

## CHEMICAL MANAGEMENT OF SOYBEAN DOWNY MILDEW CAUSED BY *PERONOSPORA MANSHURICA* IN EGYPT

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### ABSTRACT

Five soybean cultivars; Crawford, Clark, Giza 21, Giza 35 and Giza 82 were evaluated for the control of downy mildew infection under greenhouse and field conditions using three different fungicides.

*In vitro* studies show that Sandofan M-8 and Ridomil plus were more effective against the spore germination and germ tube length of *Peronospora manshurica* the causal pathogen. Complete inhibition of the spore germination and germ tube was obtained at 100 ppm of Sandofan M-8, and 200 ppm for Ridomil plus and Dithan M 45.

The fungicide application under greenhouse conditions, 2 days before or 2 days after inoculation by the pathogen spore suspension at the rate of  $1 \times 10^5$  spores/ml was also studied. It was observed that, the cvs. Giza 35 and Giza 82 showed lower infection than the other tested cvs., however Crawford gave the highest infection. The fungicide Sandofan M-8 was the most effective on downy mildew followed by Ridomil plus and Dithan M 45 either two days before or after inoculation.

Field studies at both Sakha and Itay El-Baroud in the growing season 2000 gave as similar results as the greenhouse. The obtained results indicated that three sprays of Sandofan M-8 or Ridomil plus, at the recommended rate of each fungicide when the first disease symptoms appeared and at 15 days intervals, gave low downy mildew infection and high seed yield when compared with the check plots.

### INTRODUCTION

Soybean (*Glycine max* (L.) Mer.) is one of the main either oil or protein crops all over the world, that the seeds are characterized by high nutritional values; 20% oil and 40% protein (Kassem, 1982).

Soybean plants are attacked by many pathogenic fungi which infect different parts of the plant causing different amounts of losses (Mosa, 1982; Hassanien, 1985 and Sinclair and Backman, 1989). Downy mildew disease causes appreciable economic yield loss and could be a threat to soybean plants in many countries of the world including Egypt (Sinclair, 1982).

*Peronospora manshurica* is the main fungal pathogen on soybean, causing downy mildew disease (Gaumann, 1923). It was reported to be transmitted by seeds (Nicholson and Sinclair, 1971).

The chemical control of downy mildew disease had been practiced by many investigators with good results either in the greenhouse or at the field (Dunleavy, 1987).

The present investigation was planned to minimize the seed yield loss caused by downy mildew disease through application of some promising fungicides on different soybean cultivars.

## MATERIALS AND METHODS

### A- Laboratory Experiments (*In vitro* assay) :

Three fungicides namely, Sandofan M-8, Ridomil plus and Dithan M 45 were tested for its inhibitory effect on conidial germination and germ tube length of *P. manshurica*. Six concentrations of each fungicide; 0, 10, 25, 50, 100 and 200 ppm were prepared according to the active ingredient. Five ml of each concentration were transferred to a watch glass.

Conidiospores of soybean downy mildew *P. manshurica*, collected from artificially inoculated plants (cv. Clark) under greenhouse conditions were brushed using sterilized brush and spores were added to each watch glass. One drop of cotton blue was added to increase microscopic visibility of the germinated conidiospores. One drop of each spore concentration (containing about 300 spores/ml suspension using hemocytometer slide), was then put on a glass slide in petri-dish at 100% relative humidity. The prepared dishes were incubated at  $20 \pm 1$  °C for 24 hrs. Five replicates were used for each concentration. The average percentage of germinated conidia and germ tube length were estimated (Attia et al., 1997).

### B- Pot Experiment (Greenhouse test) :

The reaction of five soybean cultivars; Crawford, Clark, Giza 21, Giza 35 and Giza 82 to the infection with *P. manshurica* was tested at the seedling stage, under greenhouse conditions, moreover the performance of the tested fungicides over the disease control was also studied.

