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EVALUATION OF SOME COMMERCIAL FUNGICIDES FOR CONTROLLING LATE BLIGHT DISEASE OF POTATO IN EGYPT

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ABSTRACT : *Nowadays, the successful management of potato late blight disease under Egyptian conditions requires evaluating the effectiveness of fungicides continuously. A total of 419 isolates of Phytophthora infestans were isolated during three growing seasons in Egypt. Under laboratory conditions, all active ingredients treatments significantly reduced the mycelium growth compared to the untreated control plates. Cymoxanil and Dimethomorph have the highest effect on the mycelium growth reduction. Results obtained from the field experiment showed that fungicides contain mixtures of active ingredients were superior in their effect than other fungicides which contain only one active ingredient. In addition, fungicides contain mixture of contact with trans- laminar active ingredients reduced potato late blight incidence more than contact fungicide with one active ingredient. Triomax has the highest effect on foliar blight reduction.*

Key words: *Late blight, Phytophthora infestans, Fungicides application.*

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important starchy edible tuber crops with high nutritive value. About 329 million tons of potato is produced in the world over an area of about 19.13 million hectares. It is considered a very important crop as staple food in the Mediterranean Basin, occupying an overall area of about 1 million ha and produces 28 million tons of tubers (FAO, 2008).

However, the production of potato has been hindered by several diseases, among them late blight disease caused by the Oomycete *Phytophthora infestans* (Mont.) de Barry that considered one of the most destructive and recurring diseases wherever potato is grown and has a potential to annihilate the entire crop within a week time. Economists at the International Potato Centre estimated the annual costs of control efforts and lost production due to late blight were approximately US $3.2 billion (Raman *et al.,* 2000; Judelson and Blanco 2005; Haldar *et al.* 2006).

Control of *P. infestans* has relied on intensive use of fungicides often without any appropriate strategy for resistance management. There are a number of registered fungicides available for the control of late blight on potato.

Fungicides that are commonly used for managing late blight worldwide include dimethomorph/mancozeb (trade name Acrobat MZ), mancozeb (trade name Dithane and many others), mixtures of metalaxyl and mancozeb (trade name Ridomil Gold MZ), propamocarb hydrochloride plus chlorothalonil (trade name Tattoo C) and cymoxanil plus mancozeb (trade name Curzat) (Mayton *et al.,* 2001; Nel *et al.,* 2003).

Metalaxyl (mefenoxam) is the most widely fungicide used against late blight and is translocated from roots and leaves to new growth. The occurrence of resistant isolates of *P. infestans* was first observed in potato crops grown in Ireland and in the Netherlands where metalaxyl was used as a single ingredient product that was applied curatively and during high disease pressure (Davidse *et al.,* 1981; Dowley and O'Sullivan 1981). The resistance to metalaxyl may be connected with changes in *P. infestans* populations relating to the occurrence of new genotypes composed of the A1 and A2 mating types (Goodwin *et al.,* 1992; Cooke *et al.,* 2009).

Dimethomorph, a cinnamic acid derivative, has protective, curative and anti-sporulation activity toward the genus *Phytophthora.* (Albert *et al.,* 1988; Cohen *et al.,* 1995). That disrupted cell wall formation by interfering with the molecular arrangement of cell wall components and not the inhibition of component synthesis (Kuhn *et al.,* 1991). Although resistant dimethomorph *P. infestans* populations have not been detected, naturally occurring intermediate resistant isolates have been detected in China (Zhu *et al.,* 2008).

For cymoxanil, similar to dimethomorph, Zhu *et al.,* (2008) found intermediately resistant *P. infestans* isolates in China but resistant isolates were not found. Thus, cymoxanil has not lost its efficacy.

Thus, the aim of this presented study is evaluating the effect of some commercial fungicides for controlling the late blight disease in Egypt.

MATERIALS AND METHODS

Origin of *P. infestans* isolates:

Samples were collected from commercial fields (one sample per field) during heavy outbreaks of late blight, from eleven Egyptian governorates; Minufiya, Gharbia, Beheira, Kafr el Sheikh, Dakahliya, Qalubiya, Sharkyia, Fayioum, Ismailiya, Bani Sweif and Giza. Pathogen isolation and purification were conducted as described by (Forbes, 1997).

