Effect of some fungicides against early blight disease in potato cv. sponuta (*Solanum tuberosum* L.) and residual in tuber

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ABSTRACT: Four fungicides were tested to control the disease in the year 2019 at two localities at EL-Behera governorate. Kocide 2000 53.8% DF (Copper hydroxide), Speedcide 25% EC (Difenconazole), Stone 50% WDG (Dimethomorph) and Byroxanil 45% SC (Propamocarb HCl, Cymoxanil) gave the best results for reducing the growth of *A. solani* in both laboratory and field conditions compared with check. Also, the use of chemical fungicides increased both peroxidase and polyphenoloxidase that were reflected through increasing in resistance of plants. The residues of fungicide in potato tuber were undetectable after 2 hours (Initial) of application for cymoxanil and promocarb HCl. while it reached maximum residue 1 day (1.4 ppm) and (0.83 ppm) for promocarb-HCl and cymoxanil respectively. The half life were 6.5 days and 6.2 days for promocarb-HCl and cymoxanil respectively.

keywords: potato, early blight, fungicides, enzymes, residues in tuber.

1.INTRODUCTION

Potato is the most important vegetable crop in terms of quantities produced and consumed worldwide. It is the fast-growing major crop in the world with important economic impact on many resource-poor farming families. **(Binyam Tsedaley 2014).** Early blight disease of potato caused by *Alternaria solani* is distributed in most countries where potatoes are grown. It is generally recognized thasst successful control of this disease can be obtained by the use of foliar fungicides. Early blight is caused by *Alternaria solani* and *A. alternata*, which is also the causative agent for brown spot. Early mainly affects potato foliage and leads to leaf necrosis and premature defoliation **(Leiminger and Hausladen, 2011, Aqleem Abbas 2017).** The early blight disease has become the most destructive in all over the world and yield losses up to 80%.The disease in severe cases can lead to complete defoliation and is most damaging on tomato in regions with heavy dew, rainfall, high humidity and fairly high temperatures. All above ground parts of the plant can have symptoms of this disease. **(Sreenivasulu *et al.*, 2019)**.

Saad and Stino (1982) found that the early blight infection was 70.08 % in the un-sprayed potato plants, and the infection decreases according to number of sprays applied by fungicide.

Growers control early blight with fungicides applied to the foliage up to 8-10 times per growing season **(Stevenson,1993)**. On early blight-susceptible cultivars, responses to fungicide applications can be highly significant with yield increases reported of as much as 127 % **(Teng and Bissonnette 1985)**.

Pesticides occupy a unique position among the many hazardous chemicals that man and animals encounter daily. Pesticides are intentionally introduced into the environment to enhance agricultural production, to reduce pest damage on crops and to control disease vectors **(Krol, *et al.* 2000).** Pesticide residue analysis plays an important role in food quality for evaluating food safety and possible risks to human health **(Fayza 2008)**.

The aim of present work:

1- determined the effective concentration of tested fungicide against *A. solani* under laboratory and filed conditions.
2- study the effect of tested fungicides on polyphenol oxides and peroxides enzymes in treated leaves of potato.
3- study the residual fungicides in tuber after spray.

2.MATERIALS AND METHODS

2.1.Isolation and Identification:

A total of 100 potato leaves showing typical early blight symptoms were collected at two localities at El-Behera governorate in the main potato producing region province in season 2019.

The leaves infected with diseases was washed thoroughly with tap water, dried between sterilized filter paper and cut into small pieces. The pieces were surface sterilized by immersing in 2.5 % sodium hypochlorite solution for 5 minutes and then rinsed several times in sterilized water and dried between sterilized filter papers, placed on PDA medium in petri-dishes, and incubated at 25±2 ºC for 5-7 days. Purification of the isolated fungus was carried out using the single spore or hyphal tip techniques **(Hildebrand, 1938)**.

The purified fungus isolates were identified by the Plant Pathology Res. Inst., Agric. Res. Cent., Giza, Egypt.