Five seeds of each cultivar were sown in pots No. 20 and the fungicides were applied at the rate of 1.5, 1.5 and 2.5 g/L for Sandofan M-8, Ridomil plus and Dithan M 45, respectively. Five plants 30 days old from each cv. were sprayed with the fungicide. The application of the fungicides was carried out either two days before inoculation (2 DBI) with spore suspension of *P. manshurica* ( $1 \times 10^5$  spore/ml) or two days after inoculation (2 DAI). The inoculum of the pathogen was prepared by collecting the fungus conidiospores from infected leaves on plants grown at the previous season. The collected leaves were kept at refrigerator till used. Each treatment was replicated 4 times. Sprayed plants with water served as control. Infection percentages were determined 10 days after inoculation at  $23 \pm 2$  °C under greenhouse conditions.

### C- Field Experiments :

A split-plot design experiments were carried out at two locations; Sakha, Kafr El-Sheikh and Itay El-Baroud, Beheira during the growing season 2000. The five cultivars were used in the main plots, while the three tested fungicides and untreated control were allocated for the sub-plots. Each treatment was replicated 4 times in 5.25 m<sup>2</sup> plots ( $\frac{1}{800}$  feddan). Normal agricultural practices were followed. Fungicides applications were started at the first appearance of infection and at 2 weeks interval for the next two sprays, at the rate of 150, 150 and 250 g/100 L water for Sandofan M-8,

Ridomil plus and Dithan M 45, respectively. Infection percentages were estimated three times after seven days from each spray and their averages were calculated.

**Statistical Analysis :**

Data obtained were statistically evaluated according to Snedecor and Cochran (1967). Treatments averages were compared at 0.05 level of probability (Fisher, 1948).

**RESULTS**

**A. Laboratory Studies :**

**Effect of different concentration of three fungicides on conidial germination of *P. manshurica* :**

Results in Table (1) show that the three tested fungicides were significantly effective on spore germination of *P. manshurica* as the mean germination was reduced from, 71.3% in the control treatment to 9.6, 13.9 and 18.4% for Sandofan M-8, Ridomil plus and Dithan M 45, respectively.

Increasing the concentration of each fungicide from 10 ppm to 200 ppm decreased significantly spore germination from 44.05% for 10 ppm to 20.40% for 100 ppm, as a mean of all fungicides, Table (1).

Complete inhibition in the spore germination was found at 100 ppm for Sandofan M-8. While, for Ridomil plus and Dithan M 45 it was at 200 ppm, Table (1).

**Table (1). Effect of different concentrations of some fungicides on the average percentage of conidial germination of *Peronospora manshurica***

Fungicides	% Germination at concentrations (ppm)					Mean fungicides (F)
	10	25	50	100	200	
Ridomil plus	32.7	21.5	11.2	3.9	0.0	13.9
Sandofan M-8	25.0	14.7	8.4	0.0	0.0	9.6
Dithan M 45	47.2	24.5	13.8	6.4	0.0	18.4
Control	71.3	71.3	71.3	71.3	71.3	71.3
<b>Mean concentration ( C )</b>	<b>44.05</b>	<b>33.0</b>	<b>26.18</b>	<b>20.40</b>	<b>17.83</b>	

LSD 5% : Fungicides (F) = 1.23  
 Concentrations ( C ) = 0.76  
 F x C = 1.51

**1. Effect of different concentrations of three fungicides on germ tube length of *P. manshurica* :**

Results in Table (2) indicated that the three tested fungicides significantly reduced the germ tube length of the fungus *P. manshurica* than the control. Sandofan M-8 and Ridomil plus gave in average 1.96 and 2.48  $\mu\text{m}$  followed by Dithan M 45 with 8.42  $\mu\text{m}$  compared with 43.5 $\mu\text{m}$  for the untreated spores. Significant differences were also found either among concentrations and the

interaction fungicides and concentrations. Sandofan M-8 showed germ tube length of 4.1µm at 10 ppm reduced to 2.1µm at 50 ppm, while at 100 and 200 ppm no germination was observed. Ridomil plus gave 4.8 µm germ tube length at 10 ppm that reduced to .2 µm at 100 ppm, while no germination was found at 200 ppm. Dithan M 45 showed 18.9 µm germ tube length at 10 ppm and reduced to 3.8 µm at 100 ppm and no germination did occur at 200 ppm, Table (2).