Fungicides used for controlling late blight disease of potato:

Under laboratory conditions:

The objective of this trial was to evaluate the efficacy of some active ingredients which intervention in commercial fungicides composition on mycelium growth reduction *in vitro*.

Commercial formulations of fungicides were used as active ingredients (a.i.): azoxystrobin, dimethomorph, fluazinam, fosetyl-Al, cymoxanil, propamocarb-hydrochloride, zoxamide and mancozeb.

Dimethomorph, cymoxanil, mancozeb, fluazinam, fosetyl-Al, propamocarb-hydrochloride and zoxamide were first dissolved in dimethylsulfoxide (DMSO), and then a set of stock solutions with sterile distilled water was made. Each stock solution, including the control (no fungicide), contained the same concentration of DMSO. Azoxystrobin was dissolved in sterile distilled water, in order to prepare the stock solutions. The stock solutions were stored at 4◦C in the dark to maintain and preserve the active ingredient activity. Freshly-made stock solutions were prepared to give specific concentrations of active ingredient in μg mL^{-1}.

The trail consisted of a two way design: Active ingredient x Concentration in randomized complete blocks. A total of eight treatments and each one was tested in seven concentrations (0.0001, 0.001, 0.01, 0.1, 1, 10 and 100 gm/L), then replicated 3X for a total trail number of 192 including the control replicates which were established.

Volumes of stock solution were added to 100 ml molten (50^oC) sterile culture media prior to pouring, thereby, producing active ingredient concentrations ranging from 0.0001 to100 ppm (Locher and Lorenz, 1991).

Mycelial plugs (10 mm in diameter) were cut from the margins of B.K.5.4.14 isolate grown on rye A medium, placed upside down on the centers of treated and non-treated (control) pea plates, and incubated at 18◦C in the dark.

After ten days, the mean mycelium growth diameter for each concentration was measured and the percent of growth inhibition (PI) was calculated using the formula:

$$PI = [(a - b)/a] \times 100$$

Whereas, a = the mean growth diameter of the control plate, and b = the mean growth diameter of the fungicide amended plate (Finney, 1971).

Under naturally infected field:

The objective of this trial was to determine the effect of some commercial fungicides, which recommended for controlling this disease, this will provide an indicator for existing any resistance developed to these tested fungicides, and whether the risk is low, medium or high.

Experimental site, plot description and maintenance:

The trial was located in Tamalay-Minufiya governorate (N: 30° 30`; E: 30° 50`), an area that is used for potato production on a yearly basis and a history of late blights infection. The trial was located along the southern edge of the field and had been planted with peanut and harvested prior to the planting of the trial. Adjacent crops were sugarcane and clover. The soil in the trail area was free draining medium loams with low clay and organic matter contents.

The land was firstly ploughed with a power tiller and was exposed to sunlight for at least 7days. Then the land was ploughed and cross ploughed until the soil had a good tilth followed by laddering to level to remove weeds and break up clods. After each plough, weeds and rubbish were removed. Finally, spade was used to prepare plots.

Trial, design and application of products:

In order to evaluate the efficiency of fungicides for controlling late blight, the trial consisted of a one-way design with randomized complete blocks to reduce systematic errors in evaluation.

For this trial, certified potato tubers (Diamont) were planted by hand on November 1, 2014 in plots with 0.30 m spacing between them. All plots were 4.5 m wide x 7 m long and 0.75 m for single row wide, for a total plot area of 31.5 m^2 which consisted of 6 rows.

A total of twenty- three treatments (Table 1) and each treatment was replicated 3X for a total trail number of 72 including three replicates for the control trail was established.

General Field sanitation, fertilization and irrigation as recommended in this area were maintained throughout the growing period.

Fungicide treatments were applied as foliar sprays using a SOLO backpack sprayer equipped with nozzles hand pumped to maintain full pressure. Treatments application began on December 9, 2014 and continued until January 27, 2015, these consisted of eight sprays with 6 day interval and application doses were applied as mentioned in Table (1).

