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Evaluation of two Formulations of the Fungicide Mixture Azoxystrobin and Metalaxyl-M against four Soil Borne Diseases

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Abstract

Two formulations of azoxystrobin + metalaxyl-M, Premium© 39.1% SC and Uniform© 44.5% SE were evaluated *in vitro* by food poisoning technique against *Fusarium solani*, *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotium rolfsii*. These fungi were isolated from infected tomato plant and were identified. Data indicated that IC_{50} (mg/L) of Premium was 32.1, 2.5, 1215.5 and15.2, as well as for Uniform was 40.6, 5.4, 84.4 and 80.7 on *F. solani*, *F. oxysporum*, *R. solani*, *S. rolfsii*, respectively. The obtained results showed that the efficiency of the Premium against *F. solani*, *F. oxysporum* and *S. rolfsii* was significantly more toxic than Uniform, while *R. solani* was significantly more sensitive to Uniform although both formulations have the same active ingredient. It may be concluded that the difference in the efficacy may be due to the difference in the type of the formulation between SC and SE.

Key words: azoxystrobin, metalaxyl-M, Premium, Uniform, *Fusarium solani*, *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotium rolfsii.*

Introduction

Pesticide are chemical compounds that are used to kill pests including insect, fungi, rodent and weeds, (Algimants et al.,2018) that chemical compound contain both active and inert ingredirnts. An “active ingredient” prevents, destroys, repels, or mitigates a pest, and the "inert ingredients" are responsible for product performance and usability. Since the discovery of the active ingredients, it had to be delivered to the target pest. In the past, most of the agrochemical formulation technologies were based on simple solutions in these conventional formulations generally create safety hazards in use and have a negative impact on the environment. The off-target loss is also a crucial problem for inefficient usage of conventional pesticide formulations (Knowles, 2008).

These conventional formulations based on 'old technology' with increased dose rate or repeated applications to get desired bio-efficacy endanger the safety of mankind as well as environment (Green and Beestman, 2007).

Soil borne fungal pathogens are important determinants of the dynamics of plant populations in natural environments and in agricultural environments. Examples of economically important soilborne fungal pathogens include *Fusarium* spp., *Sclerotium rolfsii* and *Rhizoctonia solani*.

Despite low initial densities of inoculum in soil, these pathogens can cause complete destruction of plants and occasionally, total loss of yield. *Sclerotium rolfsii*, is a destructive stem and crown rot of tomato.

White mycelium spread over stems of infected plants and formed sclerotia on the old lesions nearby soil surface. *Fusarium oxysporium* f. sp *lycoperisici* causes soil borne vascular wilt disease in the tomato plant (Van der Does *et al*., 2018). This study aims to evaluate two different formulations on four soli born disease.

Materials and Methods

1. Isolation, purification and identification of the tested fungi

1.1 Isolation and purification

Naturally- diseased tomato plants, sufferied from root rot and wilt symptoms, were collected from two different governorates, *i.e* Kalubia and BaniSweif. Each plant sample was washed carefully with tap water to remove the adhering soil particles then cutted into small pieces (1 x1 cm) and surface-sterilized by immersing in 5 % sodium hypochlorite solution for 5 min according to the method of Burr *et al*. (1978). The segments were rinsed three times in sterilized distilled water, dried between two fold of sterilized filter papers and transferred under aseptic condition to sterilized Petri dishes containing potato dextrose agar medium (PDA) (Potatoes, peeled and diced 200g, Dextrose 20g, Agar 20g and Distilled water 1000 ml, Difco,1984). Plates were incubated at 27 º C and developed colonies were examined after 5 days. The emerged fungi were isolated on PDA plates then purified using the single spore method and / or hyphal tip technique (Dhingra and Sinclair, 1984).

1.2 Identification

The isolated fungi were identified microscopically to species level according to Nelson *et al*. (1983) as well as Barnett and Hunter (1987) to *Fusarium solani*, *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotium rolfsii*. Stock cultures were maintained on PDA slants and kept at 10 ºC in refrigerator for further experiments. The identification was verified

in Plant Pathology Department, Agriculture Research Center, Giza.

