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Studying the Stability of Some Metalaxyl Wettable Powder Formulations Currently Applied in Egypt

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ABSTRACT

Three commercial metalaxyl wettable powder (WP) formulations 15% were collected from different sources in Egypt as one of them was locally formulated and the other sources of formulations were ready-made from China and Jordan. The formulations under investigation were a mixture of metalaxyl and copper oxychloride currently applied in Egypt where this study was conducted to investigate the stability of metalaxyl as well as studying the formation of its relevant impurity 2, 6-dimethylaniline after storage at $54 \pm 2^\circ\text{C}$ and exposure to sunlight for different intervals. Identification and determination of metalaxyl and its relevant impurity content were achieved using GC-FID. From the obtained results metalaxyl showed that, high stability after storage at $54 \pm 2^\circ\text{C}$, which resulted in a decrease in the rate of the formation of relevant impurity 2,6-dimethylaniline, on the other hand metalaxyl revealed that, slightly affected after exposure to sunlight compared to storage at $54 \pm 2^\circ\text{C}$ and the formation rate of relevant impurity was relatively higher than storage at $54 \pm 2^\circ\text{C}$, but the amount of relevant impurity 2,6-dimethylaniline was in allowed limits for all sources before and after storage, in addition to that there was no a variation in the rate of degradation of metalaxyl and the amount of its relevant impurity 2,6-dimethylaniline for all sources under investigation.

Keywords: Metalaxyl, 2,6-dimethylaniline, Degradation, Impurities, GC-FID.

INTRODUCTION

Metalaxyl (methyl N-(methoxyacetyl)-N-(2,6-xylyl)-DL-alaninate) is one of the most important fungicides used in agriculture to control many fungal diseases such as downy mildew, early blight, and late blight in many crops. Metalaxyl is a systemic fungicide with protective and curative action classified from a member of phenylamide: acylalanine group which inhibit protein synthesis in fungi (MacBean, 2011). Metalaxyl is classified according to the Globally Harmonized System (GHS) acute toxicity Class 4, as harmful if swallowed and harmful in contact with the skin (WHO, 2009). In water, metalaxyl has low hydrophobic properties and it greatly soluble which led to low adsorption by soils and the probability of leaking to reach groundwater. Metalaxyl has lately been monitored in groundwater at values up to $0.49 \mu\text{g/L}$, which surpasses the $0.1 \mu\text{g/L}$ EU maximum (Hildebrandt *et al.*, 2008). Many investigators have reported the significance of OM and clay content in soils to adsorb metalaxyl (Andrades *et al.*, 2001). Biodegradation is the main factor for degradation of metalaxyl in soil (Sukul and Spittler, 2000). In sewage sludge, metalaxyl has been found to break down faster than metalaxyl-m, while the opposite happened in soil as metalaxyl-m was less stable than metalaxyl and it degraded rapidly than metalaxyl (Müller and Buser 1995; Buser *et al.*, 2002; Chen and liu, 2009). Metalaxyl was rapidly degraded in sunflower (Marucchini and Zadra 2002; Zadra *et al.*, 2002). Metalaxyl is not affected by thermal storage at $54 \pm 2^\circ\text{C}$ and storage for a long period in a sunny and dark place (Ibrahim and Ramadan, 2011). According to (FAO,

1995) the 2,6-dimethylaniline is the main relevant impurity in metalaxyl due to its high toxicity compared to metalaxyl and its maximum limit should not be higher than 0.1% of metalaxyl content.

In the current study, three commercial metalaxyl (WP) formulations 15% are a mixture of metalaxyl and copper oxychloride currently applied in Egypt of different origin from Egypt, China and Jordan were investigated to study the effect of sunlight exposure and storage at $54 \pm 2^\circ\text{C}$ for different intervals 0, 1, 3, 7, 14, 21 and 28 days on the stability of metalaxyl content and formation of its relevant impurity 2,6-dimethylaniline. The choice of these intervals depended on the degradation rate of metalaxyl after the accelerated storage at $54 \pm 2^\circ\text{C}$ for 14 days according to FAO specifications. The identification and determination of metalaxyl content and its relevant impurity 2,6-dimethylaniline were confirmed using gas chromatography equipped with flame ionization detector (GC-FID) using external standard.

