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FORMULATION AND DETERMINATION THE NEMATICIDAL ACTIVITY OF ALUM AND CITRIC ACID AGAINST ROOT-KNOT NEMATODE, *Meloidogyne incognita*

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ABSTRACT

Citric acid and alum were prepared as soluble powder (SP) formulations and tested against the second stage juveniles of *Meloidogyne incognita* under laboratory conditions. The prepared formulations passed successfully all physico-chemical properties for soluble powder formulation. The results showed that the two prepared SP formulations were effective against second stage juveniles of *M. incognita*. The highest effect was noticed after 96 hours. On the other hand, both SP formulations were more effective than their active ingredients. The effectiveness of the two prepared SP formulations was found after 96 hours to be greater than their active ingredients by 67 % for alum and 88 % for citric acid.

INTRODUCTION

In Egypt, as in many warm countries, root-knot nematodes *Meloidogyne* spp., are serious pests to many field vegetable crops (El-Morshedy *et al.*, 1993). The efficacy of a number of different inorganic salts and organic acids on different stages of *M.* spp., had been studied by several workers (Oteifa,1953; Fademi, 1988; Mousa *et al.*, 1994; Paul and David, 1993; El-Kadi *et al.*, 2004).

The successful use of any active ingredient depends on its correct formulation into a preparation, which can be applied for crop protection with safety to those applying materials to animal life and to the environment. In general, formulation plays an important role in the agriculture field, it makes a very small amount of active component to spread over a very large area. Also, it facilitate the penetration of the active ingredient to reach its target and achieve its action.

Soluble powder formulations "SP" are the simplest formulations. The pesticide which can be formulated, is limited by solubility and hydrolytic properties (FAO , WHO Meeting, 2002).

The purpose of this work is to formulate of inorganic salts (alum) and organic acid (citric acid) as SP formulation and testing the efficiency of those formulations against root-knot nematode.

MATERIALS AND METHODS

A) Tested chemical :

1- Citric acid (2-hydroxypropane 1, 2, 3-tricarboxylic acid, molecular weight (192.193 g/mol) that were supplied by El-Gomheria Co., Cairo, Egypt.
2- Alum (potassium aluminium sulfate), molecular weight (474.00) that were supplied by El-Gomhoreia Co., Cairo, Egypt.

3- Surface active agent : Tween-20 and Tween-80 that were supplied by El-Gomhoreia Co., Cairo, Egypt.

B) Physico-chemical characteristics of the basic formulation ingredients :

*** Surfactants :**

1- Hydrophilic-lipophilic balance (HLB) : The solubility of surfactant in water is considered as approximate guide to its hydrophilic-lipophilic balance (HLB) (Lynch and Griffin, 1974).
2- Critical micelle concentration (CMC) : CMC is the concentration at which the surface tension of the solution does not decrease with further increase in surfactant concentrations. CMC of the tested surfactants was determined according to Osipow (1964).
3- Surface tension : It was measured by Du-Noüy tensiometer for solutions containing 0.5 % (w/v) active ingredient.
4- Free acidity or alkalinity : It was determined according to the method of WHO specifications (1979).

*** Active ingredients :**

1- Free acidity or alkalinity : it was determined according to WHO specifications (1979) as mentioned before.
2- Solubility : It was determined by measuring the volume of distilled water, acetone and xylen for complete solubility or miscibility of one gram of active ingredient at 20°C (Nelson and Fiero, 1954). Then % solubility was calculated according to equation : % solubility = W / V x 100 (where; W : active ingredient weight, V : volume of solvent required for complete solubility).

C) Preparation of soluble powder formulations :

This type of formulation is suitable for compounds which are soluble in water. This formulation was prepared by mixing surfactant with active ingredient in three forms (95 % + 5 %), (92.5 % + 7.5 %) an (90 % + 10 %), respectively.

D) Physico-chemical properties for the prepared soluble powder formulations :

The following physico-chemical properties were determined for the above three mixtures of the prepared formulation before and after heat storage (54±1°C) for three days .

1- Surface tension : The SP's should have low surface tension when diluted with water for achieving wettability and spreading on treated surface which is irreversely correlated to surface tension value. The surface tension was determined using (Du Noüy tensiometer).
2- Solubility : It was determined as mentioned before.
3- Acidity or alkalinity : It was determined as mentioned before.

