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Evaluation of the Herbicidal Efficiency of The Newly Formulated Urea Citrate As 20 % Soluble Liquid Under Laboratory and Greenhouse Conditions

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ABSTRACT

Urea citrate was synthesized using standard procedure, and its effect on the germination, root and shoot growth of wheat and radish as models for mono and dicotyledonous plants were examined under laboratory conditions. It effectively inhibited the growth of radish roots and shoots; its respective EC_{50} values for root and shoot growth was 91.4 and 48.5 ppm. It was formulated as 20% soluble concentrate (SL) and tested in accordance with guidelines established by the WHO and FAO for soluble concentrate formulations, it passed all tests successfully. The formulation was then tested on radish under greenhouse conditions using serial concentrations. It demonstrated a very noticeable effect that appeared as yellowing, dryness, and shrinkage with all concentrations related to exposure periods, and all plants died entirely after 96 hours from treatment in comparison to control.

INTRODUCTION

One of the biggest issues a farmer faces is the presence of weeds or plants that were not sown amid the crops and are harmful to getting the best results. Any crop that is sown must be accompanied by the assurance that further plants will emerge, either from seeds that have already been buried in the soil or from seeds that were brought in with the crop. The practical effects of weed and crop competition are widely understood (Winifred, 1917). Competition is a biological interaction between at least two plants for a limited resource (mainly light, water and nutrients) (McNaughton and Wolf, 1973). Actually, competition between weeds and crops affects both types of plants; nevertheless, weeds almost have a deleterious effect on crops (Pitelli, 1985). The weeds consume nutrients from the soil, and when they are above ground, they shade the crop and rob it of a lot of the sunshine that is necessary for its proper development. However, it is frequently questioned whether this simple "vegetative competition," if such a thing exists, is the sole component at play or whether the weeds actively suppress the growth of the crops by excreting harmful compounds from their roots (Winifred, 1917). Various trials were conducted in Egypt and India (Fletcher, 1908) and he claims that some species actively harm others due to toxic excretions from the root. Damages caused to cultivated plants by weeds can be great. According to some opinions, damages caused by weeds are greater than those caused by diseases and pests together (Kojic and Janjic, 1996). So, weeds pose a serious risk to the

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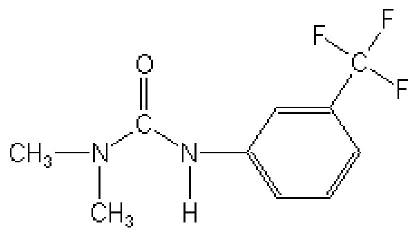
availability of food. Before the first herbicides were launched in the late 1940s, weed management in agriculture was labor-intensive and only moderately effective (Oerke, 2006). The most successful weed-control methods ever established are herbicides, which eliminate 90 to > 99% of the target weeds (Foster *et al.*, 1993). A diversity of herbicides is currently used by growers.

This golden age of herbicides was quickly cut short, however, by the detection of the first herbicide-resistant weeds in 1957s (Hilton, 1957). Today, herbicide resistance has been reported in 217 weed species in more than 670 000 fields worldwide (a conservative estimate). Moreover, the number of cases collated at http://www.weedscience.org is continuously rising.

Herbicide resistance is now commonly acknowledged as the outcome of weed populations adapting to the strong selection pressure applied by herbicides (Jasieniuk *et al.*, 1996; Neve *et al.*, 2009).

A sizable class of non-selective herbicidal chemicals is the substituted urea herbicides (SUHs). Some SUHs were first introduced in the 1960s, while others were not really introduced until 2003. The SUHs are frequently used to manage invasive broadleaf weeds and grasses in non-crop areas including drainage ditches and roadside margins, but many also have registered pre- and post-emergence treatments for specific crops. The soil actively absorbs the ureas before the roots do. They work by preventing photosynthesis, the synthesis of plant sugars, and, indirectly, the Hill reaction (The Pesticide Book, 2004). One relatively newer type of SUHs is the sulfonylurea herbicides (Battaglin *et al.*, 2000), derived from urea and sulfonic acid.