The foliage infection was by natural air-borne inoculum, no artificial inoculum was used.

Foliar assessment:

Plots were assessed weekly for incidence and severity of late blight infection, starting on December 2, 2014 and continuing until February 3, 2015 (one-week post last treatment).

Incidence was determined by counting the number of infected plants out of the total number of plants within each plot. A single severity score was assigned to each plot by visually determining the total percentage of infected tissue for all plants infected with late blight in the plot. The percentage of infected tissue was then converted to a severity grade using the James scale (James, 1971).

% late blight incidence in potato =
$$\frac{\text{No.of infected plants per plot}}{\text{Total number of plants per plot}} \text{x}100$$

% late blight severity of potato =
$$\frac{\text{No.of blighted leaves per plant}}{\text{Total No.of leaves per plants}} \text{x}100$$

Table (1): Twenty three commercial fungicides assigned for potato late blight management.

No.	Commercial name	Active ingredient	Concentration Formulation type	Mobility in the plant	Dose/100L
1	Alectis	Mancozeb + Zoxamide	75 % W.G.	Contact + Contact	200g
2	Bravotop	Chlorothalonil + Difenoconazole	55.5 % S.C.	Contact+ Systemic	500ml/ Fadden
3	Chemotrobin	Azoxystobin	23 % S.C.	Systemic	50ml
4	Flash	Dimethomorph + Flopet	71.3 % W.G.	Translaminar + Contact	250g
5	Kaiman	Mancozeb	80 % W.P.	Contact	250g
6	Kapreido	Dimethomorph + Pyraclostrobin	11.2 % E.C.	Translaminar	250ml
7	Koma	Copper oxychloride	50 % W.P.	Contact	250g
8	Kopar	Copper oxychloride	33.6% W.P.	Contact	250g
9	Lemay	Amiuslbrom	20 % S.C.	Contact	40ml
10	Mamort	Benthiavalicarb – isopropyl	15 % W.G.	Translaminar	50g
11	Manfil	Mancozeb	75 % W.G.	Contact	200g
12	Medomil	Mancozeb + Metalaxyl	72 % W.G.	Contact + Systemic	200g
13	Metalman	Mancozeb + Metalaxyl	72 % W.P.	Contact + Systemic	250g
14	Nando	Fluazinam	50 % S.C.	Translaminar	50ml
15	Orvigo	Ametoctradin + Dimethomorph	52.2 % S.C.	Translaminar	80ml
16	Prado	Chlorothalonil	72 % S.C.	Contact	875ml/Fadden
17	Prwiet	Fosetyle Aluminum	80 % W.G.	Systemic	250g
18	Rado Alfer	Mancozeb + Metalaxyl	72 % W.P.	Contact + Systemic	300g
19	Revastop	Mandipropamid +Difenoconazole	50 % S.C.	Systemic + Systemic	200ml/ Fadden
20	Rival	Propamocarb – HCL	72.2 % S.C.	Systemic	250g
21	Supermanco	Mancozeb + Dimethomorph	69 % W.P.	Contact +Translaminar	250g
22	Triomax	Cymoxanil + Mancozeb + Copper oxychloride	45 % W.P.	Translaminar + Contact + Translaminar	250g
23	Zignal	Fluazinam	50 % S.C.	Translaminar	50g

Efficacy of each fungicide was computed according to the following formula adopted by Rewal and Jhooty (1985).

$$\% \text{ Efficacy} = \frac{\% \text{ Disease severity of control plants} - \% \text{ Disease severity of treatmented plants}}{\% \text{ Disease severity of control plants}}$$

Data analysis and statistics:

All measurement data in the current study were analyzed according the methods described by Snedecor and Cochran (1980).

The differences among the means of different treatments were tested using the analysis of variance (ANOVA) test, and then the means were compared by the Least Significant Differences (LSD) at probability 5%. Statistical analysis was done using the CoStat package program, version 6.311 (cohort software, USA).

Results:

Collection of blighted samples and Isolation:

Potato late blight disease was surveyed from different cultivars grown in commercial fields at eleven governorates representing different growing areas of potatoes in Egypt during three growing seasons 2014-2016.

