Herbicidal Activity of Acid Mixture 20 % Soluble Concentrate Formulation on Dicotyledonous plants

Abd-Alla; Hisham I., N. S. Abd Elattif and S. E. S. Hamouda*

Formulation Research Department, Central Agricultural Pesticides Lab. (CAPL), Agriculture Research Center (ARC), Dokki, Giza, Egypt

*To whom correspondence should be addressed: saad_capl@yahoo.com

ABSTRACT: A mixture of acetic acid and citric acid (1:1) was tested as weed killer on radish as pattern for dicotyledonous plants by serial concentrations under laboratory conditions. It showed good inhibition on germination. Their mixture was formulated as 20 % soluble concentrate formulation (SL), the physio-chemical properties of the new formula showed complete consistence with the specifications of WHO and FAO for soluble concentrates. The herbicidal activity of this formulation was then tested on radish under laboratory conditions; it increased the inhibition of seed germination by 33.2 % compared with its active ingredient. Also it was tested on radish under greenhouse conditions before and after germination. It showed on treatment before germination, good inhibition on germination, root and shoot growth. The effect on germination was the highest followed by root growth and then shoot growth. After germination, the effect appeared as yellowing, dry, shrinkage and wilt of leaves which resulted in complete death of all plants under study.

Keywords: Acetic acid, Dicotyledonous Plants, Formulation, Post and Pre-emergence Herbicides.

1.Introduction

When defining a weed, it should be emphasized that there is no simple and precise definition. Weeds are plants growing among cultivated plants and interfere with man's activity **(Kojic *et al*., 1996). Zekic (1983)** stated that weeds are plants growing in places they are not wanted. Weeds are undesirable plants, which infest different crops and inflict negative effect on their yield. There are innumerable reports on the inhibitory effects of weeds on crop plants **(Bhowmik & Doll, 1992; Javaid *et al*., 2007).** Generally weed-crop competition is complicated as weeds compete with the crop plants by occupying a space, which would otherwise be available to the crop plant. Anything that reduces this space reduces the plant growth **(Wright *et al*., 2001).** In addition, the competition of weeds for moisture that may result in such obvious responses as dwarfing in plant size, nutrient starved conditions, wilting and actual dying out of plants **(Anderson and Streibig,) 1996**. Research indicated that, worldwide, over 10 % of agricultural production is lost as a result of crop weed competition for the resources light, water and nutrients **(Parker and Fryer, 1975).** Weeds also serve as alternate hosts to insects, nematodes and pathogenic fungi **(Jenkinson & Parry, 1994)**. **Akobundu (1987)** reported loss estimates of 5 % in developed countries, 10 % in the less developed countries and 25 % in the least developed countries. This clearly indicates problems associated with weed control in tropical crops. When weeds are left uncontrolled, yield losses range from 20 -100 % depending upon the crop and its environment.

Worldwide, herbicides remain the most efficient technology for large-scale weed control **(Walsh and Powles, 2004)**, but it is used extensively. One consequence of the extensive use of herbicides has been the appearance of herbicide resistance in weed species. Currently herbicide resistance has appeared in more than 140 weed species and many thousands of crop fields worldwide **(Heap, 1999)**. Herbicide resistance is known in all areas where herbicides are used intensively **(Powles *et al.,* 1997).**

Evant *et al.*, 2011 reported that, acetic acid does not persist in the environment. According to **Webber and Shrefler (2006)** acetic acid was absorbed into the plant and translocated to other plant parts of the inflict damage, therefore, it was considered to be a contact and as post-emergence herbicide as glyphosate. **(Pujisiswant *et al.,* 2012)** found that the pre-emergence application at 10 % and 20 % of the glacial acetic acid solution on maize inhibited seed germination, no shoots and roots growth. Acids are most effective at killing weeds when applied as a foliar spray at concentrations ranging from 10 % to 20 % acetic acid, citric acid, or blends of the two and when the weeds are about 6 to 9 inches tall or less **(Doll, 2002; Radhakrishnan *et al.,* 2003).**

The active ingredients in a pesticide are the chemicals that control the target pest. Most pesticide products also have inert (inactive) ingredients, which are used to dilute the pesticide or to make it safer, more effective, easier to measure, mix, or apply, and more convenient to handle. These are the pesticide formulations which are broken-down into active ingredients and inert ingredients (Arizona Agricultural Pesticide Applicator Training Manual, 2000).

