about ten fungicide treatments to *S. littoralis* significantly reduced the larvae feeding on treated leaves, reduced larval weight, increased larval and pupal duration (days), reduced pupation %, and reduced pupal weight. A negligible effect on fecundity was noticed, but reduction in number of eggs per female was recorded for Mancozeb, Metalaxyl, Difenocenazole, and Thiram recorded by Rahouma and El-Kholy (2018). An example of using fungicide formulation mixed with insecticide results in increases in toxicity to *S. littoralis* found by Abdel-Hamid (2014) for toxicity by Benconazol, Daifeniconazol, and Anisol-hydroxybioteylated. Also, Jayasekharan *et al.*

(2018), tested a mixture of insecticide with a fungicide to control this pest, such as Cabendazim, Azoxystrobin, and Mrtalaxyl which had a good effect. Several fungicide mixtures were found to be highly effective against the two common pathogens of Fusarium, such as the mixtures Azoxystrobin + Difenoconazole, Fluopyram + Trifloxystrobin, or Fluazinam compounds, in a big survey experiment by Degani *et al.* (2022).

Table (4): Responses of *Spodoptera littoralis* to unstored insecticide formulation.

Fungicide	Slope ±SE	Intercept	LC_{50} (95% CI)	LC_{90} (95% CI)	$\chi 2$
Ortho-phenylphenol (Ortosol)	1.477-0.134	4.96	1.1(0.57-1.93)	7.77(4.2-14.2)	0.96
Thianendazole TBZ (Fruit Guard)	1.7-0.12	4.36	2.37(1.37-4.1)	13.8(8.0-23.8)	0.77
Imazalil+Ortho-phenylphenol (Citrocil plus)	1.7-0.117	4.6	1.94(1.2-3.1)	10.0(5.9-16.9)	0.68
Fludioxonil (ScholaR 230)	1.7-0.118	4.6	1.7(0.99-2.9)	9.8(5.8-16.7)	0.997
Imazalil (Imacid)	2.0-0.11	5.74	0.43(0.26-0.73)	1.85(1.1-3.1)	0.87
Pyrimethanil (Decco)	2.36-0.1	3.9	2.97(1.9-4.7)	10.4(6.6-16.3)	0.95

Table (5): Responses of *Spodoptera littoralis* to the stored insecticide formulation at heat conditions 54 °C.

Insecticide	Slope ±SE	Intercept	LC_{50} (95% CI)	LC_{90} (95% CI)	$\chi 2$
Ortho-phenylphenol (Ortosol)	1.22-0.162	5.03	0.95(0.46-1.97)	10.9(5.2-22.6)	0.935
Thianendazole TBZ (Fruit Guard)	1.1-0.163	4.5	2.6(1.26-5.4)	39.3(18.8-81.8)	0.993
Imazalil+Ortho-phenylphenol (Citrocil plus)	1.12-0.156	4.74	1.68(0.82-3.45)	23.8(11.6-48.8)	0.995
Fludioxonil (ScholaR 230)	1.5-0.12	4.55	1.9(1.1-3.4)	13.9(8.0-24.1)	0.98
Imazalil (Imacid)	1.7-0.137	5.2	0.76(0.41-1.42)	4.47(2.4-8.3)	0.89
Pyrimethanil (Decco)	1.94-0.1	3.5	5.9(3.7-9.6)	27.3(17.1-43.7)	0.87

The tested fungicides are already used for many varieties of fungus toxicity all over the world (Kanetis and Förster, 2007), tested Azoxystrobin, Fludioxonil, and Pyrimethanil for postharvest management of citrus green mold in the United States. But Overton *et al.* (2021), said that chemical compounds are used to reduce pest populations in Australian grain farming systems within an integrated pest management (IPM) framework of spiders, hoverflies, rove and cucumber beetle and carabid beetles. Beginning from seed, treatments to broad-spectrum of chemicals were adopted as pyrimethanil for high toxicity. Margus *et al.* (2023), found that fungicide can control the Colorado potato beetle because of its effect on life history traits, in the case of mutation (Acetylcholinesterase-2 gene) and the high survival in different populations that produce high offspring numbers. The fungicide exposure can have both negative and positive, long-lasting effects on beetles. Then most agricultural pests can be controlled by fungicide products. Also, Chen *et al.* (2022) study the combined seed treatment of neonicotinoids and fungicides, which offers a potential control measure for pest management of wheat seeding by a seed-coating agent prepared by thifluzamide and fludioxonil, as active components and other additives. Moreover, the distribution of

residues in the wheat plants at harvest was below 0.05 mg/kg at the recommended dosage.

The bioassay toxicity in earthworms revealed a low level of toxicity at LC_{50} > 10 mg/kg. Physical properties also may enhance the approach of the high chemical product toxicity features used to control pests. El-Nahhal (2014) studied the controlled release formulation of thiabendazole for reduced contaminations to soil, water, and agricultural products and showed that the absorption reaction in clay increased with temperature or pH increases.