MATERIALS AND METHODS

Chemicals

Analytical standard of metalaxyl and 2,6-dimethylaniline were obtained from Sigma Aldrich with 99% purity for both of them. The commercial formulations of metalaxyl 15% WP were obtained from three different sources in Egypt as the first source I was locally formulated, but Source II and III were ready-made from China and Jordan. Methanol HPLC Grade (Merck) was used. Chemical structure of metalaxyl and 2,6-dimethylaniline are shown in Fig. 1.

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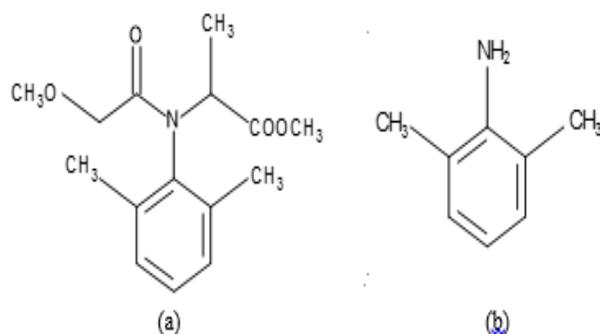


Fig. 1. Chemical structure of (a) metalaxyl and (b) 2,6-dimethylaniline

Accelerated storage procedures (CIPAC MT 46.1.1 1995)

Wettable powder formulations of metalaxyl 15% WP were placed in beakers and spread it (about 20 g). Place the disc on the surface of powder in the beaker. This disk is a loose fit in the beaker, and of such dimensions that even pressure 25 g/cm² can be produced on the surface of the sample in the beaker. These beakers were divided into two groups and subjected to different treatments as follows: The first group was exposed to storage at 54 ± 2 °C for different intervals 0, 1, 3, 7, 14, 21 and 28 days, while the second group was exposed to sunlight for the same intervals. Samples from the two tested groups were taken for study investigations.

Column	capillary column HP-50+ (30 m x 0.53 mm I.D., 1 µm film thickness)
Temperature program	held at 120 °C for 1 min, then ramp 20 °C/min to 260 °C and kept at that temperature for 2 min
Detector	FID at 250 °C
Injector	250 °C with splitless mode
Carrier Gas	Nitrogen
Flow rate	8 ml/min.
The injection volume	1 µl
Retention times	2,6-dimethylaniline : 3.165 min. metalaxyl : 7.767 min.

2. Kinetic study

According to (FAO, 1995) the content of metalaxyl after storage should not be lower than 97% relative to the content of metalaxyl before storage. Shelf life of metalaxyl was calculated according to (Moye et al., 1987) and (Anderson and Scott 1991).

$$t_{97} \text{ for metalaxyl} = 0.0305/K$$

$$K = (1/t_x) \ln (a/b_x)$$

Where,

t_{97} = the time required to reach 97% of the initial metalaxyl concentration.

K = rate of degradation $0.0305 = \ln (0.97)$

a = initial concentration t_x = time in days

b_x = concentration at x time

Table 1. Effect of storage stability on metalaxyl 15% WP and the amount of 2,6-dimethylaniline in metalaxyl of three different manufactured sources at 54 ± 2 °C for 28 days.