The objective of this study was to evaluate the efficacy of acetic acid and citric acid mixture (1:1) on dicotyledons and formulating it in appropriate formulation form, in the hope of finding a new active ingredient to be used in the field of weed control after completion of the other needed technical studies in the future.

2.MATERIALS AND METHODS

2 .1.Tested chemicals:

1) Acetic acid (ethanoic acid, molar mass 60.05 $g.mol^{-1}$), was supplied by EL-Gomhoria Co., Cairo, Egypt.
2) Citric acid (2-hydroxypropane-1,2,3-tricarboxylic acid, molar mass 192.12 $g.mol^{-1}$), was supplied by EL-Gomhoria Co., Cairo, Egypt.
3) Surface active agents: Tween 20, Tween 40 and Tween 80 were supplied by EL-Gomhoria Co., Cairo, Egypt.

2.2.The physico-chemical properties of basic formulation constituents:

2 .2.1.Active ingredient:

The physico-chemical properties of acetic acid and citric acid mixture (1:1) as active ingredient were:

a) Solubility: It was determined by measuring the volume of distilled water, acetone and xylene for complete solubility or miscibility of one gram of active ingredient at 20 ˚C **(Nelson and Fiero, 1954)**. The % Solubility was calculated according to the following equation:

% solubility = W/V x 100

[Where; W= active ingredient weight, V= volume of solvent required for complete solubility].

b) Free acidity or alkalinity: It was determined according to the method described by **WHO specification (1979).**

2.2.2.Surface active agents:

a) Hydrophilic-lipophilic balance (HLB): The solubility of surfactant in water is considered as approximate guide to its hydrophilic-lipophilic balance **(Lynch and Griffin, 1974)**.

b) Surface tension: It was determined by using Du-Nouy tensiometer for solutions containing 0.5 % (W/V) surfactant according to **ASTM D-1331 (2001)**.

c) Critical micelle concentration (CMC): The concentration in which the surface tension of solution doesn't decrease with further increase in surfactant concentration, (CMC) of the tested surfactants was determined according to the method described by **(Osipow, 1964)**.

d) Free acidity or alkalinity: It was determined by the same method described before.

2.2.3.Local prepared soluble concentrate formulation:

The following physico-chemical properties of the newly prepared local soluble concentrate formulation were determined:

a) Surface tension: It was determined as mentioned before.

b) Free acidity or alkalinity: It was measured as mentioned before.

2.2.4.Spray solution at field dilution rate.

Determination of physico-chemical properties for the spray solution:

a) PH: It was determined by using Cole-Parmer PH conductivity meter 1484-44 according to **Dobrat and Martijn (1995).**

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

c) Electrical Conductivity: It was determined by using Cole-Parmer PH / Conductivity meter 1484-44, where µmhos is the unit of electrical conductivity measurements according to **Dobrat and Martijn (1995)**.

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

2.3.Bioassay:

2.3.1.Under laboratory conditions:

Inhibition effect of acid mixture (a.i) and its soluble concentrate (SL) formulation on seed germination, root and shoot growth was carried out according to the procedure described by **Powel and Spencer (1988)** with some modifications as described below:

Serial concentrations from active ingredient and its soluble concentrate (SL) formulation were prepared by dissolution in water. The calculated amount from each concentration was pipette on thirty seeds of radish as pattern for dicotyledonous plants and agitated to coat the seed surface. Each ten seeds were transferred to Petri dish (90 mm diameter), lined with a dry filter paper, 6 ml distilled water were pipette on the filter paper, Petri dish was then sealed with (PVC) electrical insulating tape. After complete germination of control (Petri dishes containing untreated seeds), the number of germinated and non-germinated seeds and radical length were recorded. Three replicates were done for each treatment **(El-kady *et al.*2000 ,)**

2.3.2.Under greenhouse conditions:

Three plastic pots for each concentration were filled till their lower surface by sand, ten radish seeds were planted in each pot and filled with water, at the second leaf stage, sprayed by the calculated concentration of spray solution of the soluble concentrate formulation, irrigated with water daily according to need, then compared with untreated pots taken as control for post- emergence treatment, and sprayed by the calculated concentration of spray solution before germination for the pre-emergence treatment (soil treatment) **(Hussein, 1989)**.

2.4.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)**.