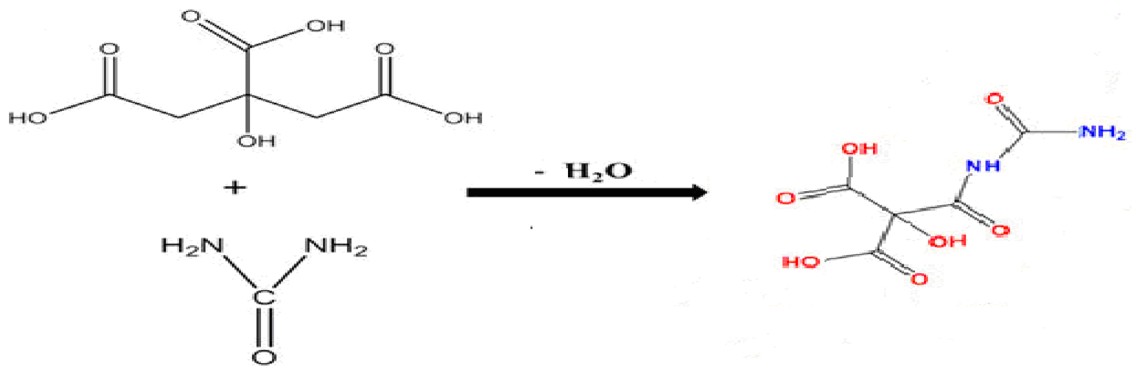
Structure of herbicide comprising urea.
N-dimethyl-N′-[3-(trifluoromethyl) phenyl] urea
(FLUOMETURON)

Depending on what was mentioned above the main aim of this research paper was to prepare one of the urea derivatives as in the case of sulfonic acid and urea in order to make use of the property of solubility of the salt obtained and promotion of formulation processes (relation between solubility and the kind of formulation) as a primary stage in developing novel active substances for use in the field of herbicide control after conducting the complementary research in the future.

MATERIALS AND METHODS

Synthesis of Urea Citrate:

According to the method outlined by Paleckiene *et al.*, 2005, urea citrate was synthesized by the reaction of citric acid and urea in an aqueous solution at a molar ratio of 1:1.



Tested Chemicals:

1-**Citric acid** (2- Hydroxypropane-1,2,3-tricarboxylic acid, molar mass 192.12 g.mol^{-1}), was obtained from EL-Gomhoria Co., Cairo, Egypt.

2-**Urea** (Carbonyl diamide, molar mass 60.06 g·mol^{-1}), was purchased from EL-Gomhoria Co., Cairo, Egypt

3-**Surface active agents**: Sodium lauryl sulfate (SLS), Triton X-100 and Tween 20 were obtained from EL-Gomhoria Co., Cairo, Egypt.

The Physico-Chemical Properties Of All Formulation Components:

1. Active Ingredient:

The physico-chemical properties of urea citrate as an active ingredient were

a) **Solubility**: 1g of the active component was tested for complete solubility or miscibility at 20 degrees Celsius by measuring the volume of distilled water, acetone, and xylene (Nelson and Fiero, 1954). The following equation was used to compute the percentage of solubility:

$$\text{Percentage solubility} = W/V \times 100$$

[Where; W is the active ingredient weight and V is the volume of solvent required for complete solubility].

b) **Free Acidity or Alkalinity**: It was calculated according to the method described by WHO specification (1979).

2. Surface Active Agents:

a) **Surface Tension**: For solutions containing 0.5% (W/V) surfactant, it was determined by utilising the Du-Nouy tensiometer according to (ASTM) D-1331 (2001).

b) **Hydrophilic-Lipophilic Balance (HLB):** Surfactant solubility in water is used as a rough indicator of its hydrophilic-lipophilic balance (HLB) (Lynch and Griffin, 1974).

c) Critical Micelle Concentration (CMC): The method outlined by (Osipow, 1964) was used to identify the concentration of the tested surfactants at which the surface tension of the solution does not decrease with additional increases in surfactant concentration.

d) **Free Alkalinity or Acidity**: It was measured as mentioned before.