2.2.Chemical Control:

2.2.1. Laboratory experiments:

2.2.1.1.Effect of different concentration of various fungicides on radial growth of *Alternaria soloni* on PDA medium:

The effect of four different fungicides each with Kocide 2000 53.8% DF (Copper hydroxide), Speedcide 25% EC (Difenconazole), Stone 50% WDG (Dimethomorph) and Byroxanil 45% SC (Propamocarb HCl, Cymoxanil) concentrations, i.e. (0, 50, 100, 200, 400, 600, 800, 900, 1000 ppm) on the radial growth of *Alternaria solani* was studied on PDA medium. Four petri-dishes were used for each concentration and four petri-dishes free from fungicides were used as control for fungus. Different concentrations were added before solidification of the medium. After solidification, a disk of 6 mm in diameter of the tested fungus was inoculated in the middle of each plate. After inoculation, the dishes were incubated at 25±2 ºC. The radial growth was recorded after 5 to 7 days according to **EL-Desoki (1993)**.

2.2.2. Field experiments:

The effectiveness of leaf treatments with fungicides in controlling *A. solani* was determined in a naturally infested field at two localities at kom-Hamada EL-Behera gavernorate. Four fungicides treatments and a control of non-treated plants, sponuta variety, were arranged in a complete randomized block design with four replicates. Each fungicide was tested as previously described and shown in rows, each tuber in one holes, in 4-row (length of 4 m and wides for 0.80 m) with approximately 25 cm between holes. The experiments were conducted during successive summer season of 2019.

Percentages of disease incidence and severity were recorded after 7 days from the end spray (75 days) after sown, the formula suggested by **Crowe and Hall (1980)** as follows:

$$\text{Disease incidence (D.I.\%)} = \frac{\text{No. of infected plants}}{\text{No. of total plants}} \times 100$$

As well as, disease severity (D.S) was estimated based on the 0-100 scale earlier suggested by **Abd El-Moity (1976) and Shatla *et al*. (1980)**, which was also applied in the virulence test. To evaluate efficacy of the tested control agents, percentage of the reduction rate of both data obtained from disease incidence (D.I) and disease severity (D.S) in relative to control was represented by the following formulas:

$$\text{Reduction rate of D.i \%} = \frac{\text{D.I \% of control} - \text{D.I \% of treatment}}{\text{D.I \% of control}} \times 100$$

$$\text{Reduction rate of D.s \%} = \frac{\text{D.S \% of control} - \text{D.S \% of treatment}}{\text{D.S \% of control}} \times 100$$

D.I = Disease index
D.S= Disease severity

2.2.3. Enzyme activities:

Antagonists effect on activities of some disease defense responsible enzymes polyphenol oxidase (PPO) and peroxidase (PO), were estimated 80 days after sowing in the leaves. Therefore, crude enzyme extract. was prepared according to the methods described by **Maxwell and Bateman (1967)**.

2.2.3.1. Assay of polyphenol oxidase:

Activity of polyphenol oxidase was determined according to the Colorimetric procedure adopted by **Matt and Dimond (1963)**.

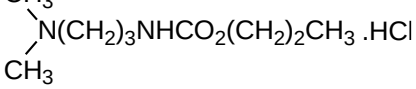
2.2.3.2. Assay of peroxidase:

Peroxidase activity was determined according to the method of **Srivastava (1987)**.

2.4. Chemical and physical composition:

2.4.1. Propamocarb HCl:

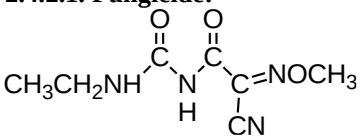
2.4.1.1. Fungicide:



IUPAC name: propyl 3-(dimethylamino) propylcarbamate hydrochloride
chemical abstracts name: propyl [3-(dimethyl amino) propyl]carbamate monohydrochloride
Common name: propamocarb (BSI, E-ISO, ANSI); propamocarbe (*m*) F-ISO)
Solubility: In water >500 g/l (20 °C).

2.4.2. Cymoxanil:

2.4.2.1. Fungicide:



IUPAC name: 1-(2-cyano-2-methoxyiminoacetyl)-3-ethylurea

Chemical Abstracts name: 2-cyano-*N*-[(ethyl amino)carbonyl]-2-(methoxyimino)acetamide

Common name: cymoxanil (BSI, ANSI, draft E-ISO, (*m*) draft F-ISO)

Solubility: In water 890 mg/kg.