176 isolates were obtained during 2014 growing season, 134 isolates during 2015 growing season, and 109 isolates during 2016 growing season.

Evaluation of some commercial fungicides against late blight disease:

Under laboratory conditions:

All active ingredients (a.i.) treatments used in this experiment significantly reduced the mycelium growth on Petri dishes compared to the untreated control plates.

Data presented in Fig. (1) show that cymoxanil and propamocarb hydrochloride have the highest effect on mycelium growth inhibition with average of (6.7, 6.8% LSD, respectively), followed by azoxystrobin and dimethomorph (7.5, 8.3% LSD, respectively); while, fluazinam and mancozeb were less effective compared to other active ingredients used in this experiment (12.25, 12% LSD, respectively).

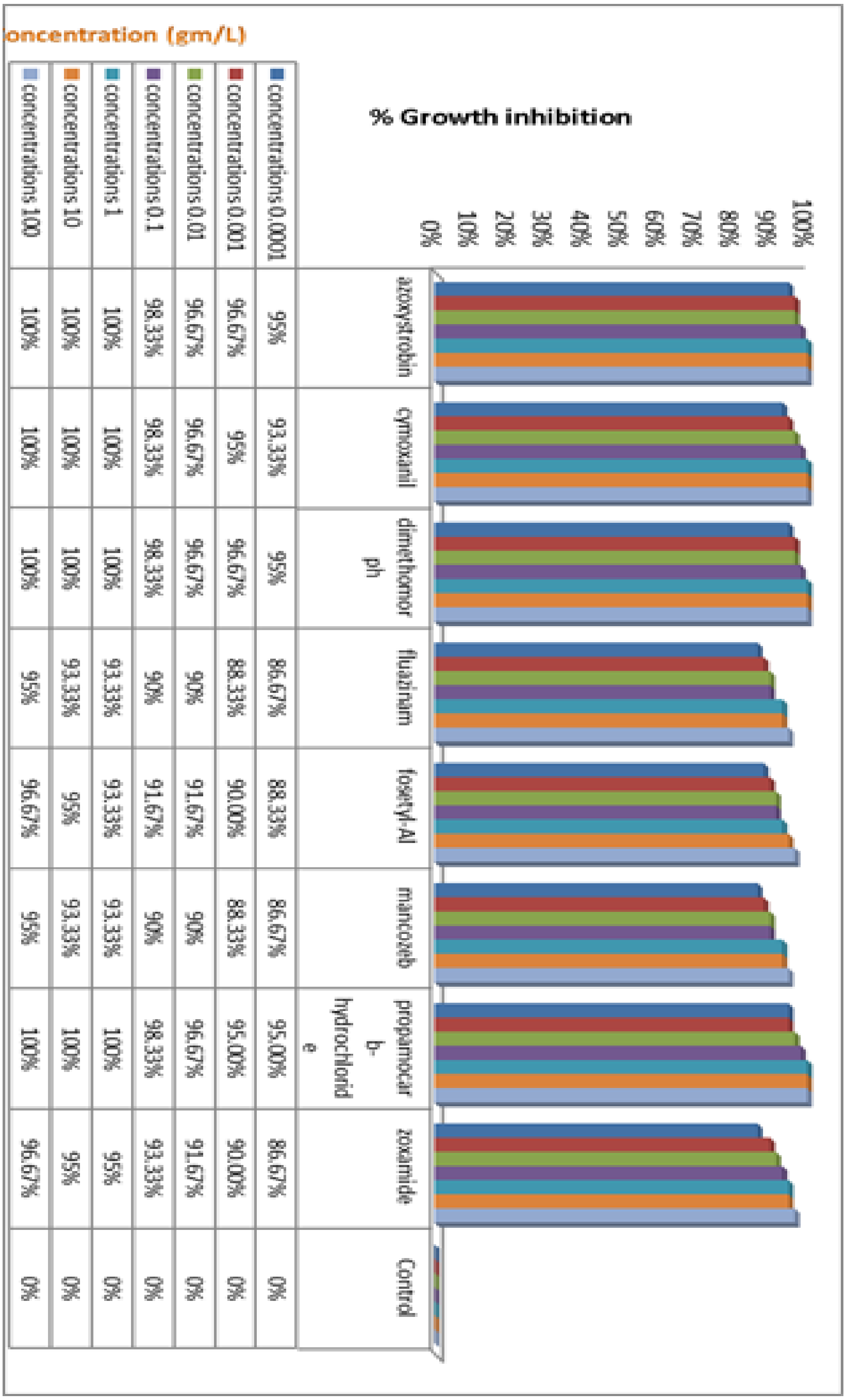
Under naturally infected field:

The percentages of fungicides effectiveness and the foliage blight at the end of the growing season are given in Table (2). All fungicide treatments significantly reduced the incidence of foliage blight compared to the untreated control plants. Fungicides that contain mixtures of active ingredients have the superior effect compared with others which contain only one active ingredient.

Also, in the same table, Triomax show the highest effect on the reduction of the disease severity (3.33%) compared to untreated control plants (30%). While, Zignal and Nando have the less effect on reducing the disease severity (10.67, 10%, respectively) compared to other fungicides. No significant differences in the mean final late blight score among the contact fungicides i.e., Manfil, Kopar, Koma and Kaiman, which disease severity score ranged from 6.33 to 8.33%.

Mixtures of contact and trans-laminar active ingredients reduced late blight severity more than contact fungicide with one active ingredient.

The obtained results showed that the effective chemical active ingredients in controlling late blight disease are: Mandipropamid+Difenoconazole, Cymoxanil+Mancozeb+Copper oxychloride, Propamocarb hydrochloride, Mancozeb+ Metalaxyl, Dimethomorph + Mancozeb, Azoxystobin and Mancozeb+Zoxamide, , while Mancozeb, Fluazinam, and Copper oxycloride which considered contact active ingredients are significantly less effective in controlling this disease.



	azoxystrobin	cymoxanil	dimethomorph	fluazinam	fosetyl-Al	mancozeb	propamocarb-hydrochloride	zoxamide	Control
concentrations 0.0001	95%	93.33%	95%	86.67%	88.33%	86.67%	95.00%	86.67%	0%
concentrations 0.001	96.67%	95%	96.67%	88.33%	90.00%	88.33%	95.00%	90.00%	0%
concentrations 0.01	96.67%	96.67%	96.67%	90%	91.67%	90%	96.67%	91.67%	0%
concentrations 0.1	98.33%	98.33%	98.33%	90%	91.67%	90%	98.33%	93.33%	0%
concentrations 1	100%	100%	100%	93.33%	93.33%	93.33%	100%	95%	0%
concentrations 10	100%	100%	100%	93.33%	95%	93.33%	100%	95%	0%
concentrations 100	100%	100%	100%	95%	96.67%	95%	100%	96.67%	0%

Fig. (1):The effect of eight active ingredients on mycelium growth inhibition under lab conditions:

Table (2): The effect of twenty three commercial fungicides against potato late blight disease under naturally infection in the field:

No.	Commercial name	Effectiveness (%)	Foliar blight severity (%)
1	Alectis	80	6
2	Bravotop	82.23	5.33
3	Chemotrobin	80	6
4	Flash	81.1	5.67
5	Kaiman	78.9	6.33
6	Kapreido	83.33	5
7	Koma	73.33	8
8	Kopar	72.23	8.33
9	Lemay	75.6	7.33
10	Mamort	71.1	8.67
11	Manfil	75.6	7.33
12	Medomil	82.23	5.33
13	Metalman	78.9	6.33
14	Nando	66.67	10
15	Orvigo	72.23	8.33
16	Prado	80	6
17	Prwiet	76.7	7
18	Rado Alfer	80	6
19	Revastop	81.1	5.67
20	Rival	76.7	7
21	Supermanco	83.33	5
22	Triomax	88.9	3.33
23	Zignal	64.43	10.67
24	Control	_	30
L.S.D. $_{(0.05)}$		6.88	4.445

Discussion:

Potato late blight caused by *Phytophthora infestans* is the most destructive disease threats potato in Egypt and worldwide. The importance of the genus *Phytophthora*, both to humanity and to the development of the science of plant pathology has been obvious ever since *P. infestans* devastated the potato crop in Western Europe in 1845. Its greatest impact was the potato blight epidemic in Ireland (Gregory, 1983). In all, one million people died of famine-related diseases (Clarkson, 1989) and up to 1.5 million more emigrated (Alexopoulos *et al.,* 1996).