3. Local Prepared Soluble Concentrate Formulation (SL):

a) Surface Tension: It was determined as before.

b) Free Alkalinity or Acidity: It was measured by the same method as described before.

4. **Spray Solution at Field Dilution Rate**:

a) PH: According to Dobrat and Martijn, it was calculated using the Cole-Parmer PH conductivity meter 1484-44. (1995).

b) Surface Tension: It was determined as mentioned before.

c) Viscosity: It was calculated using a Brookfield viscometer Model DVII+Pro, where the unit of measurement is the centipoise according to (ASTM D-2196) (2005).

d) Electrical Conductivity: It was calculated using a Cole-Parmer PH/Conductivity measuring device 1484-44, where m µmhos is the unit used to measure electrical conductivity according to Dobrat and Martijn (1995).

Bioassay Test:

1- Under Laboratory Conditions:

According to the approach outlined by Powel and Spencer (1988), the inhibition effect of urea citrate and its soluble concentrate formulation (SL) on seed germination, root and shoot growth was conducted. However, certain changes were made for this work as detailed below:

By dissolving urea citrate in water, serial concentrations of the active ingredient were prepared. Thirty seeds of wheat (for monocotyledons) and radish (for dicotyledons) were pipetted with the determined amount from each concentration, and the seeds were then stirred to coat the surface. Ten seeds were placed in each Petri dish (90 mm in diameter), which was then lined with dry filter paper and pipette-filled with 6 ml of distilled water. The Petri dish was then sealed with (PVC) electrical insulating tape. The number of germinated and non-germinated seeds as well as the radical length was counted after the control seedlings in the Petri plates had fully grown. For each treatment, three replicates were performed (El-kady *et al.*, 2000).

2- Under Greenhouse Conditions:

Three plastic pots, one for each concentration, were filled to the bottom with sand, teen radish seeds were planted in each pot, and water was added. After waiting until the seeds had grown, the pots were sprayed with the calculated concentration of the soluble concentrate formulation spray solution, irrigated with water as needed daily, and the results were compared to the pots that had not been treated, which served as the control (Hussein, 1989).

Statistical Analysis:

Abbott formula (1925) was used to correct inhibition percentages, and Finney method (1952) was used to generate the concentration inhibition regression lines.

RESULTS AND DISCUSSION

A solution results in dissolving (the solute) in a liquid (the solvent). The solute can be a solid or a liquid. The solute may be either a liquid or a solid. A true solution constituent is inseparable mechanically. A true solution does not require agitation after it has been mixed to prevent its constituent parts from settling. Most of the time, solutions are apparent. The active ingredient of the herbicide Roundup PRO, glyphosate (solute), which is dissolved in water, is an example of a solution (Anderw *et al.*, 2011).

Urea citrate was considered an active ingredient and prepared as a soluble concentrate (SL) after carrying out the following physico-chemical properties, in addition to that of the used surface-active agents.

1. Physico-Chemical Features of Urea Citrate as An Active Ingredient:

Table (1) showed that urea citrate had moderate solubility in acetone, soluble in water and completely insoluble in xylene; their respective values were 14.8, 30.4 and 0 %. Since the solubility of the active ingredient as physical property is one of the main factors that determine the type of formulation, urea citrate can be prepared as a soluble concentrate (SL). In addition, it showed an acidic property, which means that the required surface-active agents for the formulation should be acidic.

Table 1: Physico-chemical features of urea citrate as an active ingredient.

Solubility % (W/V)			**Free acidity as % H_2SO_4**
Water	Acetone	Xylene	
30.4	14.8	Insoluble	7.06

2. Physico-Chemical Properties of Surface-Active Agents:

Data in Table (2) showed physico-chemical properties of three surface active agents, sodium lauryl sulfate (SLS), Tween 20 and Triton X-100. All of them lowered the value of surface tension from 72 for water to 34.2, 36.0 and 29.0 dyne/cm for the three formerly mentioned surface active agents respectively. Also, all of them showed an HLB value, greater than 13, meaning that they are considered dispersing agents in addition Tween 20 and Triton X-100 have the same CMC whereas sodium lauryl sulfate (SLS) showed a higher CMC value. On the other hand, sodium lauryl sulfate (SLS) and Triton X-100 showed weak alkaline properties, while Tween 20 showed the weak acidic property. Although an acidic adjuvant is required in this case depending on the nature of the active ingredient, also it could be possible to use an alkaline adjuvant with the weak alkaline property.