2.5. Chemicals and reagents:

Acetonitrile were purchased from Merck (Darmstadt, Germany) in HPLC grade quality. Ultra-pure water was prepared by a Millipore system.

anhydrous magnesium sulfate was purchased from merck (darmstadt, germany). anhydrous magnesium sulfate was activated by heating at 400 ºC for 4 hrs in a muffle furnace, cooled and kept in desiccators before use. Primary secondary amine (PSA, 40 µm Bondesil) and graphite carbon black (GCB, 40 µm) sorbents were purchased from Agilent Technologies (Santa Clara, CA). Sodium chloride and sodium sulphate in analytical grade were purchased from El Nasr Pharmaceutical Chemicals Company (Cairo, Egypt).

Reference standards of Propamocarb hydrochloride and Cymoxanil were obtained from Dr. Ehrensdorfer (Augsburg, Germany), with purities >98%.

2.6. Apparatus and equipment:

The equipment; PTFE 15 ml and 50 ml with screw cap tubes, vials 2ml with screw top, blender HGB55E, vortex shaker, desiccator, analytical balances, rotary evaporator, syringe filters PTFE and high-speed centrifuge were used in this study.

High Performance Liquid Chromatography (HPLC): Agilent Model 1260 (Agilent Technology, Waldbronn, Germany), with quaternary pump, auto sampler injector, thermostat compartment for the column and photodiode array detector.

2.6.1. Preparation of standard solution:

The stock solution containing 1,000 µg /ml^{-1} of analyte was prepared using acetonitrile as solvent. The standard solutions used for fortification of the matrices and instrument calibration purposes were prepared by serial dilution. All standards solutions were stored at 4°C before use. Standard calibration .

curve of propamocarb hydrochloride and cymoxanil was constructed by plotting analyte concentrations versus peak area

2.6.2. analytical procedures for fungcticides residues:

2.6.2.1. Sampling of tuber:

After spray of the tested fungcticides, samples of tuber were taken randomly from each replicate at intervals of zero time (2 hrs after application), 3, 5, 7, 10 and 15 days, and stored at –20 ºC until using for analysis.

2.6.2.2. Extraction and Clean-up:

Propamocarb hydrochloride and Cymoxanil weigh 10 g of into a 50 ml centrifuge tube (with screw cap). Add 10 ml of acetonitrile and e.g. 100 µl of the ISTD solution. Shake vigorously for 1 min (first extraction step). Add 4 g of $MgSO_4$, 1 g of NaCl, 1 g of Na_3Citrate dihydrate and 0.5 g of Na_2HCitrat sesquihydrate shake each tube directly after the salt addition shortly. Shake vigorously for 1 min (second extraction with phase separation). Centrifuge for 5 min at > 3000 g. Transfer X ml of the extracts into a PP single use centrifugation tube, which contains X*25 mg of PSA and X*150 mg of $MgSO_4$ (for samples with high amounts of chlorophyll or carotinoids add GCB as well, see 5.4.3). Shake for 30 sec. (when using GCB 2 min.). Centrifuge for 5 min at >3000. Transfer Y ml of the extracts into screw cup vial, and acidify with Y*10 µl of 5 % formic acid in acetonitrile (10 µl/ml extract). The cleaned and acidified extracts are transferred into auto sampler vials to be used for the multi-residue determination by GC or LC techniques (**DIN, 2009)**

2.6.3. Cleaning-up:

An aliquot of 4 ml was transferred from the supernatant to a new clean 15-ml centrifuge tube containing 100 mg PSA and 600 mg anhydrous magnesium sulfate. The samples were again vortexed for 3 min and then centrifuged for 10 min at 4,000 rpm. An aliquot of 2 ml was filtered through a 0.2 µm PTFE filter (Millipore, USA). The sample was then ready for the final analysis in LC system **(Anastassiades, *et al.* 2003)**.

Recovery of the efficiency of the chromatographic analysis for determination of Propamocarb HCl, Cymoxanil, residues in Tuber of potato was run by adding known amount of each fungicide as alone to untreated tuber samples which then put through the extraction, clean-up and residue determination as followed in the applied methods.