3. RESULTS AND DISCUSSION

Soluble concentrates (liquids, SLs) are usually water-based products that contain a dissolved active ingredient. This is one of the formulation types that actually contain dissolved molecules, not suspended particles. SLs tend to have low viscosities (closer to that of water). They mix easily in water and require minimal agitation after dilution, though some actives are dense enough to settle out over time.

According to the physico-chemical properties of active ingredient (acid mixture), it could be prepared as SL after carrying out the following physico-chemical properties for both active ingredient and additives.

3.1.Physico-chemical properties of acid mixture as active ingredient:

Table (1) showed that, the acid mixture was completely soluble in water, medium in acetone but completely insoluble in xylene. It showed acidic property which appeared from its free acidity (6.08). Taking these results into account, it could be prepared as soluble concentrate and needs acidic surface active agents for complete compatibility.

3.2.Physico-chemical properties of surface active agents:

Data in Table (2) showed the physico-chemical properties of three Tweens (polysorbates) 20, 40 and 80 as surface active agents. All of them showed very close surface tension values, their values were 36, 37.02 and 39.2 dyne/cm for Tween 20, 40 and 80 respectively. Also all tested surfactants had the same hydrophilic-lipophilic balance, over 13, but there were clear differences between them in CMC values as they showed 0.2, 0.4 and 0.5 % respectively. Also for the free acidity or alkalinity, all showed acidic property, Tween 80 showed the highest value (0.49), followed by Tween 40 (0.13) and then Tween 20 (0.0196). Depending on the values of free acidity for the three surface active agents, any of them can be used for formulating this active ingredient in the form of soluble concentrate, but the main factor that determined the best surfactant for this formula was their stability and compatability with the required properties of soluble concentrates.

Table (1) :Physico-chemical properties of acid mixture as active ingredient

Solubility % (W/V)			Free acidity as % H_2SO_4
Water	Acetone	Xylene	
100	32.5	Insoluble	6.08

3.3.Physico-chemical properties of the local 20 % soluble concentrate formulation before and after accelerated storage:

Table (3) showed physico-chemical properties of the 20 % soluble concentrate formulation before and after accelerated storage (50±3˚C for three days). Except for surface tension, all physico-chemical properties of the formulation did not showed any valuable changes, it showed acidic property before and after storage relatively by the same value; in addition it was completely soluble, clear with no sedimentation in both cases, while surface tension showed decrease after storage than before storage , an inverse relationship with temperature, the same result that was reported by **Osipow (1964)**. Generally there were no effective changes in the physico-chemical properties of the new formula before and after accelerated storage.

Table (2) :Physico-chemical properties of the tested surface active agents.

Surface active agent	Surface tension dyne/cm	HLB	CMC %	Free acidity as % H_2SO_4
Tween 20	36	>13	0.2	0.0196
Tween 40	37.02	>13	0.4	0.13
Tween 80	39.2	>13	0.5	0.49

3.4.Physico-chemical properties of spray solution at field dilution rate:

The biological activity of a pesticide to the target pest species is greatly influenced by its physical and chemical properties. The physical properties of a pesticide in particular determine the pesticide mode of action, dosage, mode of application and the subsequent environmental chemodynamics **(Zacharia, 2011)** .The physical properties of pesticides vary greatly according to their chemical nature and formulation. The spray solution showed a decrease in surface tension and pH, while an increase in electrical conductivity and viscosity was observed. Decreasing in surface tension of spray solution cause improving in wettability and spreading on the treated surface then increasing deposit and activity of pesticide **(Osipow, 1964)**. The decrease in PH value with increasing electrical conductivity can result in an increase in pesticide efficacy according to **Tawfik and El-Sisi (1987)** who stated that, the retention and effectiveness of pesticides spray solution increased with decreasing in pH values and with increasing its conductivity. The relation between increasing viscosity and increasing pesticidal efficiency could be explained according to **Richardson (1974)** who reported that, increasing viscosity of spray solution caused a reduction in drift and an increase in the retention and sticking of spray solution on the surface of plant.

