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Herbicidal Efficiency of Pharmaceutical Material Potassium Salicylate Alone and Prepared as 20 % Soluble Liquid Formulation on Mono and Dicotyledonous Plants

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ABSTRACT: The herbicidal efficiency of potassium salicylate was tested on wheat and radish as pattern for mono and dicotyledonous plants. Three plant parameters, germination, root and shoot growth under laboratory conditions were assessed as indicators for herbicidal activity; it inhibited markedly root and shoot growth of wheat and root growth of radish. Therefore it was considered as active ingredient and consequently formulated as 20 % soluble Liquid (SL). The new formula passed successfully all physico-chemical tests identified by the specialized organizations in the field of pesticides. The local formulation was also tested on mono and dicotyledonous plants under laboratory conditions. The formulated product enhanced the effectiveness by 98.7 and 100 % for shoot growth and germination in case of wheat. While in case of radish, it increased the effectiveness by 90.2 and 100 % for root and shoot growth respectively on comparison to its active ingredient. As a confirmatory step to the results obtained under laboratory conditions, the formulated solution was sprayed on both crop plants under greenhouse conditions. Both plants died completely after seven and three days from treatment for mono and dicotyledons respectively.

Keywords: Salicylate, Weeds, Soluble Liquid and Formulation.

1. INTRODUCTION

Weeds are unwanted plants growing along with agricultural crops. As cropland is an artificial ecosystem where the plants desired by man (crops) are cultivated but the weeds do come up and compete with crops mainly for space, sunlight, moisture, nutrients and reduces the quantity as well as the quality of production. The competition of weeds for nutrients may result in such obvious responses as dwarfing in plants size, nutrient starved conditions, wilting and actual dying out of plants **(Anderson *et al*., 1996).** Weed seeds germinate earlier to agricultural crops, their seedlings grows faster and aggressive so that they crowed out all other plants which possesses more valuable properties and establishes a kingdom of their own within a short period of time. Weed species mature ahead of crops through their seeds that collected with the crop harvest and get distributed to other places. Some species of weed caused damage to crops by harboring pests and disease factors and nematodes that cause diseases in crop plants. Weeds possess allelopathic effects on agricultural crops by secreting allelochemicales that inhibit their growth and germination (**Oudhia *et al.*, 1998).** The weedy crop may sometime leads to complete failure. The cost of removing weeds adds to the cost of production of crops, thus producer's losses part of their investment and the country suffers a reduction in agricultural products (**Lakhi *et al*., 2011).**

It has been reported that salicylic acid and its salicylate salt has biological activity in activation or increase defense or resistance of plants to viruses. The mechanisms through which salicylic acid mediates these effects are varied and can involve alterations in the activity or synthesis of certain enzymes (**D'Maris *et al*., 2010).** Similarly salicylates were reported to induce PR-1a protein to increase resistance of plants of tobacco mosaic virus and to potentiate plant protective activity of systemic acquired resistance (SAR) inducers (**Best, 1940).** So, on studying the biological activity of salicylic acid and its salt, if both showed the same degree of biological activity it will be expected that the salt will have an advantage over the parent compound, solubility (salicylic acid is partially soluble in water, while potassium salicylate is soluble), the property that could promote the process of formulation.

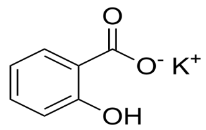

Fig.1: Structure of potassium salicylate.

Regardless of their source, pesticide active ingredients have a range of solubilities some dissolve readily in water, others only in oils. Some active ingredients may be relatively insoluble in either water or oil. Solubility characteristics and the intended use of the pesticide generally define which formulations best deliver the active ingredient **(Pesticides and Formulation Technology, 2004).** Based on the above, our main goal from this research is an attempt to find a new characteristic safe group of active ingredients and formulating it in a suitable formulation type to be used as alternatives for conventional herbicides, so potassium salicylate is considered as the best safe material for this purpose.

2. MATERIALS AND METHODS

2.1 .Tested chemicals:

2.1.1.Potassium salicylate (potassium -2- hydroxyl benzoate, molar mass 176.21 $g \cdot mol^{-1}$), was supplied by EL-Gomhoria Co., Cairo, Egypt.

2.1.2.Surface active agents: Sodium lauryl sulfate (SLS), Triton X-100 and Tween 40 were supplied by EL-Gomhoria Co., Cairo, Egypt.