Table 2: Physico-chemical characteristics of the tested surfactants.

Surface active agent	**Surface tension dyne/cm**	**HLB**	**CMC %**	**Free alkalinity as % NaOH**	**Free acidity as % H_2SO_4**
Sodium lauryl sulfate	27.8	>13	8	0.48	-
Tween 20	36.0	>13	0.2	-	0.0196
Triton X-100	29.0	>13	0.2	0.02	-

3. Physico-Chemical Characteristics of Local Urea Citrate 20 % Soluble Concentrate Formulation Before And After Accelerated Storage:

Many trials were carried out to find the correct percentages for the components of the target soluble concentrate formulation (based on the solubility of urea citrate). Table (3) showed physical features of the soluble concentrate formulation before and after hot storage, some changes were observed for the formulation before and after accelerated storage, which appears clear from the values of surface tension and free acidity in both cases, in addition, to complete solubility without any sedimentation.

Table 3: Physico-chemical properties of urea citrate 20 % soluble concentrate local formulation before and after accelerated storage.

Before storage				**After storage**			
Surface tension dyne/cm	Free acidity as % H_2SO_4	% Solubility	Sedimentation	Surface tension dyne/cm	Free acidity as % H_2SO_4	% Solubility	Sedimentation
40.5	0.76	soluble	Nil	29.7	1.078	soluble	Nil

4. Physico-Chemical Properties of Spray Solution at The Field Dilution Rate:

Table (4) showed the physico-chemical properties of the spray solution, it showed high viscosity, high electrical conductivity, acidic PH value and low surface tension, these properties show how far the spray solution is expected to be effective. Spray solution viscosity increases resulting in less drift and increased pesticidal effectiveness by retention sticking (Spanoghe *et al.*, 2007). According to Twifik and El-Sisi (1987), an increase in electrical conductivity and a decrease in the PH value of the spray solution would also result in the deionization of the insecticide, increase its deposits and penetration in the treated surface, and as a result, increase its insecticidal efficacy. According to Ryckaert *et al.*, (2007), a decrease in the surface tension of a pesticide spray solution predicts increased spreading across the treated surface and an increase in pesticidal efficacy as a result.

Table 4: Physico-chemical features of spray solution at field recommended dilution rate

Viscosity Centipoise	Electrical conductivity µ mhos	PH	Surface tension dyne/cm
18.78	4700	3.95	33.06

Data in Table (5) showed the effect of urea citrate with serial concentrations on the root, shoot growth and germination of wheat and radish as a pattern for mono and dicotyledonous plants. In the case of wheat, there is no relation between concentration and percentage of inhibition, the effect was increased with certain concentrations and returns to decrease with other concentrations. Contrary to radish, a direct proportion was found between concentration and inhibition as it inhibited markedly root growth by 28.5, 34.7, 57.8 and 78.6 % on treatment by 10, 20, 200, and 2000 ppm respectively and shoot growth by 16.9, 38.0, 61.9 and 70.6 % on treatment by 20, 200, 2000 and 4000 ppm respectively. The EC_{50} values for root and shoot growth were 91.4 and 48.5 ppm respectively. But it showed no inhibition effect on germination.