In general, the commercial botanical-based insecticides Agroneem, Ecozin, and Neemix had a good effect as deterrents, and antifeedants on larvae of the beet armyworm, *Spodoptera exigua* and direct contact showed a decrease in the beet armyworm egg survivals. Survival of larvae fed for 7 days on treated leaves was reduced by 61% (Greenberg *et al.*, 2005). Briefly, the toxicity and biological effect of fungicides to control numerous kinds of invertebrates were studied by some researchers, such as Huang *et al.* (2022), who found that Imazalil and its metabolite are fungicides detected in aquatic environments tested against kind of fish (Danio rerio) embryos that showed toxicity signs leading to death and cell apoptosis of larvae. In another experiment, Imidacloprid plus Imazalil induced high cytotoxicity against distinct human cell line models (Silva *et al.*, 2022).

From the review of literature, we can briefly discuss the consequences of the status of fungicide product exposures used in IPM programs for pest control corporations with insecticides like Mello *et al.* (2021), speak about communities of aquatic insects, like the family Chironomidae insect group diversity affected by pyrimethanil fungicide that completely degraded and found increase in Richness and Shannon-Wiener Index (H'). But Shinn *et al.* (2015), studied the effects of pyrimethanil on the growth of the freshwater green microalgae *Selenastrum capricornutumas* a non-target pest because induced cell growth inhibition at one day after treatments thus considered an environmental disruptor.

6. Identification of Imazalil, Ortho-phenylphenol, Fludioxonil, Thiabendazole and Pyrimethanil by chemical ionization GC/MS Spectroscopy:

Tables (6, 7, 8, 9, and 10) and Figures (1-10) present the results of the GC-MS study, which classify the degradation products of Imazalil, Orthophenylphenol, Fludioxonil, Thiabendazole, and Pyrimethanil before and after storage. In Figure (1) and Table (6), the following degradation products were observed: Imazalil m/z = 297.18, which was identified as the pathway for the degradation of Imazalil that was initiated: 1-{2-(2-chlorophenyl)-2-[(prop-2-en-1-yl) oxy] ethyl}-1H-imidazole. However, the main breakdown product of Imazalil is 1-(2-ethoxy-2-phenylethyl)-1H-imidazole.

Imazalil is attacked by OH• radicals either on the benzene ring or on the double bond, leading to three monohydroxylated products, which reach their maximum concentrations after ca. 12 min of degradation and then decrease progressively to disappear from solution after ca. 120 min. Hazime *et al.* (2012). On the other hand, in Figure (2) and Table (7), the following degradation products were observed: Ortho-phenylphenol m/z = 170.21, which was identified as the pathway for the degradation of Oortho-phenylphenol initiated by 1,1'-biphenyl. However, the main breakdown product of Ortho-phenylphenol is [(3E)-penta-1,3-dien-3-yl] benzene.

Conjugates of OPP were acid-hydrolyzed to free OPP, derived to the Pentafluorobenzoyl ester derivative, and analyzed via negative-ion chemical ionization gas chromatography-mass spectrometry. Two stable isotope analogues of OPP were shown to be suitable as internal

standards for this method (D2-phenol ring, 13C6-phenyl ring) by Bartels *et al.* (1997).

However, the following degradation products of fludioxonil in Figure (3) and Table (8) were fludioxonil m/z = 248.18, which were identified as the pathways for the degradation of Fludioxonil that were initiated: 4-ethenyl-2,2-difluoro-2H-1,3-benzodioxole; however, the main breakdown products of 2,2-difluoro-2H-1,3-benzodioxole.

The fludioxonil degradation pathway was initiated by successive hydroxylation and carbonylation of the pyrrole moiety and disruption of the oxidized cyanopyrrole ring at the NH-C bond by Zografina *et al.* (2022). But in Figure (4) and Table (9), the following degradation products were observed: thiabendazole m/z = 201.25, which was identified as the pathway for the degradation of thiabendazole, was initiated: 1,3-thiazole-4-carboximidamide. However, the main breakdown product of thiabendazole is (E)-2-(1H-benzimidazol-2-yl) ethene-1-thiol. The possible direct cleavage of the TBZ molecule by the two N atoms in the imidazole ring. The pathway initiated by the hydroxylation of the TBZ molecule was not identified by Melisa *et al.* (2022).

Results, in Figure (5) and Table (10), the following degradation products were observed: pyrimethanil (PYR) m/z = 199.25, which was identified as the main breakdown product of pyrimethanil, which is 4,6-dimethylpyrimidin-2-amine.