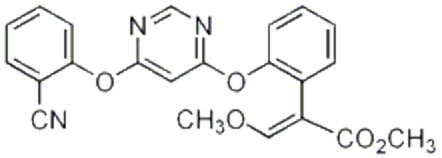
2. Fungicides used

2.1 Fungicide common name, trade name, formulation, recommended dose and manufacturer:

Table 1. Fungicide common name, trade name, formulation, recommended dose and manufacturer

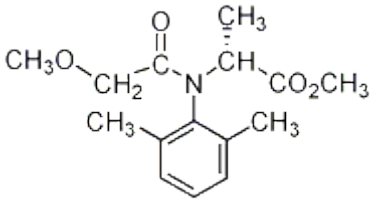
Common name	Trade name	Formulation (w/v)	Recommended dose	Manufacturers
Azoxystrobin + Metalaxyl M	**Premium© 39.1% SC**	39.1% SC Azoxystrobin 28.2% + Metalaxel M 10.9%	650 ml/feddan	The tower for the agricultural and veterinary pesticides industry
	Uniform© 44.5% SE	44.5% SE Azoxystrobin 32.17% + Metalaxel M 12.37%	650 ml/feddan	Syngenta Agro

2.2 Chemical structure of fungicides used:



Azoxystrobin

methyl (E)-2-[[6-(2-cyanophenoxy)-4-pyrimidinyl]oxy]-α-(methoxymethylene) benzeneacetate



Metalaxel M

methyl N-(2,6-dimethylphenyl)-N-(methoxyacetyl)- D –alaninate

3. *In vitro* determination effect of fungicides on fungal growth:

The efficiency of the chemical fungicides was tested on the *in vitro* linear growth of the pathogenic fungi. Different concentrations milligram/liter Table 2 based on recommended dose of each fungicide, were evaluated as described by Sharvell (1961). The food posion technique proposed by Al-Hassan and Neslah (1982) was applied.

The required quantity of respective fungicide was incorporated in 100 ml of PDA in 250 ml flask. The medium was shaken well to give Uniform© 44.5% SE dispersal of the fungicides. Twenty ml medium was poured separately into each sterilized Petri plates, replicated three times and centrally inoculated with 5 mm mycelial disc of the pathogen and incubated at 28±2 ºC for seven days. A suitable control was maintained by growing the pathogen on fungicides free PDA medium. Observation on radial mycelial growth / colony diameter of the fungi was recorded at an interval of 24 hours and continued till untreated control plates were fully covered with mycelial growth. Percent mycelial growth inhibition of the pathogen with the test fungicides over the untreated control was calculated by using the formula Abd El-Ghany, 2001.

$$Percent\ inhibition = (C\text{-}T / C) \times 100$$

Where, C = Growth of the test fungus in untreated (control) plates.

T = Growth of the test fungus in treated plates.

Table 2. Fungicide concentrations (ul of fungicide formulation/L of agar nutrient) against the pathogenic fungi

Fungi	Fungicide concentration (ul/L)	
	Premium© 39.1% SC	**Uniform© 44.5% SE**
F. solani	20, 40, and 80	20, 40, and 80
F. oxysporum	20, 40, and 80	20, 40, and 80
R. solani	1600, 2000, and 2400	80, 120, and 160
S. rolfsii	20, 40, and 80	80, 140, and 200

Evaluation the effect of fungicide was carried out by estimating the following parameters:

IC_{50}: half-maximal inhibitory concentration.

Toxicity index (T.I) = (Lowest IC_{50} of tested fungicides / IC_{50} for other fungicides) × 100

4. Statistical analysis

Data were subjected to analysis of variance (ANOVA) (Gomez and Gomez, 1984), followed by Duncan' s multiple range tests to compare means (Duncan, 1955).

Results and Discussion

A. Inhibition effect of the two fungicide formulations (azoxystrobin + metalaxyl-M) against the tested phytopathogenic fungi

The two fungicide formulations, premium© 39.1 % SC and Uniform© 44.5% SE, which contain the same active ingredient azoxystrobin+ metalaxyl-M were evaluated *in vitro* against the four pathogenic fungi, *Fusarium solani*, *Fusarium oxysporum*, *Rhizoctonia solani and Sclerotium rolfsii* at different concentrations Table 3.

Data shows that the concentrations used of the active substances in milligram (mg) per Potato Dextrose Agar (PDA) liter (L) and what each concentration achieved in terms of inhibition of pathogenic fungi. Results revealed that *F. oxysporum* was the most sensitive fungus to both fungicide formulations tested, which recorded 61 and 60% reduction in mycelium growth with Uniform© 44.5% SE and Premium© 39.1% SC at 35.5 and 31.5 ml/L, respectively.