(F) Bioassay : It was carried out according to method described by Feldmesser (1972) as follow :

Egg-masses of *M. incognita* were isolated from infested tomato roots obtained from pure culture prepared and propagated in greenhouse. The second stage juveniles were obtained by incubating egg-masses in distilled water. Newly hatched juveniles were collected using a micropipette. To evaluate the effectiveness of active ingredient and the prepared formulation,

about 100 second stage juveniles were used for every diluted active ingredient or prepared formulation. The final volume of diluted active ingredient or prepared formulation and nematode solution was 5 ml in 10 ml clean glass vials. Water was served as control and each treatment was replicated 5 times. The number of mobile and immobile nematodes were counted after 48 and 96 h.

(G) Statistical analysis :

Inhibition percentages were corrected using Abbott's formula (1925) and the concentration inhibition regression lines were drawn according to the method of Finney (1952). Increasing in effectiveness of active ingredient as resulting to formulation was determined by using the following formula :

$$\frac{EC_{50} \text{ of active ingredient} - EC_{50} \text{ of formulation}}{EC_{50} \text{ of active ingredient}} \times 100$$

RESULTS AND DISCUSSION

A- Physico-chemical properties of active ingredients and the tested surfactants :

Table (1) showed that, both two active ingredients (citric acid and Alum) were insoluble in xylen, whereas citric acid showed highly solubility in water (100 %) and low solubility in acetone (11.1 %). On the other hand, Alum recorded 25 % solubility in water and it was insoluble in acetone. According to FAO, WHO Meeting (2002) on pesticides specification. The pesticide which can be formulated is limited by solubility and hydrolytic ability, therefore, soluble powder formulation is suitable form for the two tested materials.

Table (1). Physico-chemical properties of active ingredients.

Materials	Solubility at 25°C % (wt/v) in			% Free acidity as H_2SO_4
	Water	Acetone	Xylen	
Citric acid	100	11.1	Insoluble	45.6
Alum	25	Insoluble	Insoluble	24.9

From another point of view, both active ingredient were acidic. The % free acidity was 45.6 and 24.9 for citric acid and Alum, respectively. Therefore, both two active ingredients required acidic medium for maximum chemical stability. So, the two nonionic surfactants Tween-20 and Tween-80 that have an acidic properties and possess low surface tension values as found in Table (2), are suitable as wetting agents to prepare the soluble powder formulations.

Table (2). Important characteristics of experimented surfactants.

Surfactant	Type	Dispersibility	HLB	CMC	Free acidity as H_2SO_4	Surface tension (dyne/cm)
Tween-80	Nonionic	Clear solution	> 13	0.5	0.61	48.0
Tween-20	Nonionic	Clear solution	> 13	0.2	0.049	41.5

Surfactants applied to soluble powder will reduce the surface tension of spray droplets that spread over the treated surface providing more coverage for toxicant by decreasing contact angle of spray drops on solid surface (El-Sisi, 1985).

B- Physico-chemical properties of spray solution for the locally prepared SP formulations :

In Table (3), the mixtures of the two active ingredients under study and Tween-20 gave low surface tension when diluted at 0.5 % in water than when they mixed with Tween-80, and the surface tension decreased by increasing the concentration of surfactant. The mixture of active ingredients and Tween-20 should be used since it improved spreading, wettability and retention of sprayed solution. Bioactivity increased as surface tension of spray solution decreased (Osipow, 1964). Also, all prepared SP formulations are completely soluble in water without any sedimentation, and these SP gave clear solution when added to water.

Table (3). Preparation and physico-chemical properties of soluble powders.

Material	% Wetting agent	Tween-20			Tween-80		
		Surface tension 0.5 % dil. in water	Solubility	Sedimen-tation	Surface tension 0.5 % dil. in water	Solubility	Sedimen-tation
Citric acid	0.0	74.3	-	-	74.3	-	-
	2.5	48.9	Soluble	Nil	52.6	Soluble	Nil
	5.0	44.9	Soluble	Nil	52.6	Soluble	Nil
	10.0	42.1	Soluble	Nil	50.7	Soluble	Nil
Alum	0.0	76.0	-	-	76.0	-	-
	2.5	41.5	Soluble	Nil	48.9	Soluble	Nil
	5.0	40.2	Soluble	Nil	48.9	Soluble	Nil
	10.0	39.1	Soluble	Nil	45.6	Soluble	Nil

According to data presented in Table (4), no changes were found in solubility and sedimentation of the prepared SP formulations before and after heat storage, whereas a slight changes were found in their acidity as a result to heat storage. According to Anonymous (1973), no any precipitation or flocculating should be occurred when SP diluted with water at field application rate.

Table (4). Effect of heat storage on physico-chemical properties of locally prepared SP formulations.