In the present study, infected potato leaves, stems, and tubers were collected from potato commercial fields during 3growing seasons 2014-2016 from different 47 locations at eleven governorates, Minufiya, Qalubiya, Gharbia, Behira, Kafr El-Sheikh, Dakahliya, Sharkyia, Fayioum, Esmailiya, Bani Sweif and Giza, resulted in 419 *P. infestans* isolates were collected.

Because of the Egyptian climate favors late blight epidemics and the susceptible potato cultivars, such as Spunta, Lady Rosetta, Cara and many others, are widely grown for their attractive edible or processing characteristics, controlling this disease depends mainly on fungicide application. Also, studies were focused on development of resistant or tolerant *P. infestans* strains following frequent use of different fungicides as it became a major limitation in disease management.

In this study, all fungicides reduced the disease severity on foliar blight but varied in their effectiveness. In general, the results partially support our first hypothesis that fungicide treatments can reduce late blight development if applied before the occurrence of infection. The obtained results are in harmony with the findings of Schepers and van Soesbergen (1995) they reported that not all fungicides are equally effective in controlling tuber blight via foliar application. Different ways in mobility of fungicides active ingredients affected on reducing disease developments. Systemic fungicides were more effective than contact ones but they were not necessary to apply at the early age of plants while the contact fungicides were essential. Contact fungicide treatments applied after the appearance of late blight symptoms or after disease establishment may be effective for disease control.

Other late blight research reports showed that Mancozeb applied as a protectant can be effective in reducing the impact of late blight under tropical conditions (Namanda *et al.,* 2004).

The use of systemic fungicides was obligatory with abundant foliage whereas the humidity rose around contacted plants and favorable conditions became more available. Applications of fungicides which contain trans-laminar active ingredients were more effective than contact ones at the appearance of initial symptoms.

In conclusion, the most effective fungicide application schemes start with contact fungicides (protectant) followed by translaminal fungicides (super protectant) then end by systemic fungicides. This is in accordance with Inglis and Vestey (1998), who reported that fungicide application schemes that included cymoxanil, dimethomorph, or propamocarb hydrochloride provided slightly better control of late blight in a North American fungicide trial than schemes that did not include those fungicides.

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تقييم بعض المبيدات التجارية الفطرية المستخدمة في مكافحة مرض اللفحة المتأخرة في البطاطس في مصر

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الملخص العربي

تتطلب المكافحة الناجحة لمرض اللفحة المتأخرة في البطاطس تحت الظروف المصرية تقييم كفاءة المبيدات الفطرية المستخدمة لمكافحة المرض بإستمرار. تم عزل ٤١٩ عزلة من المسبب المرضي خلال ثلاث مواسم. أظهرت النتائج المتحصل عليها من تقييم ثمان مواد فعالة كتجربة معملية كفاءتها معنوياً في الحد من النمو الميسليومي للمسبب المرضي مقارنة بالأطباق الغير معاملة، وكان لكل من (الأزوكستروبين و الداي ميثومورف) التأثير الأعلي علي تثبيط النمو الميسليومي للمسبب المرضي.أما بالنسبة للتجربة الحقلية، أظهرت التائج أن كل المبيدات الفطرية كان لها قدرة علي التقليل من سرعة تقدم المرض بدرجات متفاوتة عند المعاملة بها قبل حدوث الإصابة (كرشات وقائية)، حيث كان المبيد ترايوماكس أكثر المبيدات تأثيرآ في الحد من إنتشار المرض، أيضاً تفوقت المبيدات الفطرية المحتوية علي خليط من المواد الفعالة علي تلك المبيدات التي تحتوي علي مادة فعالة واحدة، وكان الخليط من المواد الفعالة المتلامسة والجهازية المحلية أكثر قدرة علي خفض شدة المرض عن المبيدات التي تعمل بالملامسة وتحتوي علي مادة فعالة واحدة.