Storage Periods (Days)	Source I			Source II			Source III		
	Metalaxyl content %	Loss %	2,6-dimethyl aniline (%of metalaxyl)	Metalaxyl content %	Loss %	2,6-dimethyl aniline (%of metalaxyl)	Metalaxyl content %	Loss %	2,6-dimethyl aniline (%of metalaxyl)
0	14.98*	0	0.00136	14.95	0	0.00141	14.97	0	0.00139
1	14.95	0.20	0.00151	14.94	0.067	0.00153	14.95	0.13	0.00154
3	14.90	0.53	0.00175	14.91	0.27	0.00164	14.92	0.33	0.00169
7	14.85	0.87	0.00201	14.87	0.54	0.00182	14.84	0.87	0.00179
14	14.73	1.67	0.00263	14.74	1.40	0.00254	14.70	1.80	0.00236
21	14.62	2.40	0.00275	14.59	2.41	0.00289	14.60	2.47	0.00303
28	14.50	3.20	0.00352	14.44	3.41	0.00360	14.47	3.34	0.00355
t_{97} (days)	***26.30			24.83			25.14		

* Samples before and after storage stability test analyzed together to reduce the analytical error., ** Maximum level of 2,6-dimethylaniline in tested samples = 0.1% of metalaxyl content., ***The time required to reach 97% of the initial metalaxyl content.

Preparation of samples

1. Metalaxyl

Standard Preparation

Weight 10 mg of metalaxyl analytical standard of the known purity into a 25 ml grade (A) measuring flask, dissolve and complete with methanol.

Sample Preparation

A specific weight equivalent 10 mg of metalaxyl analytical standard was taken from each formulation and transferred into 25 ml grade (A) measuring flask and complete with methanol.

2,2,6-dimethylaniline

Standard Preparation

Ten mg of 2,6-dimethylaniline analytical standard was weighed into a 25 ml grade (A) measuring flask, dissolve and complete to volume with methanol.

Sample Preparation

One gm from the formulation sample of metalaxyl was weighed into a 25 ml grade (A) measuring flask, dissolve and complete to volume with methanol.

Identification and determination of metalaxyl and its impurity 2,6-dimethylaniline

1. Gas Chromatography Determination

The procedures were according to CIPAC 365/TC/M/(1992), using Agilent 7890B gas chromatograph with autosampler 7693. The conditions of GC were as follows:

RESULTS AND DISCUSSION

1. Effect of storage stability at 54 °C ± 2 °C on metalaxyl 15% WP content and the amount of relevant impurity 2,6-dimethylaniline.

Table 1 and Fig. 2 explained the effect of storage at 54 ± 2 °C for different intervals 0, 1, 3, 7, 14, 21 and 28 days on stability of the three commercial metalaxyl formulations of different origin. respectively where these values did not exceed the maximum allowed limits after storage for all sources.

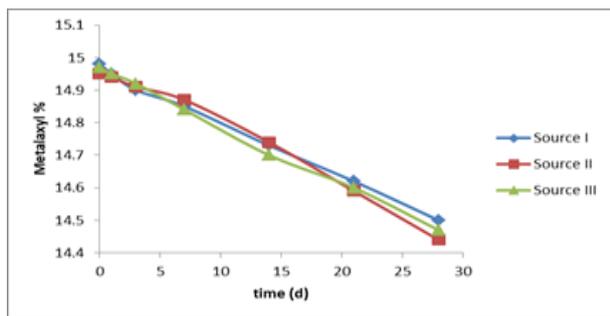


Fig. 2. Effect of storage stability on metalaxyl 15% WP from three different manufactured sources at 54 ± 2 °C.

The data of the three samples showed that no significant differences in metalaxyl and its impurity content before and after storage for 14 days at 54 ± 2 °C, suggesting that the formulation is stable at 54 ± 2 °C for 14 days where specifications were not affected, and also it was noticeable even after increasing the period of storage, that metalaxyl showed high stability after storage at 54 ± 2 °C for 28 days and the loss percentages increased slightly to reach 3.20, 3.41 and 3.34%, also shelf-lives were 26.30, 24.83 and 25.14 days for source I, II and III, respectively. The stability of the degradation rate of metalaxyl resulted in a decrease in the rate of the formation of relevant impurity 2,6-dimethylaniline where data presented in Table 1 cleared that, the effect of storage on the amount of 2,6-dimethylaniline at 54 ± 2 °C for the different intervals of study. The amount of 2,6-dimethylaniline was within the FAO limits for all sources before storage, where the

maximum level of 2,6-dimethylaniline in metalaxyl samples is 0.1% of metalaxyl content, and the results assured that the amount of 2,6-dimethylaniline before storage was 0.00136, 0.00141 and 0.00139% for source I, II, and III, respectively, and there was a slight increase in the amount of 2,6-dimethylaniline after storage for 28 days and the amount of 2,6-dimethylaniline increased to reach 0.00352, 0.00360 and 0.00355% for sources I, II, and III

2. Effect of exposure to sunlight on stability of metalaxyl 15% WP content and the amount of relevant impurity 2,6-dimethylaniline.