Firstly, hydroxylation can occur via the H-abstraction on the aromatic ring of PYR due to the electron-donating resonance effect through π orbitals (Walse and Karaca, 2011 and Zheng *et al.*, 2019). Meanwhile, the presence of amino and alkyl substituents on the pyrimidine ring of PYR directs the electrophilic attack of reactive radicals to the same site, leading to the insertion of a hydroxy group in the C5 position by Walse and Karaca (2011). Consequently, the hydroxylation of aromatic and/or pyrimidine rings could result in the generation of the monohydroxylated compound Int 1, which has two kinds of isomers depending on the binding positions of the hydroxy group. Similarly, as successive attacks of hydroxyl radicals and positive holes.

Data in Tables (6, 7, 8, 9 and 10) show that the Retention time (R.T.) of breakdown products of Imazalil, Orthophenylphenol, Fludioxonil, Thiabendazole, and Pyrimethanil before storage were 20.79, 14.48, 25.52, 21.17, and 16.89 min., respectively, and after 14 days of storage were 20.46, 14.27, 25.54, 20.83, and 16.87 min., respectively.

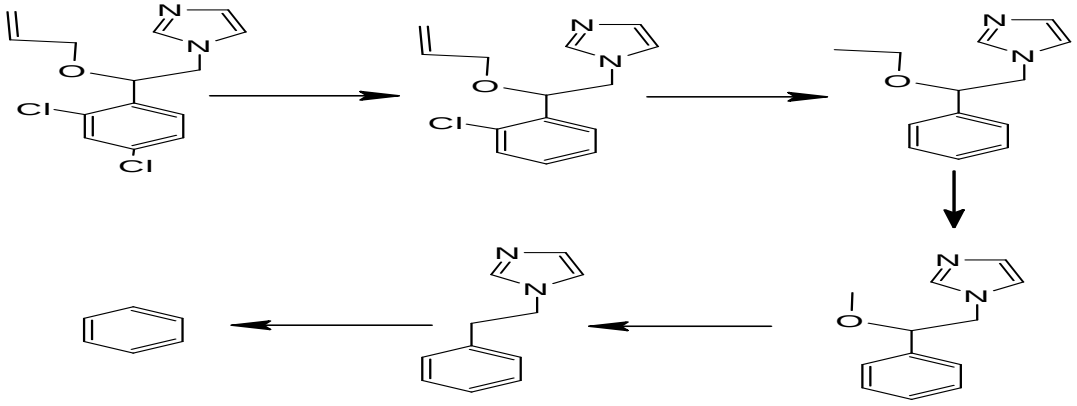


Figure (1): Degradation pathway of Imazalil.

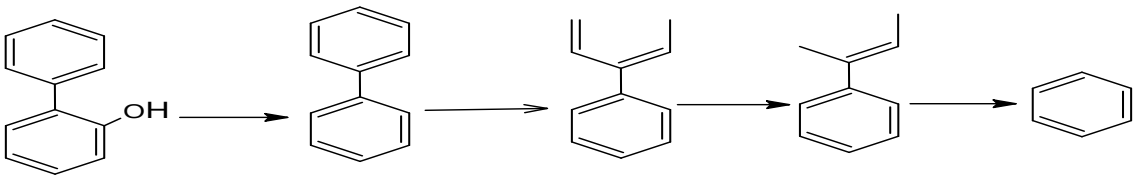


Figure (2): Degradation pathway of Ortho-phenylphenol.

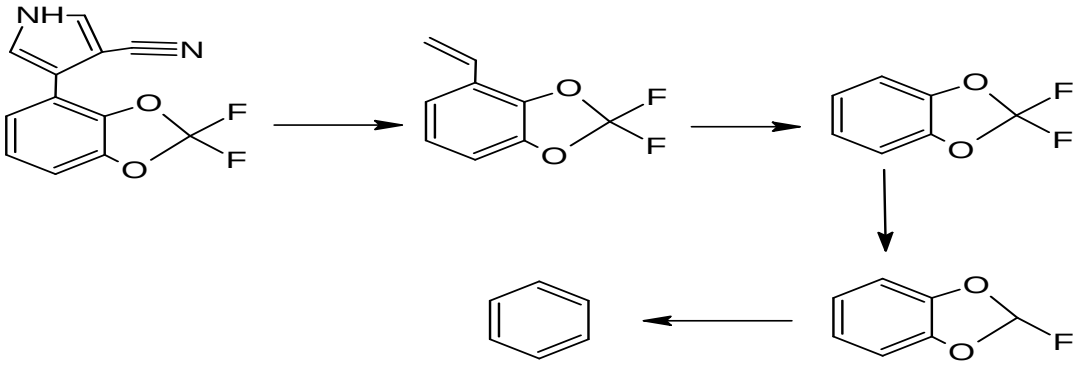


Figure (3): Degradation pathway of Fludioxonil.