Table (5) and Fig. (1) showed the effect of active ingredient and its 20 % soluble concentrate formulation on germination of radish as pattern for dicotyledonous plants by serial concentrations under laboratory conditions. The results showed that, the formulation has higher effect than its active ingredient which appeared clear from their percentages of inhibition. The formulation inhibited radish germination by 0, 2.1, 9.4, 54.12, 86 and 99.9 % on treatment by 3000 ,2250 ,1500 ,1225 ,750 and 6000 ppm respectively. While the acid mixture inhibited radish germination by 0.4, 3.1, 7.5, 22.4, 43.3 and 87 % for the same concentrations respectively. In addition the slope value in case of formulation (8) was much greater than that of its active ingredient (4.23). These results could be clarified by the role of inert ingredients (additives) added to the active ingredient which resulted in an increase in the efficacy of the formulation compared to its active ingredient. In addition these results showed the value of EC_{50} for formulation (2162 ppm) and for the active ingredient it was (3237 ppm), and the percentage of increase in effectiveness for formulation compared to active ingredient that was 33.2 %.

Under greenhouse conditions, radish was treated with serial concentrations from the 20 % soluble concentrate formulation at the two leaf stage Fig. (2). The effects appeared as yellowing, dry, shrinkage, wilt of leaves and complete death for all plants with all concentrations used, but there were direct proportion between concentration and effect, 20000 ppm showed the highest effect while 2500 ppm showed the lowest effect. The obtained results were consistent with what was mentioned by **Webber and Shrefler (2006)**, that acetic acid was absorbed into the plant and translocated to other plant parts of the inflict damage, therefore, it was considered to be a contact and as post-emergence herbicide as glyphosate.

Table (6) showed the effect of the 20 % soluble concentrate formulation on radish by serial concentration under greenhouse conditions as pre-emergence treatment (soil treatment). The biological activity was determined according to three plant parameters, germination, root and shoot growth. For the three parameters, there were direct proportion response between the concentration and effect. The formulation showed the highest effect on

Table (3):Physico-chemical properties of the 20 % soluble concentrate local formulation before and after accelerated storage

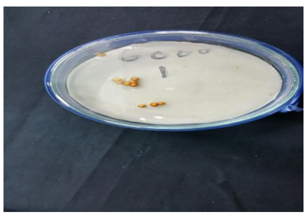
Before storage					After storage				
Surface tension dyne/cm	Free acidity as %H_2SO_4	Solubility	Sed*	App**	Surface tension dyne/cm	Free acidity as %H_2SO_4	Solubility	Sed*	App**
37.5	2.004	soluble	Nil	clear	29.62	2.403	soluble	Nil	clear

Sed*: Sedimentation App**: Appearance

Table (4) :Physico-chemical properties of spray solution at field dilution rate.

Viscosity centipoise	Electrical conductivity µ mhos	PH	Surface tension dyne/cm
8.88	1600	4.12	41.9

Control



Active ingredient by 6000 ppm

Active ingredient by 3000 ppm

Active ingredient by 1500ppm

Active ingredient by 750 ppm

Effects of active ingredient on radish under laboratory conditions by 750, 1500, 3000 and 6000 ppm.

Formulation by 6000 ppm

Formulation by 3000 ppm

Formulation by 1500 ppm

Formulation by 750 ppm

Fig. (1): Effects of the local 20 % soluble concentrate formulation on radish under laboratory conditions by 750, 1500, 3000 and 6000 ppm.

Table (5): Effect of active ingredient and its 20 % soluble concentrate formulation by a serial of concentrations on germination of radish as pattern for dicotyledonous plants under laboratory conditions.

Concentration (ppm)	% of inhibition of		% of increase in effectiveness for formulation
	Active ingredient	Formulation	
750	0. 4	0	
1225	3.1	2.1	
1500	7.5	9.4	
2250	22.4	54.12	**33.2**
3000	43.3	86	
6000	87	99.9	
Slope	**4.23**	**8**	
EC_{50}	**3237**	**2162**	
EC_{90}	**6469**	**3126**	

Table (6): Effect of the 20 % soluble concentrate formulation on radish by serial concentrations under greenhouse conditions as pre-emergence treatment (soil treatment).

Concentration (ppm)	% of inhibition of		
	Germination	Root growth	Shoot growth
375	4	3.4	3.6
750	9.6	7.8	7.5
1500	19.5	15.4	14.2
3000	34.1	27.1	23.6
6000	51.6	41.3	36.4
12000	68.8	58	50.4
Slope	**1.5**	**1.35**	**1.21**
EC_{50}	**5495**	**8317**	**11481**
EC_{90}	**38904**	**72443**	**128824**

5495 ppm for germination, 8317 ppm for root growth and 11481 ppm for shoot growth. These results are in consistence with the findings of **Pujisiswanto, *et al.*, (2012)**, who tested the pre-emergence application at 10 % and 20 % of the glacial acetic acid solution on maize, the two concentrations inhibited seed germination. No shoots and roots growth.