Data obtained in Table 2 and Fig. 3 showed that, the stability of three commercial metalaxyl formulations after exposure to sunlight for 28 days. It is observed that there was an increase in the rate of degradation of metalaxyl and showed slightly affected after exposure to sunlight for the intervals of study compared to storage at 54 ± 2 °C and the results indicated that the loss percentages after 28 days were found to be 5.01, 5.08 and 5.48%, and also the shelf-lives were 16.63, 16.37 and 15.16 days for source I, II and III, respectively, in addition to that from the results presented in Table 2 there was an increase in the amount of 2,6-dimethylaniline after exposure to sunlight for 28 days and the amount of 2,6-dimethylaniline increased to reach 0.0479, 0.0490 and 0.0494 for sources I, II, and III respectively, despite the increase in the formation rate of the relevant impurity the amount of 2,6-dimethylaniline was in allowed limits after storage for all sources.

Table 2. Effect of exposure to sunlight on stability of metalaxyl 15% WP content and the amount of relevant impurity 2,6-dimethylaniline in metalaxyl of three different manufactured sources for 28 days.

Storage Periods (Days)	Source I			Source II			Source III		
	Metalaxyl content %	Loss %	2,6-dimethyl aniline (%of metalaxyl)	Metalaxyl content %	Loss %	2,6-dimethyl aniline (%of metalaxyl)	Metalaxyl content %	Loss %	2,6-dimethyl aniline (%of metalaxyl)
0	14.98*	0	0.00136**	14.95	0	0.00141	14.97	0	0.00139
1	14.90	0.53	0.0135	14.85	0.67	0.0141	14.83	0.94	0.0161
3	14.81	1.13	0.0217	14.75	1.34	0.0238	14.72	1.67	0.0253
7	14.70	1.87	0.0305	14.69	1.74	0.0327	14.65	2.14	0.0339
14	14.50	3.20	0.0387	14.50	3.01	0.0403	14.52	3.006	0.0409
21	14.32	4.41	0.0431	14.25	4.68	0.0453	14.21	5.08	0.0465
28	14.23	5.01	0.0479	14.19	5.08	0.0490	14.15	5.48	0.0494
t ₉₇ (days)	***16.63			16.37			15.16		

* Samples before and after storage stability test analyzed together to reduce the analytical error., ** Maximum level of 2,6-dimethylaniline in tested samples = 0.1% of metalaxyl content., ***The time required to reach 97% of the initial metalaxyl content.

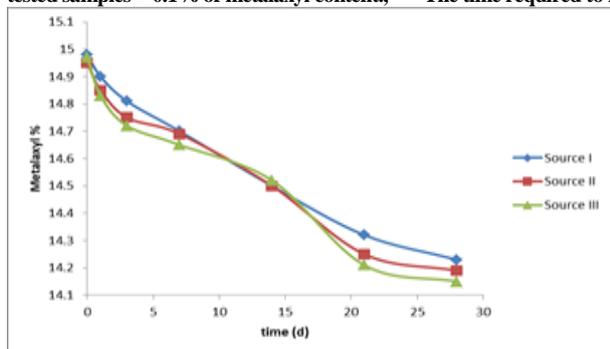


Fig. 3. Effect of exposure to sunlight on stability of metalaxyl 15% WP from three different manufactured sources.