Table 5: Effect of urea citrate with serial concentrations on germination, shoot and root growth of radish and wheat as patterns for mono and dicotyledonous plants under laboratory conditions

Plant Kind	Monocotyledonous plant			Dicotyledonous plant		
Parameter / Conc. (ppm)	Root growth	Shoot growth	Germination	Root growth	Shoot growth	Germination
10	-	-	-	28.5	-	-
20	4.41	5.47	0	34.7	16.9	-
200	1.65	3.83	4	57.8	38	-
1000	-14.6	-8.6	4	-	-	-
2000	51.2	20.54	10	78.6	61.9	-
4000	-1.4	10	10	-	70.6	-
8000	-6.59	-6.22	0	-	-	-
20000	97.9	92.05	57	-	-	-
Slope	**-**	**-**	**-**	**0.59**	**0.65**	**-**
EC_{50}	**-**	**-**	**-**	**91.4**	**48.5**	**-**
EC_{90}	**-**	**-**	**-**	**13505**	**4506**	**-**

Table (6) and Figure (1), showed the effect of soluble concentrate formulation (SL) on radish as dicotyledone under greenhouse conditions after 24, 48 and 96 hrs. The effect appeared as yellowing, drying, shrinkage and wilt of leaves in comparison to control. After 24 hours. 24000 ppm affected completely all plants, while 12000, 6000 and 3000 ppm showed 91.3, 76.0 and 0 % respectively. After 48 hrs all used concentrations completely affected all plants except the concentration of 3000 ppm which showed a 61.7 % effect, but after 96 hrs. from treatment, all concentrations used for treatment resulted in complete death of all treated plants.

Table 6: Effect of urea citrate 20 % soluble concentrate formulation on radish under greenhouse conditions

Concentration ppm	% Of affected plants after		
	24 hrs.	48 hrs.	96 hrs.
Control	0	0	100
3000	0	61.7	
6000	76.0	100	
12000	91.3	100	
24000	100	100	