Conclusion

Acetic acid and citric acid mixture was tested on dicotyledonous plants under laboratory conditions. It showed good inhibition effect on seed germination. It was considered as an active ingredient and formulated as 20 % soluble concentrate (SL) formulation .The formulation was then tested germination, followed by root growth and then shoot growth. The results that observed clearly from EC_{50} values,

on dicotyledons under greenhouse conditions before and after germination (as pre and post-emergence), it exhibited marked effect on germination, root and shoot growth on treatment before germination, and after germination, it affects plant leaves, resulted in yellowing, dry, shrinkage and complete death of all treatments. It could be used as herbicide after the completion of the other necessary studies.

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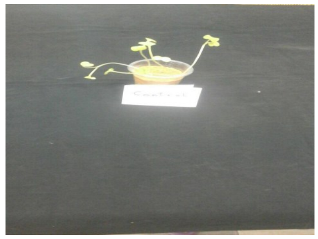

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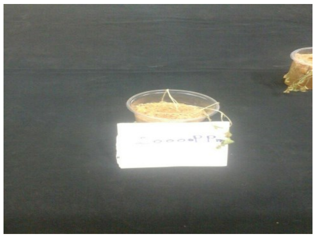
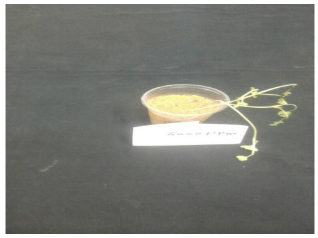
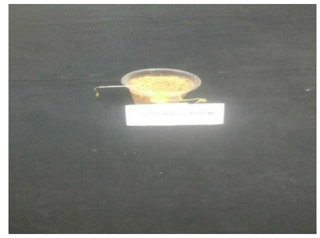


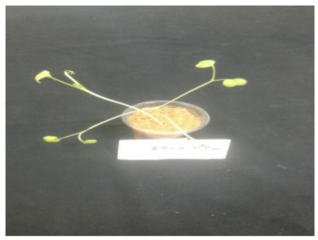
Formulation by 20000 ppm



Formulation by 10000 ppm



Formulation by 5000 ppm



Formulation by 2500 ppm

Fig. (2): Effects of the local 20 % soluble concentrate formulation on radish under greenhouse conditions by 2500, 5000, 10000 and 20000 ppm.

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دراسة كفاءة المركز القابل للذوبان في الماء بتركيز 20 % لخليط من الأحماض على النباتات ذوات الفلقتين

هشام ابراهيم عبد الله - نوسة شعبان عبد اللطيف - سعد العدوى شحاتة حموده

قسم بحوث مستحضرات المبيدات - المعمل المركزى للمبيدات - مركز البحوث الزراعية - دقى - جيزة - مصر

الملخص العربي

تم تجريب مخلوط من حمض الأسيتيك وحمض السيتريك بنسبة (1 : 1) على النباتات ذوات الفلقتين تحت ظروف المعمل بسلسلة من التركيزات. أظهر المخلوط تأثير تثبيطي جيد على الإنبات، لذا فقد تم تجهيزه في صورة مركز قابل للذوبان في الماء بتركيز 20 %. واخُتبرت الخواص الطبيعية والكيميائية للمستحضر فأظهر توافق كامل مع تلك الخواص المحددة من قبل منظمة الصحة العالمية ومنظمة الأغذية والزراعة للمركزات القابلة للذوبان في الماء. ثم تم تجريب المستحضر على النباتات ذوات الفلقتين تحت ظروف المعمل ، فأدى إلى زيادة في التأثير على الإنبات بمقدار 33.2 % مقارنة بالمادة الفعالة . كما تم تجريبه أيضا تحت ظروف الصوبة الزجاجية قبل وبعد الإنبات. فأظهر المستحضر قبل الإنبات تأثير تثبيطى جيد على الإنبات وطول الجذروطول الساق لكن تأثيرة كان الأعلى على الانبات ثم طول الجذر ثم طول الساق . وبعد الإنبات ظهر تأثيره فى صورة اصفرار و جفاف وذبول الاوراق ثم موت كليا لكل النباتات تحت الدراسة