The recovery values were calculated according to the following formula:

$$\text{Recovery value} = \frac{\text{µg fungicide /g sample found}}{\text{µg fungicide /g sample added}} \times 100$$

Table (1) The condition of high performance chromatography (HPLC).

Pesticides	Mobile phase	Flow rate (ml/min)	Detector wavelength (nm)	R. Time
Cymoxanil	Acetonitrile 80% + Water 20%	0.8	230	3.62
Propamcarb-HCL	Methanol 70% + Water 30%	0.8	260	4.13

The average recovery values of tuber samples were used to correct all obtained values of Propamocarb HCl, Cymoxxanil, residues.

2.6.4. Fungcticide residue calculation:

The residues were calculated by applying the following equation of **Mollhoff (1975).**

ppm = *ps*.B .V/ pst. G.C * F

Where: F=100/R (recovery factor)
Pst=standard peak area
R=average of recovery.
V=final of sample solution. (ml).
ps = sample peak area.
B= amount injected of standard solution (ng)
G= sample weight (gm)
C=amount of sample solution injected.

3.RESULTS AND DISCUSSION

3.1. Chemical Control.

3.1.1. *In vitro* experiment.

Four of the commerically available fungicides were chosen to study *in vitro* fungicidal effect at different concentrations on the radial growth of one isolate of *Alternaria solani* .

Data in Table (2) show that Byroxanil was the most effective fungicide used against the isolated *Alternaria solani* and no fungal growth occurred on PDA medium containing as low as 800 ppm, followed by Stone that inhibited the *A. solani* at 900 ppm. Meanwhile, Speedcide and Kocide 2000 were completely inhibited the growth of isolated *A. solani* at 1000 ppm.

Table (2) Effect of different concentrations of four fungicides on the radial growth (cm) of the tested *Alternaria solani* on PDA medium seven days after inoculations and incubations at 25±2 ºC.

Concentration (ppm)	Effect of fungicides on radial growth (cm)			
	Byroxanil	**Stone**	**Speedcide**	**Kocide2000**
0	8.5	9	9	8.5
100	3.5 × 4.0	4 × 4	5 × 4.5	5.6 × 5.2
200	3 × 3	3.5 × 3	4.2 × 4	5 × 4.8
400	2.5 × 2	3 × 2.5	3.5 × 3	3.5 × 3.2
600	1 × 1.5	2 × 2	2.3 × 2.1	3.0 × 2.8
800	0.0	1 × 1.5	2 × 1.6	2.5 × 2
900	-	0.0	1.0 × 0.8	1 × 1
1000	-	-	0.0	0.0

3.1.2.Chemical control against early blight disease on potato cv. Spounta at two localities at EL-Behera governorate during season 2019.

Results in Table (3) showed that the application of fungicides on potato cv. spounta against *Alternaria solani* was significantly effective at two localities at EL-Behera governorate during season 2019. Byroxanil was the most effective fungicide and reduced percentage of infection with *A. solani* on potato cv. spounta of 67.21 followed by Stone reach 59.21 compared with non-treated as a control at two localities during season 2019. Meanwhile, each Kocide 2000 and Speedcide gave lower reduction reach 52.46 and 40.98, respectively.

Also, the same trend was obtained in case of disease severity percentage and reduction in Table (3). The spray program trials confirmed the importance of fungicide use for the control of *A. solani* in the potato production and the results corresponded to the timing of applicatin **(Franc and Stump 2008)**. In addition to disease control, most fungicides are known to have beneficial physiological / growth - Promoting effects on plants, including delaying of leaf senescence **(Bertelsen *et al*., 2001)**, increased chlorophyll content **(Bulkute *et al*., 2008)** and greater stress tolerance **(Jabs *et al*., 2002)**. these physiological effects may contribute to greater yield, even in the absence of disease.

Table (3) Chemical control against early blight in potato cv. Spounta at two localities at El-Behera governorate during season 2019.