Table 3. Inhibition effect of the two fungicide formulations of azoxystrobin + metalaxyl-M against the tested phytopathogenic fungi

Fungicide formulation (w/v)	Pathogenic fungi	Con. (mg/L)	Azoxystrobin (mg/L)	Metalaxel-M (mg/L)	Inhibtion* (%)
Uniform© SE 44.5%	***Fusarium solani***	**8.8**	**6.4**	**2.4**	32^{c}
		17.7	**12.8**	**4.9**	41^{b}
		35.5	**25.7**	**9.8**	46^{a}
	Fusarium oxysporum	**8.8**	**6.4**	**2.4**	53^{b}
		17.7	**12.8**	**4.9**	55^{ab}
		35.5	**25.7**	**9.8**	60^{a}
	Rhizoctonia solani	**35.5**	**25.7**	**9.8**	22^{c}
		53.4	**38.6**	**14.8**	33^{b}
		71.2	**51.4**	**19.7**	44^{a}
	Sclerotium rolfsii	**35.5**	**25.7**	**9.8**	22^{c}
		62.3	**45**	**17.3**	38^{b}
		89	**64**	**24.7**	55^{a}
Premium© SC 39.1%	***Fusarium solani***	**7.7**	**5.6**	**2.1**	36^{c}
		15.5	**11.2**	**4.3**	42^{b}
		31.1	**22.4**	**8.7**	50^{a}
	Fusarium oxysporum	**7.7**	**5.6**	**2.1**	55^{b}
		15.5	**11.2**	**4.3**	58^{ab}
		31.1	**22.4**	**8.7**	61^{a}
	Rhizoctonia solani	**625.6**	**451.2**	**174.4**	28^{c}
		782	**564**	**218**	35^{b}
		938.4	**676.8**	**261.6**	41^{a}
	Sclerotium rolfsii	**7.7**	**5.6**	**2.1**	41^{c}
		15.5	**11.2**	**4.3**	50^{b}
		31.1	**22.4**	**8.7**	60^{a}

* The inhibition in control was zero

(mean±SE) a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.

B. Bioassay parameters of the two fungicide formulations of azoxystrobin + metalaxyl-M against the tested fungi.

The data obtained in Table 4 and Figure 1 proved that Premium© 39.1% SC was more toxic against *F. Solani* with IC_{50} value of 32.1 mg/L, while Uniform© 44.5% SE was less toxic against this fungus where its IC_{50} was 40.6 mg/L.

Table 4. Efficacy of azoxystrobine + metalaxyl M against *F. Solani*

Fungicide Formulation	Azoxystrobin (%)	Metalaxyl M (%)	IC_{50} (mg/L)	Confidence limits 95% (mg/L)	Slope± SD
Uniform© SE 44.5%	**32.2**	**12.4**	**40.6**	**8.94 - 184.5**	**0.69±0.3**
Premium© SC 39.1%	**28.2**	**10.9**	**32.1**	**5.57- 185.3**	**0.59±0.2**

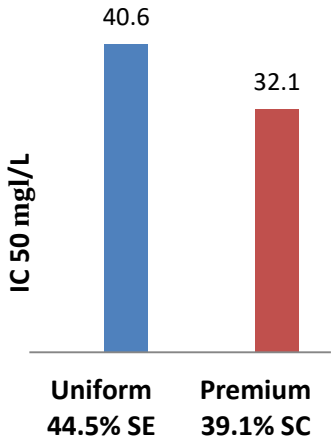


Figure 1 Efficacy of Uniform© 44.5%and Premium© 39.1% SC against *F. solani*

The results in Table 5 Figure 2 indicated that Premium© 39.1% SC was more toxic against *F. oxysporum* with IC_{50} value of 2.5 mg/L, while Uniform© 44.5% SE was less toxic against this fungus where its IC_{50} was 5.4 mg/L.

Table 5. Efficacy of Azoxystrobin + Metalaxyl M gainst *F. oxysporum*

Fungicide Formulation	Azoxystrobin (%)	Metalaxyl M (%)	IC_{50} (mg/L)	Confidence limits 95% (mg/L)	Slope± SD
Uniform© SE 44.5%	**32.2**	**12.4**	**5.4**	**0.16 - 183.1**	**0.29±0.3**
Premium© SC 39.1%	**28.2**	**10.9**	**2.5**	**0.04 - 148.6**	**0.25±0.3**

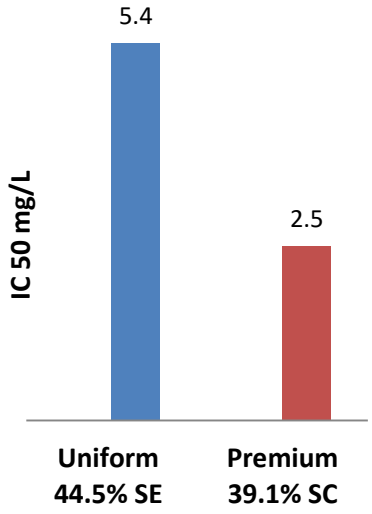


Figure 2 Efficacy of Uniform© 44.5% and Premium© 39.1% SC against *F. oxysporum*

On the other hand, the results showed that Uniform© 44.5% SE was more toxic against *R. solani* with IC_{50} value of 84.4 mg/L, while Premium© 39.1% SC was less toxic against this fungus where its IC_{50} was 1215.5 mg/L Table 6 and Figure 3.