**Table (2). Effect of different concentrations of some fungicides on germ tube length of *Peronospora manshurica* after 24 hrs. of incubation at 20 °C**

Fungicides	Average germ tube length (µm) at concentrations (ppm)					Mean fungicides (F)
	10	25	50	100	200	
Ridomil plus	4.8	3.9	2.5	1.2	0.0	2.48
Sandofan M-8	4.1	3.6	2.1	0.0	0.0	1.96
Dithan M 45	18.9	14.8	4.6	3.8	0.0	8.42
Control	43.5	43.5	43.5	43.5	43.5	43.5
Mean concentration (C)	17.83	16.53	13.18	12.13	10.88	

LSD 5% : Fungicides (F) = 2.81  
 Concentrations (C) = 0.66  
 F x C = 1.37

**B. Greenhouse Experiments :**

Five soybean cvs. were artificially inoculated with *P. manshurica* the causal organism of downy mildew under greenhouse conditions. The three tested fungicides were sprayed either at 2 days before inoculation (2 DBI) or 2 days after inoculation (2 DAI). Results in Table (3) indicated that the three tested fungicides reduced significantly downy mildew infection than the untreated pots either when sprayed before or after the fungus inoculation. Significant differences were also found between the five cvs. used, Crawford cv. showed higher infection than others. While, Giza 35 and Giza 82 showed the least infection either at 2 DBI or 2 DAI.

Statistical analysis pointed that there were significant interactions among all of the different factors; cultivars, fungicides and time of spray. In spite of that no significant difference was proved among the two times of spray (2 DBI, 2 DAI). However on 2 DBI Sandofan M-8 and Ridomil plus were more effective than Dithan M 45 on the cultivars, Crawford as the infection percentage were respectively 19.8, 20.1 and 35.6%; Giza 35 showed 14.1, 18.5 and 34.5%; Giza 21 showed 17.5, 19.9 and 32.3% and Clark showed 16.2, 20.4 and 30.2% infection for the three fungicides, respectively, compared with more than 50% infection for the untreated pots. However, Giza 82 showed some reduction in the pots treated with Dithan M 45 as 20.4% compared with 16.3% and 20.9% for Sandofan M-8 and Ridomil plus, respectively.

On the 2 DAI spray the fungicides behaved as such as the spray on 2 DBI without any marked variation. Sandofan M-8 was the most effective followed by Ridomil plus and Dithan M 45 had the least potency on the disease infection.

**Table (3). Effect of some fungicides on downy mildew infection of five soybean cvs. under greenhouse conditions**

Time of spray	Fungicides	Infection % of cultivars					Mean fungicides (F)
		Crawford	Clark	Giza 21	Giza 35	Giza 82	
2 DBI	* Ridomil plus	20.1	20.4	19.9	18.5	20.9	19.96
	Sandofan M-8	19.8	16.2	17.5	14.1	16.3	16.78
	Dithan M 45	35.6	30.2	32.3	34.5	20.4	30.60
	Control	58.5	54.6	56.7	52.2	51.8	54.76
	<b>X cultivars ( C )</b>	<b>33.50</b>	<b>30.35</b>	<b>31.60</b>	<b>29.83</b>	<b>27.35</b>	<b>-</b>
2 DAI	** Ridomil plus	23.3	21.9	21.8	20.2	22.4	21.92
	Sandofan M-8	21.6	18.4	20.6	17.4	19.8	19.56
	Dithan M 45	29.7	27.8	26.4	28.7	27.2	27.96
	Control	58.5	54.6	56.7	52.2	51.8	54.76
	<b>X cultivars ( C )</b>	<b>33.28</b>	<b>30.68</b>	<b>31.38</b>	<b>29.63</b>	<b>30.3</b>	<b>-</b>

Spray with fungicides was :

\* 2DBI = Two days before inoculation

\*\* 2DAI = Two days after inoculation

LSD 5% : Cvs ( C ) = 1.47

Time of spray (T)= N.S.

C x T = 1.27

C x F x T = 2.07

Fungicides (F) = 0.65

C x F = 1.46

F x T = 0.92

### C. Field Experiments :

Downy mildew infection were scored three times 7 days after each fungicide application, and then the mean of three scores was calculated. Results in Table (4) revealed that downy mildew infection was higher on the cultivar Crawford with 11.0 and 10.7% infection at Sakha and Itay El-Baroud, respectively. While Giza 35 was the least infected at Sakha with 7.6%, Giza 82 cv. was less infected at Itay El-Baroud with 8.1 %.