Treatments	% Disease incidence			Mean % Reduction	% Disease severity			Mean % Reduction
	Locality (1)	Locality (2)	% Mean		Locality (1)	Locality (2)	% Mean	
Speedcide	3.8 b	3.4 b	3.6 b	40.98	5.0 bc	4.6 bc	4.8 bc	83.73
Kocide 2000	3.0 bc	2.8 bc	2.9 bc	52.46	4.4c	4.2 cd	4.3 cd	85.42
Byroxanil	2.20 c	1.8 d	2.0 c	67.21	4.4 bc	3.6 d	4.00 d	86.44
Stone	2.8 c	2.2 cd	2.5 c	59.21	5.2 b	5.0 b	5.1 b	82.71
Control	6.2 a	6.0 a	6.1 a	-	31.0 a	28.0 a	29.5 a	-
L.S.D. 0.5	1.11	1.03	1.1 0	-	8.65	0.68	0.62	-

3.1.3. Effect of fungicides on polyphenol oxidase and peroxidase enzymes in leaves of potato cv. sponuta growing in field conditions at two localities at El-Behera governorate during season 2019:

Enzyme activity of both polyphenol oxidase and peroxidase of potato leaves were assayed 7days after 1st spray under field conditions.

3.1.3.1. Polyphenol oxidese (PPO) activity:

For polyphenol oxidase data presented in Table (4) showed that activity of the enzyme significantly increased in leaves potato cv . spounta compared with control (un-treated check). Meantime, the increase in the enzyme activity was significantly higher in case of application of Byroxanil which reach 1.102 (OD/Sec/ g.f.w.) compared with control (non spray) that gave 0.221 (OD/Sec/g.f.w.) during the growing in season 2019. Other fungicides increased the polyphynol oxidase compared with the control.

3.1.3.2. Peroxidase (PO) activity.

Similar trend to that of PPO was obtained for peroxidase activity in leaves of potato cv. Spounta Table (4) Data showed that the most effect of fungicides increase (PO) was obtained in case of application with Byroxanil that gave 1.565 (OD/Sec/g.f.w.) followed by Stone that gave 1.330 (OD/Sec/g.f.w) compared with the control (PO) (1.095) (OD/Sec/g.f.w.) during the growing season 2019. Other fungicides gave lower contents of (PO). Generally, it is evident that the interaction between the pathogen, fungicides and host plant induces some changes in cell metabolism, primarily activity of enzymes, particularly peroxidase (PO) and polyphenol oxidase (PPO) such plant enzymes were shown to be involved in defense reactions against plant pathogens. Peroxidase (PO) enzyme plays an important role in plant resistance against different pathogens as it is involved in the oxidative polymerization to yield lignin in cell walls **(Elad *et al.* 2004)**.

On the other hand, polyphenol oxidase (PPO) catalyzing the change of oxygen- dependent phenols to quinons which are more toxic to the microorganisms than the original phenolic compounds, on the other hands, phenolies play an important role in plant development, particularly in lignin biosynthesis and they also provide structural integrity and scaffolding support to plants **(Bhattacharya *et al.* 2012)**.

Table(4) Effect of fungicides on polyphenol oxidase and peroxidase enzymes in leaves of potato cv. spounta growing in field conditions during season 2019.

Fungicides	Activity of polyphenol oxides (OD/Sec/g.f.w)		Mean	Activity of peroxidase (OD/Sec/g.f.w)		Mean
Speedcide	0.276	0.281	0.279	1.16	1.35	1.255
Kocide 2000	0.266	0.270	0.268	1.19	1.29	1.24
Byroxanil	0.297	3.010	1.102	1.50	1.63	1.565
Stone	0.279	0.288	0.284	1.26	1.40	1.330
Control	0.219	0.223	0.221	0.99	1.20	1.095
L.S.D.0.05	0.018	0.020	0.019	0.115	0.100	0.107

3.2.Determination of probomocarb HCl and cymoxanil in tubers potato

The dissipation rate of Propamcarb-HCL and Cymoxanil in tubers potato was exhibited first order kinetics. The regression equations and half-life value are summarized in Table (5) and Fig (1). The Half-life values (T_{50}) were calculated mathematically and graphically to be 6.5 and 6.2

days Propamcarb-HCL and Cymoxanil respectively. The differences of recorded half-life could be regarded to difference in cultivation plant and/or temperature, or climate changes during spraying.

In this study, the PHI of Propamcarb-HCL and Cymoxanil were 15 and 15 respectively in tubers This observation shows a safe consumption and exportation for human after 15 days of application where the residue levels were at the maximum permissible residue level (MRL) recorded by **EU (2005).**

Table (5) Behavior Propamcarb-HCL and Cymoxail in Tubers potato.