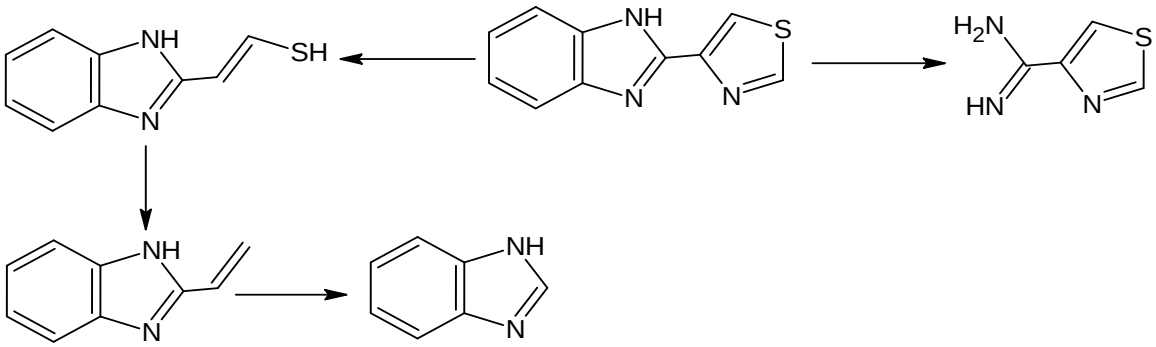


Figure (4): Degradation pathway of Thiabendazole.

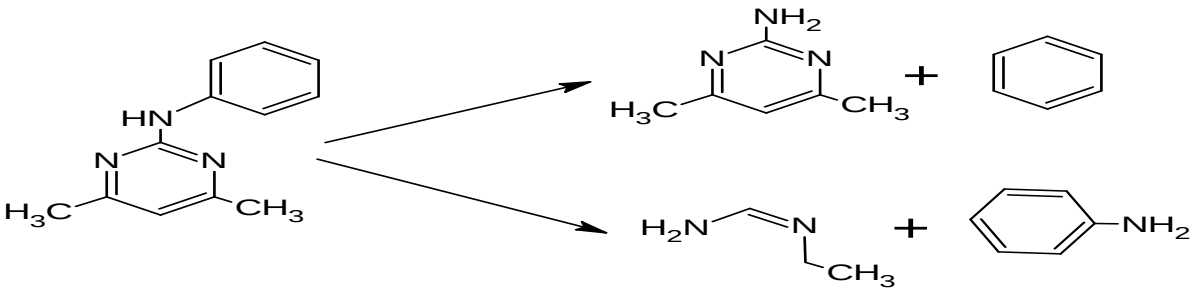
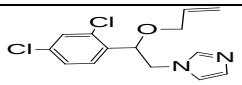
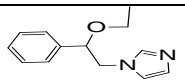
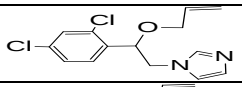
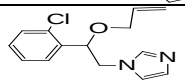
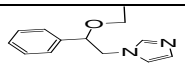
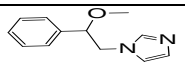
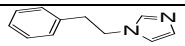


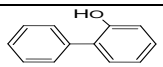
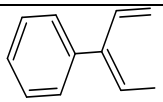
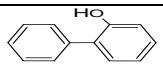
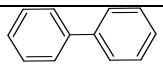
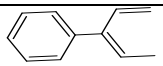
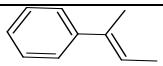
Figure (5): Degradation pathway of Pyrimethanil.

Table (6): Identification of the degradation products of Imazalil by GC-MS.

Storage Periods	Chemical Name	Chemical Structure	Molecular Formula	Molecular Weight	Retention Time
Before Storage	Imazalil		$C_{14}H_{14}Cl_2N_2O$	297.18	20.79
	1-(2-ethoxy-2-phenylethyl)-1H-imidazole		$C_{13}H_{16}N_2O$	216.28	14.48
After Storage	Imazalil		$C_{14}H_{14}Cl_2N_2O$	297.18	20.47
	1-{2-(2-chlorophenyl)-2-[(prop-2-en-1-yl)oxy]ethyl}-1H-imidazole		$C_{14}H_{15}ClN_2O$	262.74	20.20
	1-(2-ethoxy-2-phenylethyl)-1H-imidazole		$C_{13}H_{16}N_2O$	216.28	14.27
	1-(2-methoxy-2-phenylethyl)-1H-imidazole		$C_{12}H_{14}N_2O$	202.25	13.87
	1-(2-phenylethyl)-1H-imidazole		$C_{11}H_{12}N_2$	172.23	12.25

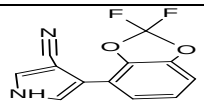
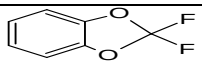
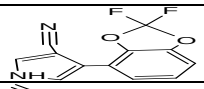
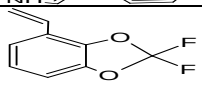
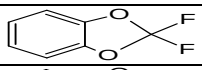
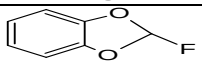
Initial: One hour before storage; Retention time (Min.)

Table (7): Identification of the degradation products of Ortho-phenylphenol by GC-MS.