Table 6. Efficacy of azoxystrobine + metalaxyl M against *R. solani*

Fungicide Formulation	Azoxystrobin (%)	Metalaxyl M (%)	IC_{50} (mg/L)	Confidence limits 95% (mg/L)	Slope± SD
Uniform© SE 44.5%	**32.2**	**12.4**	**84.4**	**70.81 – 135.43**	**2.0±0.5**
Premium© SC 39.1%	**28.2**	**10.9**	**1215.5**	**716.53 – 2061.98**	**2.0±1.0**

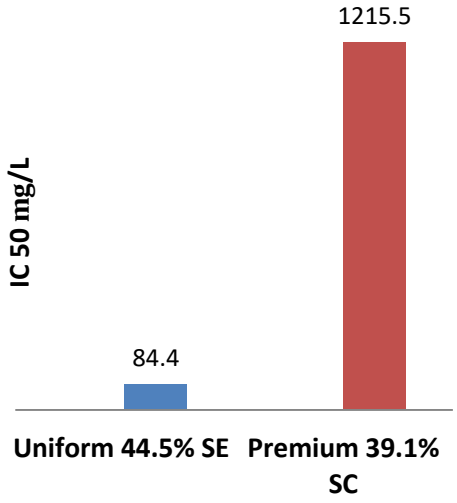


Figure 3 Efficacy of Uniform© 44.5% and Premium© 39.1% SC against *R. solani*

The data in Table 7 and Figure 4 revealed that Premium© 39.1% SC was more toxic against *S. rolfsii* with IC_{50} value of 15.2 mg/L, while Uniform© 44.5% SE was less toxic against this fungus where its IC_{50} was 80.7 mg/L.

Table 7. Efficacy of azoxystrobine + metalaxyl M against *S. rolfsii*

Fungicide Formulation	Azoxystrobin (%)	Metalaxyl M (%)	IC_{50} (mg/L)	Confidence limits 95% (mg/L)	Slope± SD
Uniform© SE 44.5%	**32.2**	**12.4**	**80.7**	**68.72 – 107.18**	**2.23±0.4**
Premium© SC 39.1%	**28.2**	**10.9**	**15.2**	**486.46 – 3256.3**	**0.79±0.3**

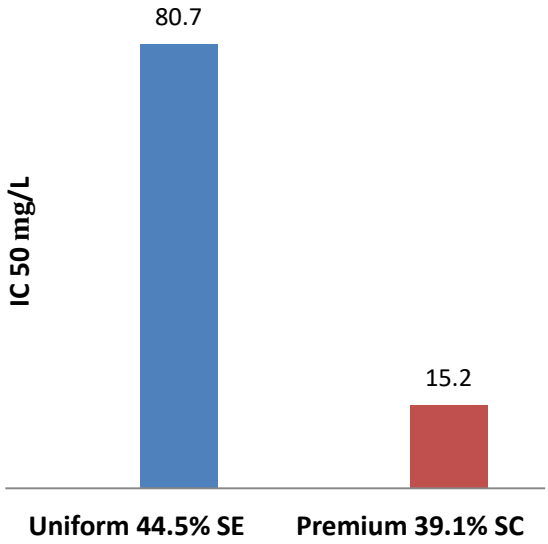


Figure 4 Efficacy of Uniform© 44.5%and Premium© 39.1% SC against *S. rolfsii*

Data presented in Tables 4 to 7 and Figures 1 to 4 revealed a comparison of the toxicity between both the two fungicide formulations under study. It was found through the values of IC_{50} mg/l of the mixture of azoxystrobine + metalaxyl on *F. solani, F. oxysporum, R. solani, S. rolfsii* that Premium© 39.1% SC was more toxic against *F. Solani, F. oxysporum* and *S. rolfsii,* IC_{50s} were 32.1, 2.5 and 15.2 mg/L, respectively. This formulation was less toxic against *R. solani* where its IC_{50} value was 1215.5 mg/L. Uniform© 44.5% SE was less toxic compared with Premium© 39.1% SC against the three tested fungi, *F. Solani, F. oxysporum, S. rolfsii* where its IC_{50} were 40.6, 5.4 and 80.7 mg/L, respectively. At the same time, it was more toxic against against *R. solani* compared with Premium© 39.1% SC and its IC_{50} was 84.4 mg/L.