Time after treatment (days)	Propamcarb-HCL			Cymoxanil		
	Residues of (ppm)	Loss %	Persistence	Residues of (ppm)	Loss %	Persistence
Initial *	N.D	-	-	N.D	-	-
1	1.4	-	100	0.83	-	-
3	1.09	22.14	77.86	1.12	-	100
7	0.65	53.57	24.29	0.45	59.82	40.16
10	0.27	80.71	-	0.09	80.00	-
15	0.07	95.00	-	0.01	88.88	-
Half life		6.5 days			6.2 days	
PHI		15 days			15 days	
MRL		0.3			0.01	

Initial *: 2 hours after application, N.D: Non detected,
PHI: Pre Harvest Interval, MRL: Maximum Residual Level (mg/kg)

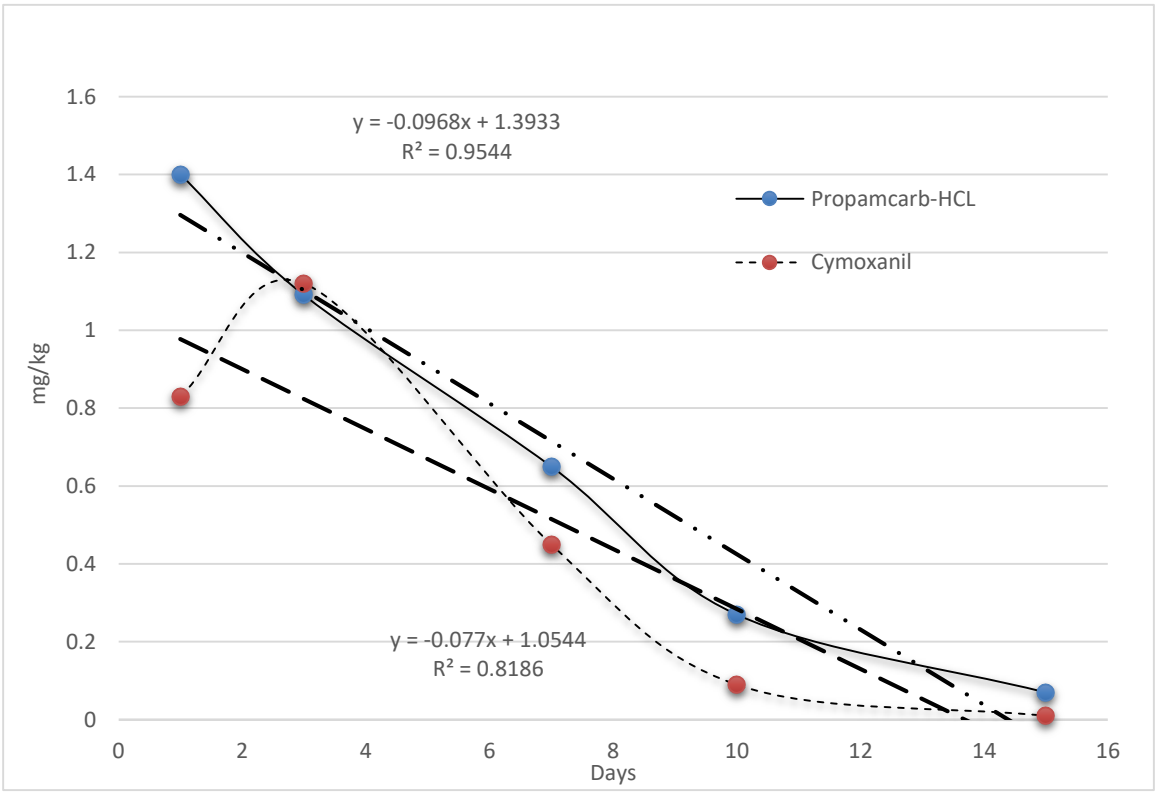


Fig (1): Degradation rate and T_{50} (half life values) of propamocarb-HCl and cymoxanil in tuber potato after its application.