Storage Periods	Chemical Name	Chemical Structure	Molecular Formula	Molecular Weight	Retention Time
Before Storage	Ortho-phenylphenol		$C_{12}H_{10}O$	170.21	14.48
	[(3E)-penta-1,3-dien-3-yl] benzene		$C_{11}H_{12}$	144.21	16.46
After Storage	Ortho-phenylphenol		$C_{12}H_{10}O$	170.21	14.27
	1,1'-biphenyl		$C_{12}H_{10}$	154.21	15.52
	[(3E)-penta-1,3-dien-3-yl] benzene		$C_{11}H_{12}$	144.21	15.98
	[(2E)-but-2-en-2-yl] benzene		$C_{10}H_{12}$	132.20	16.39

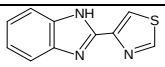
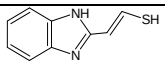
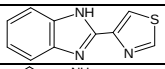
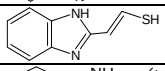
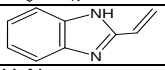
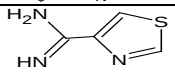
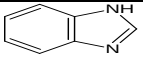
Initial: One hour before storage; Retention time (Min.)

Table (8) : Identification of the degradation products of Fludioxonil by GC-MS.

Storage Periods	Chemical Name	Chemical Structure	Molecular Formula	Molecular Weight	Retention Time
Before Storage	Fludioxonil		$C_{12}H_6F_2N_2O_2$	248.18	25.52
	2,2-difluoro-2H-1,3-benzodioxole		$C_7H_4F_2O_2$	158.1	20.16
After Storage	Fludioxonil		$C_{12}H_6F_2N_2O_2$	248.18	25.54
	4-ethenyl-2,2-difluoro-2H-1,3-benzodioxole		$C_9H_6F_2O_2$	184.14	23.78
	2,2-difluoro-2H-1,3-benzodioxole		$C_7H_4F_2O_2$	158.1	21.20
	2-fluoro-2H-1,3-benzodioxole		$C_7H_5FO_2$	140.1	20.38

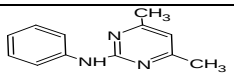
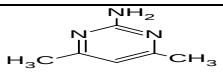
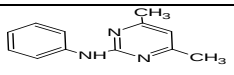
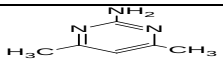
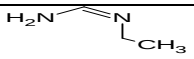
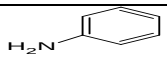
Initial: One hour before storage; Retention time (min)

Table (9): Identification of the degradation products of Thiabendazole by GC-MS.

Storage Periods	Chemical Name	Chemical Structure	Molecular Formula	Molecular Weight	Retention Time
Before Storage	Thiabendazole		$C_{10}H_7N_3S$	201.25	21.17
	(E)-2-(1H-benzimidazol-2-yl) ethene-1-thiol		$C_9H_8N_2S$	176.24	22.06
After Storage	Thiabendazole		$C_{10}H_7N_3S$	201.25	20.83
	(E)-2-(1H-benzimidazol-2-yl)ethene-1-thiol		$C_9H_8N_2S$	176.24	20.09
	2-ethenyl-1H-benzimidazole		$C_9H_8N_2$	144.17	18.98
	1,3-thiazole-4-carboximidamide		$C_4H_5N_3S$	127.17	16.78
	1H-benzimidazole		$C_7H_6N_2$	118.13	15.72

Initial: One hour before storage; Retention time (min).

Table (10) : Identification of the degradation products of Pyrimethanil by GC-MS.

Storage Periods	Chemical Name	Chemical Structure	Molecular Formula	Molecular Weight	Retention Time
Before Storage	Pyrimethanil		$C_{12}H_{13}N_3$	199.25	16.89
	4,6-dimethylpyrimidin-2-amine		$C_6H_9N_3$	123.15	21.23
After Storage	Pyrimethanil		$C_{12}H_{13}N_3$	199.25	16.87
	4,6-dimethylpyrimidin-2-amine		$C_6H_9N_3$	123.15	20.97
	N'-ethylmethanimidamide		$C_3H_8N_2$	72.10	22.71
	aniline		C_6H_7N	93.13	23.92

Initial: One hour before storage; Retention time (min)