**Table (4). Effect of some fungicides on the infection of soybean by downy mildew disease under field conditions at two location senson 2000**

Location	Fungicides	Average infection %*					Mean fungicides (F)
		Crawford	Clark	Giza 21	Giza 35	Giza 82	
Sakha	Ridomil plus	9.7	7.1	8.7	6.1	6.3	7.6
	Sandofan M-8	9.2	7.0	7.6	6.1	6.1	7.2
	Dithan M 45	10.7	7.8	9.8	6.9	6.8	8.4
	Control	14.2	12.7	12.9	11.1	12.0	12.6
	<b>X cultivars ( C )</b>	<b>11.0</b>	<b>8.7</b>	<b>9.8</b>	<b>7.6</b>	<b>7.8</b>	<b>-</b>
Itay El-Baroud	Ridomil plus	9.0	7.2	9.3	6.5	6.5	7.7
	Sandofan M-8	9.4	6.3	8.4	6.6	6.3	7.4
	Dithan M 45	10.5	8.0	9.7	7.5	8.3	8.8
	Control	13.9	11.6	12.2	12.1	11.3	10.0
	<b>X cultivars ( C )</b>	<b>10.7</b>	<b>8.3</b>	<b>9.9</b>	<b>8.2</b>	<b>8.1</b>	<b>-</b>

\*Average of infection was calculated from three scores.

The effect of each fungicide, as a mean of all cultivars, on infection percentages indicated that Sandofan M-8 was the most effective fungicide at both Sakha and Itay El-Baroud with 7.2 and 7.4%, respectively, compared with 12.6 and 10.0% for untreated plots, respectively at the two locations, Table (4).

The interaction between cultivars and fungicides were significantly different at the two locations. Giza 35 and Giza 82 showed the lowest infection percentage with Sandofan at Sakha (6.1% and 6.1%) and at Itay El-Baroud (6.6 and 6.3%). However, untreated plots of both cvs. gave 11.1 and 12.0% at Sakha and 12.1 and 11.3% at Itay El-Baroud, Table (4).

Seed yield per plot (Table 5) showed also significant differences between cultivars and fungicides, at both locations. While, their interaction effect was significant at Itay El-Baroud and not at Sakha.

The highest seed yield was obtained from Sandofan application (as a mean of all cultivars) with 1.46 and 1.43 kg/plot at Sakha and Itay El-Baroud, respectively, compared with 1.00 and 0.96 kg/plot for untreated plots. Giza 21 cv. and Giza 35 cv. gave successfully 1.38 and 1.37 kg/plot at Sakha and 1.34 and 1.31 kg/plot at Itay El-Baroud, while the lowest seed yield was obtained from Dithane M 45 application (as mean of all cvs.) with 1.30 and 1.16 kg/plot at Sakha and Itay El-Baroud, respectively.

**Table (5). Seed yield (kg/plot) of five soybean cvs. treated with three fungicides for downy mildew control at two locations, 2000**

Location	Fungicides	Yield (kg/plot) for cultivars					Mean fungicides (F)
		Crawford	Clark	Giza 21	Giza 35	Giza 82	
Sakha	Ridomil plus	1.34	1.40	1.50	1.45	1.35	1.41
	Sandofan M-8	1.34	1.41	1.55	1.53	1.45	1.46
	Dithan M 45	1.22	1.32	1.38	1.40	1.17	1.30
	Control	0.98	0.97	1.07	1.10	0.88	1.00
	X cultivars (C)	1.22	1.28	1.38	1.37	1.21	-
Itay El-Baroud	Ridomil plus	1.29	1.39	1.52	1.41	1.38	1.40
	Sandofan M-8	1.36	1.42	1.49	1.50	1.40	1.43
	Dithan M 45	1.11	1.09	1.33	1.35	0.90	1.16
	Control	0.95	1.02	1.00	0.99	0.82	0.96
	X cultivars (C)	1.18	1.23	1.34	1.31	1.13	-

LSD 5%	Sakha	Itay El-Baroud
Cultivars (C)	0.12	0.08
Fungicides (F)	0.05	0.05
C x F	N.S.	0.11

## DISCUSSIONS

Preventive and curative effect by fungicides namely; Sandofan M-8, Ridomil plus and Dithan M 45 was studied for downy mildew control on soybean plants.