CONCLUSION

The best results for reducing the growth of *A. solani* in laboratory and field conditions can be accomplished by using the four investigated fungicides: Kocide 2000 53.8% DF (Copper hydroxide), Speedcide 25% EC (Difenconazole), Stone 50% WDG (Dimethomorph) and Byroxanil 45% SC (Propamocarb HCl, Cymoxanil). The effects of these fungicides may also involve the increase of the activity of both peroxidase and polyphenol oxidase enzymes which reflects on increasing the resistance of plants to the studied disease. The study of the residual fungicide sprays in potato tuber confirms that the application of cymoxanil and promocarb HCl can be safe for consumption after 15 days.

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تأثير بعض المبيدات الفطرية على مرض الندوة المبكرة فى نبات البطاطس وتقدير متبقيات المبيدات فى الدرنات

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الملخص العربي:

١ – تم جمع عينات تظهر عليها علامات مرضيه بالندوة المبكرة علي نباتات البطاطس بمحافظة البحيره وتم عزلها والتعرف عليها بأنها فطر الترناريا سولاني مسبب المرض.

٢ – تم عمل اختبارات معمليه علي نمو الفطر تحت تأثير أربعة من المبيدات الفطريه وهي ستون- كوسيد٢٠٠٠- بروكسانيل- سبيد ساید علي بيئة PDA في الاطباق واظهرت النتائج ان مبيد بروكسانيل كان اكثر فاعليه في وقف نمو الفطر عند تركيز ٨٠٠ جزء فى المليون يليه مبيد ستون حيث توقف النمو عند ٩٠٠ جزء فى المليون.

٣- كان لتأثير رش المبيدات السابقه حقليا علي نباتات البطاطس صنف اسبونتا ابتداء من عمر ٤٥ يوم الفاعليه في تقليل الاصابه بالمرض وأيضا مدي انتشار المرض (شدة المرض) حيث كان افضل المبيدات تأثيرا هو بروكسانيل يليه ستون (٦٧,٢١ و ٥٩,٢١) , (٨٦,٤٤ و ٨٢,٧١) علي التوالي مقارنه بالكنترول.

٤- تم تقدير نشاط انزيمي البيروكسيديز والبولي فينول اوكسيديز بعد الرشه الاولي كأحد ردود النبات لمقاومة المرض فأوضحت النتائج زيادة في كلا الانزيمين في اوراق النباتات المعامله حيث وصلت الي ١,١٠٢ و٠,٢٨٤ (OD/Sec/g.f.w) بالنسبة لمبيد بروكسانيل و ستون على التوالى مقارنة بالكنترول ٠,٢٢١ (OD/Sec/g.f.w) وذلك في حالة انزيم البولي فينول اوكسيديز بينما كان لهذين المبيدين نفس التأثير فى درنات البطاطس في حالة انزيم البيروكسيديز ١,٥٦٥ و ١,٣٣٠ (OD/Sec/g.f.w) مقارنه بالكنترول ١,٠٩٥ (OD/Sec/g.f.w).

٥- تم تقدير متبقيات المبيدات للبروموكارب والسيموكسانيل فى درنات البطاطس حيث أوضحت النتائج عدم تواجد المبيدين بعد ساعتين من الرش وبدء ظهور المبيدين بعد اليوم الأول من الرش حيث بلغ المتبقى ١,٤ و ٠,٨٣ جزء فى المليون للمبيدين على التوالى وبعد ثلاثة أيام وجد متبقى المبيدين ١,٠٩ و ١,١٢ جزء فى المليون على التوالى وبعد سبعة أيام وجدت ٠,٦٥ و ٠,٤٥ جزء فى المليون على التوالى وفى اليوم العاشر كانت ٠,٢٧ و ٠,٠٩ جزء فى المليون على التوالى وبعد ١٥ يوم كانت ٠,٠٧ و ٠,٠١ جزء فى المليون على التوالى وبذلك تكون نسبة المتبقى من البروموكارب أقل من الحدود القصوى المسموح بها وفى سموكسانيل كانت ٠,٠١ وهى مساويه للحدود القصوى المسموح بها. وتم تقدير فترة ما قبل الحصاد فكانت ١٥ يوم للمبيدين وفترة نصف العمر كانت ٦,٥ و ٦,٢ يوم على التوالى