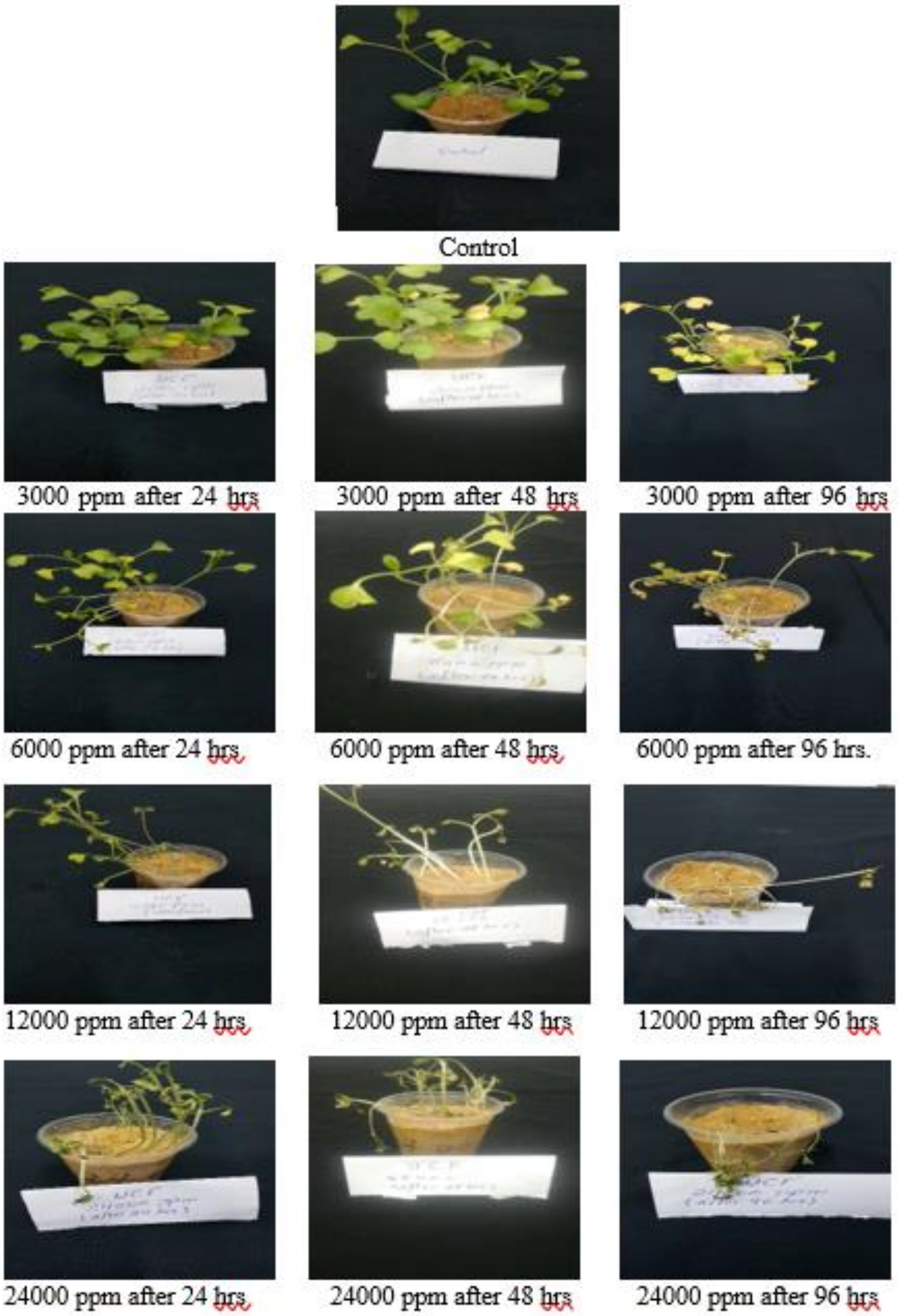


Fig. 1: Effects of urea citrate 20 % soluble concentrate formulation on radish under greenhouse conditions by 3000, 6000, 12000 and 24000 ppm after 24, 48 and 96 hrs. from treatment.

The results of treating radish with a 20% soluble concentrate formulation under greenhouse conditions were consistent with the results previously reported for the mechanism of action of ureas. Ureas are highly absorbed by roots, and their mechanism of action is to prevent the production of sugars during photosynthesis (The pesticide book, 2004). This is evidenced in the form of yellowing, dryness, and shrinkage of the leaves, and eventually leads to complete plant death.

Conclusion:

Urea citrate was tested on mono and dicotyledonous plants under laboratory conditions. It inhibited markedly root and shoot growth of dicotyledons, it was regarded as a promising compound and prepared as 20 % soluble concentrate. The formulation was tested on dicotyledons under greenhouse conditions, it showed a marked effect on plant leaves, resulting in yellowing, dryness, shrinkage and complete death after 96 hours from treatment.

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ARABIC SUMMARY

تقييم مستحضر سترات اليوريا الجديد علي صورة 20 % مركز قابل للذوبان في الماء كمبيد حشائش تحت ظروف المعمل والصوبة

سعد العدوى شحاتة حموده - نوسة شعبان عبد اللطيف - هشام ابراهيم عبد الله.
قسم بحوث مستحضرات المبيدات- المعمل المركزى للمبيدات - مركز البحوث الزراعية - دقى - جيزه - مصر

تم تحضــير ســترات اليوريا بطريقة قياســية ثم تمت تجربته تحت ظروف المعمل على الإنبات ونمو الجذر والساق للقمح والفجل كأمثلة للنباتات ذوات الفلقة الواحدة والنباتات ذوات الفلقتين. أوضح مركب سترات اليوريا تأثيرا تثبيطيا جيدا على نمو الجذر والســـاق للفجل بينما لم يظهر أى تأثير على النباتات ذوات الفلقة الواحدة. ثم تم تجهيزة على صـورة مركز قابل للذوبان فى الماء بتركيز 20 % وعُرض المستحضـر الجديد للإختبارات المرتبطة بهذا النوع من أنواع مسـتحضـرات المبيدات التي حددتها منظمة الصـحة العالمية ومنظمة الأغذية والزراعة فإجتازها بنجاح. ثم جُرب المســتحضــر على الفجل بسـلسـلة من التركيزات تحت ظروف الصـوبة الزجاجية، فأظهر المســتحضــر تأثيرا واضــحا على الأوراق ظهرت اعراضــه في صــورة اصــفرار وجفاف وذبول ثم موت النبات كليا بعد 96 ســاعة من المعاملة بالمقارنة بالكنترول.