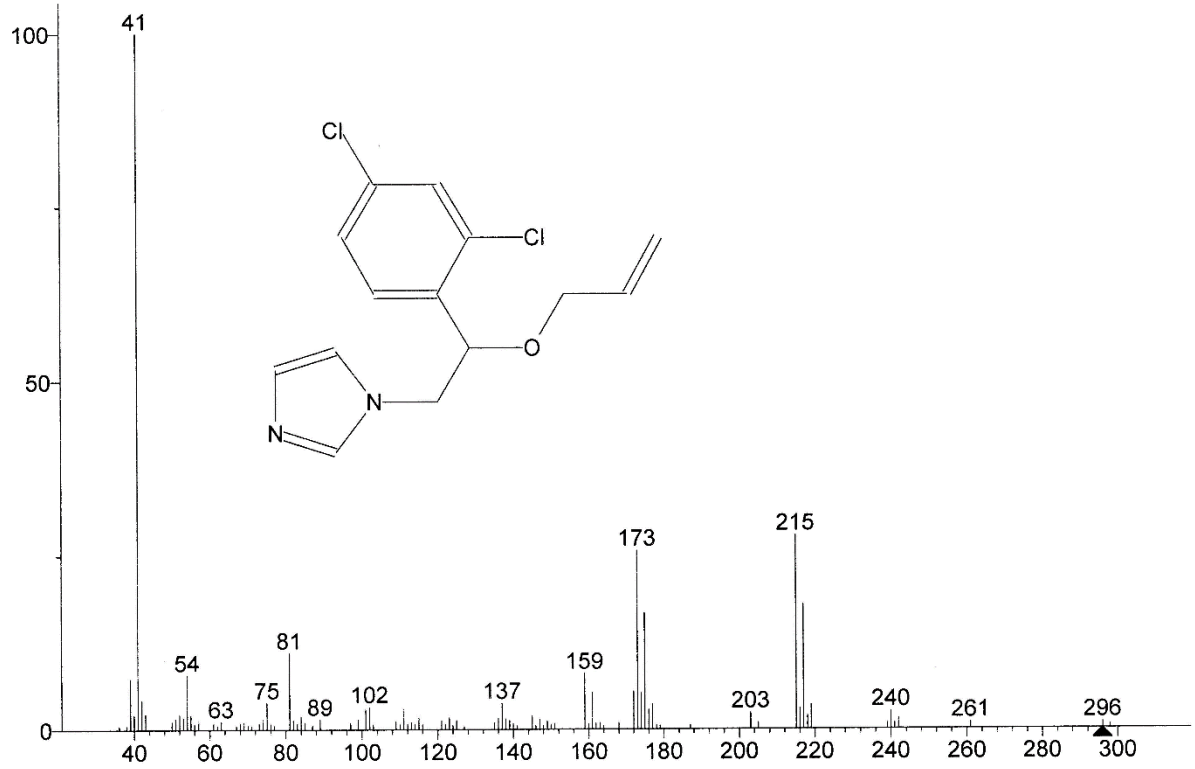


Figure (6): GC/MS Chromatogram for Imazalil.

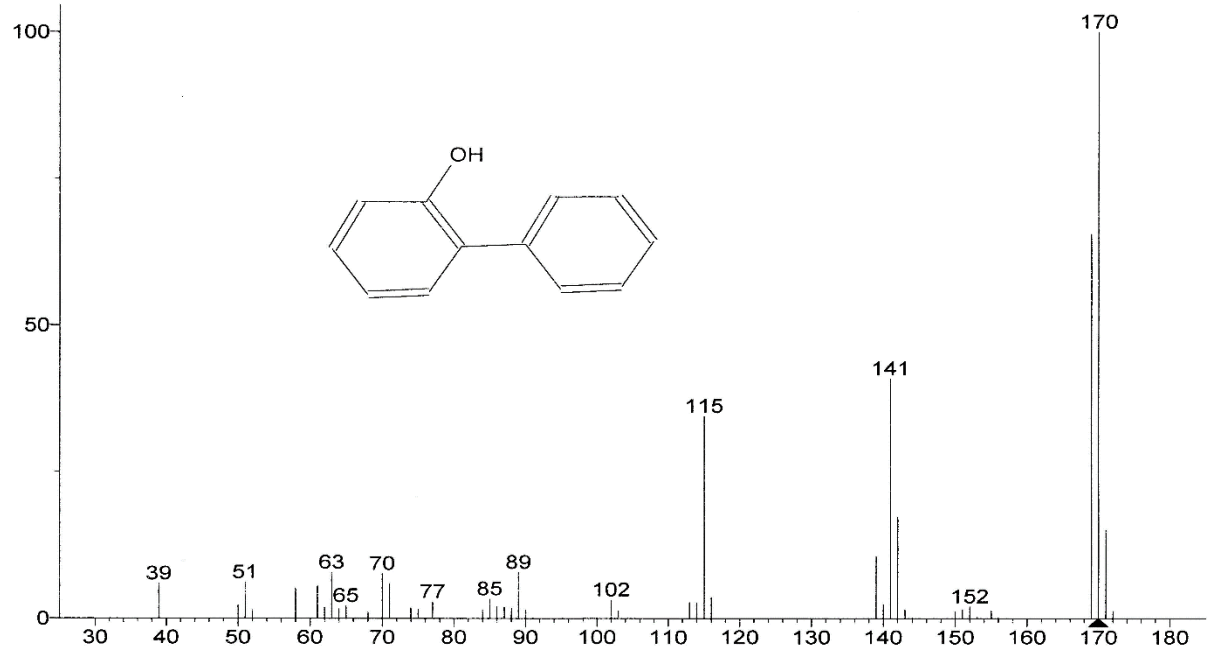


Figure (7): GC/MS Chromatogram for Ortho-phenylphenol.