The previously mentioned results from storage at 54 ± 2 °C and exposure to sunlight clearly showed that there is no significant difference in the rate of degradation of the three metalaxyl formulations and the amount of relevant impurity 2,6-dimethylaniline for all sources under investigation. Metalaxyl is thermally stable as the results showed that it was not affected by the long period of storage at 54 ± 2 °C, which led to an effect on the rate of impurity formation as the amount of 2,6-dimethylaniline was very low and was within the permissible limits according to (FAO, 1995), on the other hand, metalaxyl was less stable after exposure to sunlight and the rate of degradation increased compared to storage at 54 ± 2 °C, also the rate of formation of the relevant impurity was high compared to thermal storage but the amount of impurity 2,6-dimethylaniline was in allowed limits. The quality of

metalaxyl wettable powder formulations depend on many factors such as manufacturing process, sources of starting materials, adjuvants, wetting agents, chemical structure, exposure to air and sunlight so, all these factors could affect the degradation of metalaxyl resulting in an increase in the amount of relevant impurity 2,6-dimethylaniline. Our findings are in harmony with (Ibrahim and Ramadan, 2011) reported that metalaxyl is not affected by thermal storage at at 54 ± 2 °C and storage for a long period in a sunny and dark place, also the obtained results are compatible with several investigators (Khalil and Shaymaa, 2016; Hala *et al.*, 2016; Khozimy and Ramadan, 2018) they reported that metalaxyl is stable after storage at 54 ± 2 °C for 14 days and the amount of 2,6-dimethylaniline was in allowed limits according to (FAO, 1995).

CONCLUSIONS

Metalaxyl is thermally stable product where the rate of degradation is very low and the amount of relevant impurity 2,6-dimethylaniline was in allowed limits. Metalaxyl showed slightly affected after exposure to sunlight resulting in an increase in the amount of relevant impurity. There are many factors affected on the quality of metalaxyl formulation such as the manufacturing process, sources of technical materials, using different types of adjuvants and exposure to direct sunlight.

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دراسة ثبات بعض مستحضرات الميتالاكسيل القابلة للبلل المتداولة في مصر

اسماعيل ابراهيم اسماعيل

قسم بحوث تحليل المبيدات - المعمل المركزي للمبيدات- مركز البحوث الزراعية - الدقى - جيزه - مصر

فى هذا العمل تمت الدراسة على ثلاثة مستحضرات تجارية قابلة للبلل (WP) من المبيد الفطرى ميتالاكسيل 15%، من مصادر مختلفة فى مصر حيث كان المستحضر من المصدر الاول مجهز محليا والاخرين كانوا جاهزين التصنيع من الصين والاردن . المستحضرات قيد البحث عبارة عن خليط من الميتالاكسيل و اوكسى كلور النحاس ومتداولة فى مصر حيث اجريت هذه الدراسة لفحص ثبات الميتالاكسيل بالاضافة الى دراسة تكوين الشوائب المصاحبة 2, 6- داي ميثيل انيلين بعد التخزين عند 54 ± 2 °م والتعرض الى الشمس لفترات مختلفة. تم التعرف وتقدير محتوى مبيد الميتالاكسيل والشوائب المصاحبة باستخدام جهاز GC - FID . من النتائج المتحصل عليها اظهر الميتالاكسيل ثباتا عاليا بعد التخزين عند 54 ± 2 °م مما ادى الى انخفاض معدل تكون الشوائب المصاحبة. على الجانب الاخر اظهر الميتالاكسيل تأثرا طفيفا بعد التعرض الى الشمس وذلك بالمقارنة بالتخزين عند 54 ± 2 °م ومعدل تكون الشوائب المصاحبة كان اعلى نسبيا من التخزين عند 54 ± 2 °م ولكن محتوى الشوائب المصاحبة 2, 6- داي ميثيل انيلين كان فى الحدود المسموح بها بالنسبة لكل المستحضرات قيد البحث قبل وبعد التخزين. بالاضافة الى ذلك لا يوجد اختلاف فى معدل انهيار الميتالاكسيل ومحتوى الشوائب المصاحبة 2, 6- داي ميثيل انيلين لكل المستحضرات قيد البحث.