These results are in conformity with the earlier finding of those workers who reported that azoxystrobin 23% SC at 500, 1000, 1500 ppm inhibit 100 percent of mycelial growth of *Fusarium oxysporum* f. sp. *Udum* (Ghante *et al*., 2019). Further Uniform© 44.5% SE and Premium© 39.1% SC at 35.5 and 31.5 ml/L achieved 46 and 50 percent of inhibition *F. solani* mycelial growth, respectively, this finding is also consonance with Prince *et al*. (2020) who found that azoxystrobin 25% SC at 50, 100 and 150 ppm had 59.4, 66,7 and 74.4 percent of mycelial growth, respectively. With regard to *S. rolfsii* was more sensitive to Uniform© 44.5% SE than Premium© 39.1% SC 35.5, Shirsole *et al*. (2019) reported that azoxystrobin 35% EC at 50, 100 ppm gave 78.46 and 100 percent of *S. rolfsii* inhibition mycelium growth. On the other hand, the lowest effect was observed when used Uniform© 44.5% SE against *R. solani* at 35.5 ml/L which recorded reduction in mycelium growth only 22%. Thamarai *et al*. (2019) reported that azoxystrobin 23% SC at 200 ppm have 85 percent of inhibition mycelium growth but Uniform© 44.5% SE at 71.2 ml/L have 44 percent of inhibition mycelium growth.

The phytopathogenic fungi differ in sensitivity to the fungicides tested at different concentrations. The most sensitive fungus to these fungicides was *F. oxysporum* which recorded the lowest IC_{50} reached to (2.5 and 5.4) mg/l with Premium© 39%SC and Uniform© 44.5% SE respectively, followed by Premium© 39%SC against *S. rolfsii* which recorded 15.2.These observations were in agreement with findings of Khanna *et al.* (2021) who reported that Azoxystrobin 23% SC at 0-75 ppm recorded IC_{50}

1.57 with *F. oxysporum*. Also Raghavendra and Srinivas (2020) proved that azoxystrobin 23% SC showed 100% percent mycelia growth inhibition at concentration 500 ppm against *S. rolfsii*. On the other hand the most resistant fungus was *R. solani* which recorded IC_{50} reached to 1215.5 with Premium© 39%SC and 84.4 with Uniform© 44.5% SE fungicide.

Conclusion

From obtained data efficacy of Premium© 39.1% SC against *F. solani*, *F. oxysporum*, and *S. rolfsii* was more effective than Uniform© 44.5% SE, while *R. solani* was more affected by Uniform© 44.5% SE than Premium© 39.1% SC. Although the similarity of the active substances in both fungicide formulations, the difference in their efficacy may be due to differ in formulations between SC and SE.

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تقييم مستحضرين مبيدين فطريين خليط من الازوكسى ستروبين و ميتالاكسيل ام ضد اربعة من فطريات التربة الممرضة للنبات

احمد شحاتة عبد الخالق، محمد محمد عزب، نجلاء فكرى عبد الحميد
قسم وقاية النبات كلية الزراعة جامعة بنها مصر

تم تقييم فاعلية مستحضرين من الازوكسى ستروبين و ميتالاكسيل ام، بريميام 39.1% مركز معلق و يونيفورم 44.5% معلق مستحلب فى المعمل ضد فطريات ***Fusarium solani, Fusarium oxysporum, Rhizoctonia solani*** and ***Sclerotium rolfsii.*** . تم عزل و تعريف الفطريات من جذور نباتات الطماطم المصابة من محافظتى القليوبية و بنى سويف.

اشارت النتائج الى ان الجرعة النصف مميتة بالمليجرام / لتر للبريميام 39.1% كانت 32.1، 2.5، 1215.5، 15.2 و لليونيفورم كانت 40.6، 5.4، 84.7 ضد ***Fusarium solani, Fusarium oxysporum, Rhizoctonia solani*** and ***Sclerotium rolfsii.*** على التوالى.

تدل النتائج على كفاءة مبيد بريميام 39.1% عن مبيد يونيفورم 44.5% ضد فطريات ***Fusarium solani, Fusarium oxysporum*** and ***Sclerotium rolfsii.*** بينما يزيد كفاءة مبيد يونيفورم 44.5% عن مبيد بريميام 39.1% ضد ***Rhizoctonia solani.*** على الرغم من احتواء كل من مبيد بريميام 39.1% و يونيفورم 44.5% على نفس المادة الفعالة، يمكن استنتاج ان الاختلاف فى النتائج يرجع الى الاختلاف بين المستحضر المركز المعلق و المعلق المستحلب.