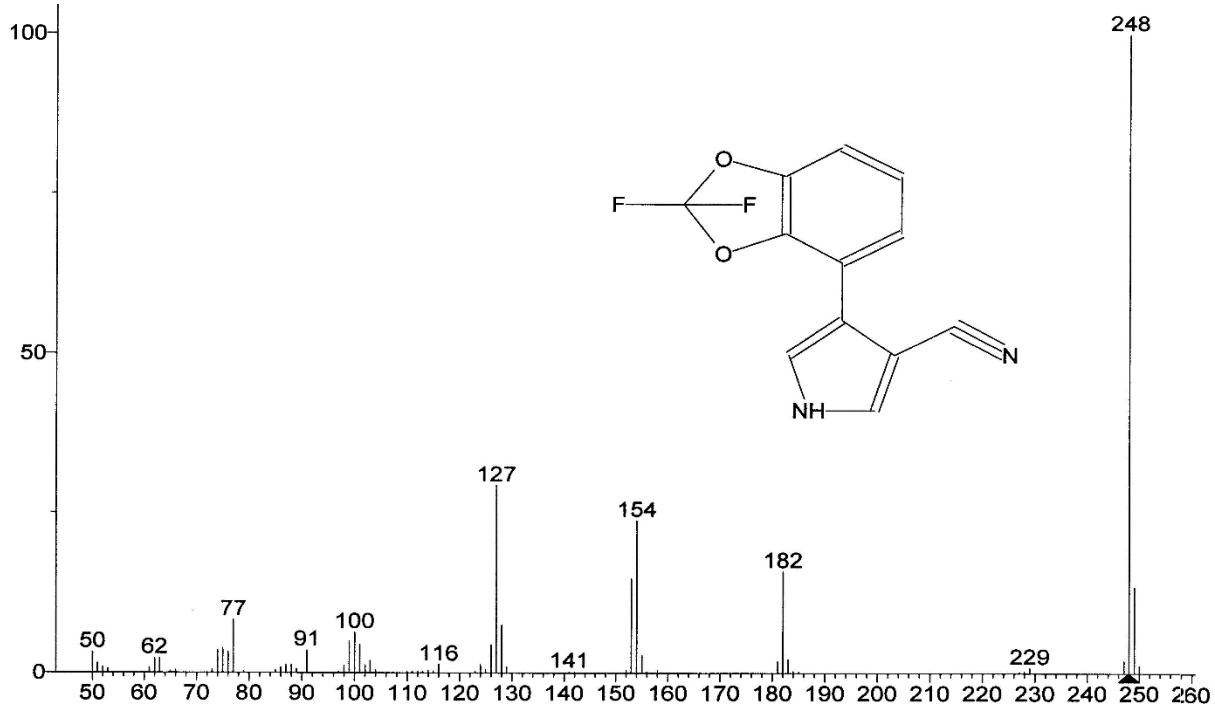


Figure (8): GC/MS Chromatogram for Fludioxonil.