Material	Before storage			Heat storage		
	Solubility	Sedimen-tation	Free acid % as H_2SO_4	Solubility	Sedimen-tation	Free acid % as H_2SO_4
Citric acid	Soluble	Nil	50.96	Soluble	Nil	50.75
Alum	Soluble	Nil	61.74	Soluble	Nil	61.00

C- Evaluation of nematicidal effect of active ingredients (citric acid and Alum) and their SP formulations :

Because of the tested active ingredient and their SP formulations showed higher level of acidity than WHO recommendation (1979), it was evaluated as soil pesticides against plant parasitic nematode, *M. incognita* to avoid expected phytotoxicity.

The effectiveness of active ingredient and their SP formulations was recorded initially as stimulation in movement, but then gradually slows down; thereafter, nematodes are partially paralyzed and the number of body undulations decreased, whereas abnormal stylet protrusions are made. All these reactions are concentration dependent. In high concentrations, nematodes become paralyzed and seem to be dead. Finally, the part between head region and overlapping seems as spotting in case of Alum, whereas all body was as mummification in case of citric acid.

Data in Tables (5 & 6), compared between the effectiveness of tested active ingredients and their SP formulations against second stage larvae of *M. incognita* under laboratory conditions. Generally, there are a positive relationship was found between tested concentrations and percentage inhibition with both active ingredients and their formulations. In addition, the effectiveness was noticed as slight inhibition percentages after 48 hours and increased to give the highly effectiveness after 96 hours with both active ingredients and their formulations. Depending on EC_{50} values, both SP formulations were more effective than their active ingredients.

The increasing in effectiveness at 96 hours was 67 % in case of Alum SP and 88 % in case of citric acid SP. The above indication may be due to the role of wetting agent that reduced the surface tension of spray droplet that spread on the body surface of nematode providing more coverage for toxicant by decreasing contact angle of spray drops on body surface. With other view wetting agent may be facilitate the penetration of active ingredient to reach its target and achieve its action.

Table (5). Nematicidal effect of Alum and it's SP formulation against second stage larvae of root-knot nematode *M. incognita* under laboratory conditions.

Conc. mg/ml	% Inhibition of active ingredient at:		% Inhibition of SP formulation at :		Increase in effectiveness at 96 h
	48 h	96 h	48 h	96 h	
0.06	13.1	13.7	2.9	2.0	67 %
0.13	13.9	22.7	9.7	15.9	
0.25	14.8	32.7	24.2	29.5	
0.50	15.8	42.1	46.1	58.0	
1.00	16.8	53.9	69.2	78.9	
2.00	*	65.4	*	*	
EC_{50}	-	0.6	0.6	0.2	
EC_{90}	-	6.6	2.5	0.9	
Slope	-	1.2	2.0	0.2	

- : non calculated
*** : non tested**

Table (6). Nematicidal effect of citric acid and it's SP formulation against second stage larvae of root-knot nematode *M. incognita* under laboratory conditions.

Conc. mg/ml	% Inhibition of active ingredient at :		% Inhibition of SP formulation at :		Increase in effectiveness at 96 h
	48 h	96 h	48 h	96 h	
0.06	9.9	7.8	2.8	1.5	88.2 %
0.13	10.6	11.4	4.0	29.2	
0.25	15.9	20.5	25.7	58.0	
0.50	19.7	30.4	40.1	82.9	
1.00	22.7	41.1	56.0	95.6	
2.00	29.0	53.0	70.9	*	
EC_{50}	-	1.66	1.6	0.21	
EC_{90}	-	33.11	12.6	0.70	
Slope	-	1.00	1.5	2.50	

- : non calculated
*** : non tested**

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تجهيز وتقييم فاعلية الصورة التجهيزية لكل من الشبه وحمض الستريك ضد نيماتودا تعقد الجذور (*ميلودجين إنكوجنيتا*)

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تناول هذا البحث تحضير صورة تجهيزية لكل من الشبه وحمض الستريك على صورة مساحيق قابلة للذوبان فى الماء، ودراسة تأثيرها ضد الطور اليرقى الثانى لنيماتودا تعقد الجذور تحت ظروف المعمل. وقد إجتازت هذه المستحضرات الإختبارات الفيزيقية والكيميائية لها بنجاح، وكذلك أظهرت فاعلية ضد الطور اليرقى الثانى لنيماتودا تعقد الجذور. حيث ظهر أعلى تأثير لها بعد ٩٦ ساعة من المعاملة.

من ناحية أخرى قدرت الزيادة فى الفاعلية لهذه المستحضرات عن المواد الفعالة لها بـ ٦٧ % فى حالة الشبة، ٨٨ % فى حالة حمض الستريك.