It was found that conidial germination and germ tube length were completely inhibited at 200 ppm concentration with Ridomil and Dithan M 45 and at 100 ppm with Sandofan M-8. These results are in agreement with those obtained by Attia *et al.* (1997) with *Peronospora pisi* the causal organism of downy mildew of pea.

Disease percentage on the tested cultivars was reduced when the fungicides were applied either 2 days before or two days after inoculation. These results are in agreement with those reported by Heweidy (1998) and Ibrahim *et al.* (1965) who stated that a protective control measure by fungicidal treatment would be feasible practices for controlling the disease. Meanwhile Guy *et al.* (1989) noted that Metalaxyl (Ridomil) was effective in reducing lesion length caused by *Phytophthora megasperma* (the incident of root and stem rot of soybean) in all cultivars of soybean.

Evaluation of the aforementioned fungicides was run under the field conditions during 2000 growing season at Sakha and Itay El-Baroud locations. In the light of the obtained results, it was found that any of the tested fungicides was unable to prevent the infection of downy mildew on soybean. However Sandofan M-8 was the most effective fungicide, followed by Ridomil plus and Dithan M 45. Significant interactions were found among cultivars and fungicides at each location.

This is in line with Fry (1977) who stated that the significance of chemicals used in a disease management program depends on the crop, its diseases, and the environment. He added that chemicals become essential to successful crop production only when cultural practices, host resistance, alterations of the environment or alteration of the associated biota are inadequate to suppress the pathogen sufficiently.

Korets-Kii (1971) demonstrated that downy mildew of soybean depended on local climatic and soil conditions. Also, reported that intensity of soybean downy mildew decreased with increasing age of plant. Dunleavy (1982) indicated that symptoms of soybean downy mildew were most severe at 25 °C, but decreased at higher and lower temperatures. Dunleavy (1971) indicated that downy mildew was favored by high humidity.

Concerning seed yield, fungicides significantly increased seed yield when compared with unsprayed treatment in both locations. Highest seed yield was from plants sprayed with Sandofan M-8, then Ridomil plus and Dithan M 45. These results are in agreement with those obtained by Dunleavy (1987) who found that foliar application with Metalaxyl (Ridomil) decreased downy mildew infection and increased seed yield.

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## المقاومة الكيماوية للبياض الزغبي في فول الصويا المتسبب عن الفطر بيرونوسبورا مانشيوريكا

محمد أحمد هويدى ، نجوى محمد أحمد محمود ، قدرى مصطفى مرسى ، إسماعيل عبد المنعم إسماعيل  
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تم دراسة مقاومة البياض الزغبي على الأصناف كراوفورد ، كلارك ،  
جيزة ٢١ ، جيزة ٣٥ وجيزة ٨٢ باستخدام مبيدات ساندوفان م ٨ وريدميل بلاس  
ودايتين م ٤٥ سواء فى الصوبة أو الحقل .

وفى الدراسة المعملية وجد أن مبيد ساندوفان أحدث تثبيط كامل لنمو جراثيم  
الفطر بيرونوسبورا مانشيوريكا وكذلك على طول أنبوب الإنبات عند تركيز ١٠٠ جزء  
فى المليون - أما مبيد ريدوميل بلاس ودايتين م ٤٥ كان التثبيط عند ٢٠٠ جزء فى  
المليون .

وفى دراسة المقاومة فى الصوبة وجد أن استعمال مبيد ساندوفان م ٨ أكثر تأثيراً  
سواء كان استعمال هذه المبيدات قبل إجراء العدوى الصناعية بجراثيم الفطر المسبب أم  
بعدها بيومين .

أما فى دراسة المقاومة فى الحقل تم إجراء هذه الدراسة عام ٢٠٠٠ فى محطات  
بحوث سخا (محافظة كفر الشيخ) وإيتاى البارود (محافظة البحيرة) وأظهرت الدراسة أن  
إجراء ثلاث رشات تبدأ عند ظهور أول أعراض الإصابة ثم رشه كل إسبوعين بمبيد  
ساندوفان م ٨ وريدميل بلاس بالتركيز الموصى به أعطت أقل نسبة إصابة وأعلى وزن  
فى محصول البذرة يليهم استعمال مبيد دايتين م ٤٥ .