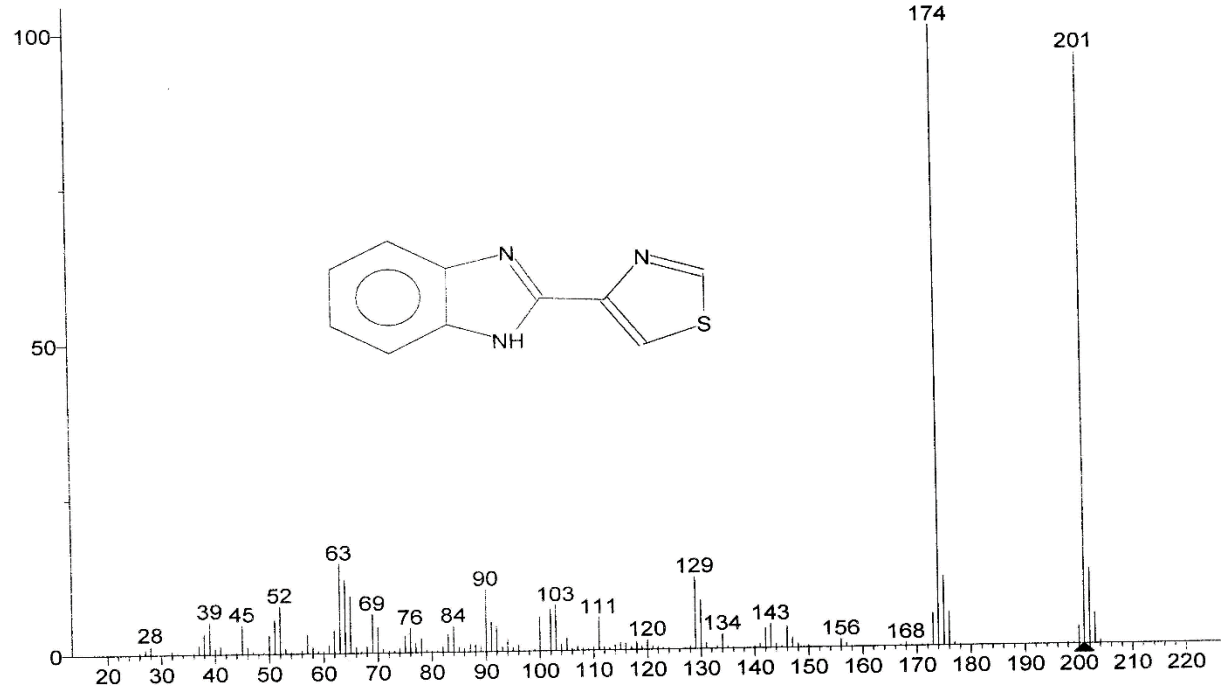


Figure (9: GC/MS Chromatogram for Thiabendazole.

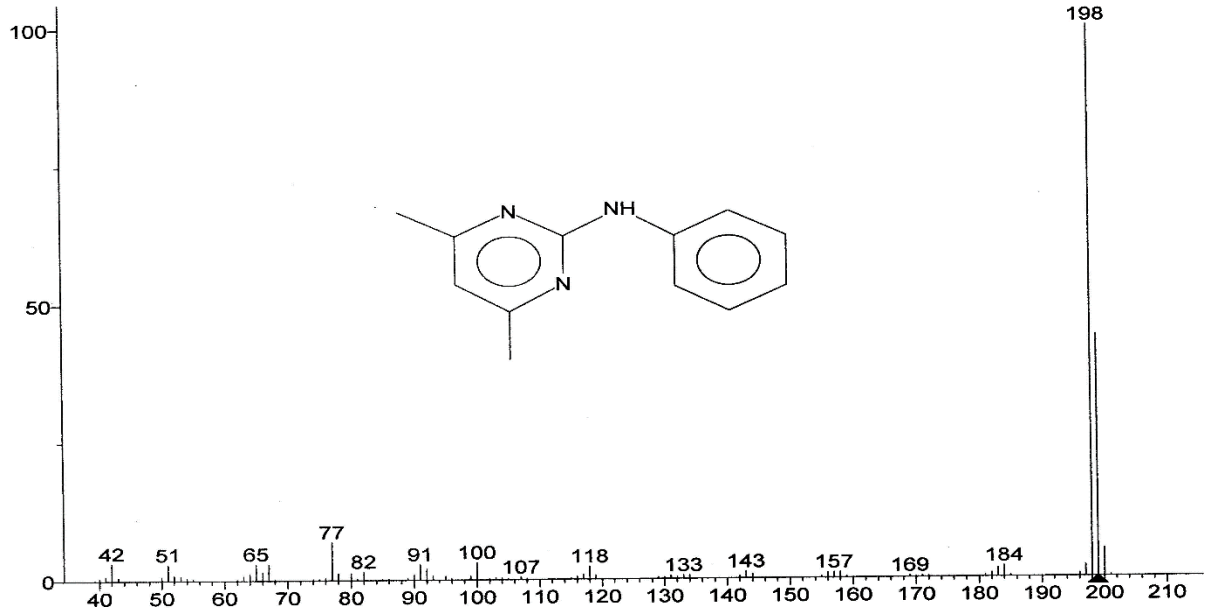


Figure (10): GC/MS Chromatogram for Pyrimethanil.

Generally, we evaluated the effects of storage at 54 ± 2 ºC for 14 days on fungicides (Imazalil) Imacid 7.5% LS (Imazalil and Ortho-phenylphenol) Citrocil Plus 27.4% EC (Ortho-phenylphenol), Ortosol 20% EC (Fludioxonil), Scholar 23% SC (Thiabendazole) Fruitgard 45% flow SC and (Pyrimethanil) Decco 36% SC and determined physical properties by HPLC and fingerprint GC/MS, along with the evaluation of their efficacy by testing them against *S. littoralis*, the most important cotton pest. GC/MS was used for the degradation of Imazalil, Ortho-phenylphenol, Fludioxonil, Thiabendazole, and Pyrimethanil fungicides. The major degradation products of Imazalil, Ortho-phenylphenol, Fludioxonil, Thiabendazole, and Pyrimethanil fungicides were 1-(2-ethoxy-2-phenylethyl)-1H-imidazole; [(3E)-penta-1,3-dien-3-yl] benzene; 2,2-difluoro-2H-1,3-benzodioxole; (E)-2-(1H-benzimidazol-2-yl) ethene-1-thiol; and 4,6-dimethylpyrimidin-2-amine, respectively.

In biological tests, most of the tested fungicide compounds showed promising data and high toxicity. But generally, Imazalil was the most effective.

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