2.2 .physico-chemical properties of basic formulation constituents:

2.2.1. Active ingredient:

Physico-chemical properties of active ingredient potassium salicylate (potassium -2- hydroxyl benzoate) 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 equa-

tion: % 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) Surface tension: It was determined by using Du-Nouy tensiometer for solutions containing 0.5 % (W/V) surfactant according to **ASTM D-1331 (2001)**.
b) 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).**
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 as mentioned before.
2.2.3. Local prepared soluble concentrate formulation:
Formulation was carried out by mixing active ingredient with surfactant in water in three forms (20 % + 2.5 % +77.5 %), (20 % + 5 % +75 %) and (20 % + 10 % +70 %), these three mixtures were subjected to many tests to select the best mixture. Then surface tension for the prepared mixtures was determined at field dilution rate (0.5 %) the mixture which showed the lowest surface tension was considered to be succeeded as it showed the best wetting, spreading and then the expected pesticidal efficiency when sprayed on the treated surface.
a) Surface tension: It was determined as mentioned before.
b) Free acidity or alkalinity: It was measured by the same method as described before.
c) Accelerated storage: It was carried out to check the chemical and physical stability of local formulations at 45±2 ^{o} C for three days.
2.2.4. Spray solution at field dilution rate:
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) Viscosity: It was determined by using Brookfield viscometer Model DVII+Pro, where Centipoise is the unit of measurement according to **ASTM D-2196 (2005).**
d) 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).**

2.3 Bioassay

2.3.1 Under laboratory conditions:
Inhibition effect of potassium salicylate and its soluble liquid formulation on seed germination, root and shoot growth was carried out according to the procedure described by **Powel and Spencer (1988)**, some modifications for this work were made as described below:
Serial concentrations from active ingredient (potassium salicylate) and its 20 % soluble liquid formulation were prepared by dissolution in water. The calculated amount from each concentration was pipette on thirty seeds of wheat as pattern for monocotyledons and radish as pattern for dicotyledons 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 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 grains of wheat or radish seeds were planted in each pot and filled with water, left until the seeds grown up then the pots were sprayed by the calculated concentration of the formulated solution, left for about ten days, irrigated with water daily according to need, then compared with untreated pots taken as control **(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 liquid formulation consists of water soluble active ingredients dissolved in water, wetting and spreading agents which are prepared for end user. They will obviously form a true solution in spray tank and require no agitation after they are thoroughly diluted. There are several major herbicides with wide **-** scale use prepared as soluble liquid formulation. They include paraquat, glyphosate and 2.4-D.

Potassium salicylate was prepared in the form of soluble liquid formulation (SL) after carrying out the following physico-chemical properties for active ingredient and surface active agents.

3.1. Physico-chemical properties of potassium salicylate as active ingredient:
Data in Table (1) showed that, potassium salicylate was insoluble in acetone and xylene, but it recorded 33 % solubility in water. Depending on the previously mentioned rule for the relation between solubility and the kind of formulation, potassium salicylate can be prepared in the form of soluble liquid formulation (SL). On the other hand, it showed an alkaline property, which means that it requires an alkaline adjuvant for formulation.

Table (1): Physico-chemical properties of potassium salicylate as active ingredient.

Solubility % (W/V)			Free alkalinity as % NaOH
Water	Acetone	Xylene	
33	N.S*	N.S*	1.20

N.S*: means insoluble.

3.2. Physico-chemical properties of surface active agents:
Table (2) showed physico-chemical properties of three surface active agents, two nonionic namely Triton X-100 and Tween 40 and an anionic surfactant sodium lauryl sulfate (SLS). Their properties were studied to choose the suitable surface active agent that is compatible with physico-chemical properties of potassium salicylate as active ingredient. According to HLB values, the three surfactants have values greater than 13, so they were considered as dispersing agents. On the other hand

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	Free alkalinity as % NaOH
Tween 40	53.12	>13	0.3	-	1.53
Triton X-100	29	>13	0.2	-	0.02
Sodium lauryl sulfate	27.8	>13	8	-	0.48

all of them decreased the surface tension of water from 72 to 27.8, 29 and 53 dyne/cm for sodium lauryl sulfate, Triton X-100 and Tween 40 respectively. In addition all of them showed alkaline properties, as they recorded 0.02, 0.48 and 1.53, for Triton X-100, sodium lauryl sulfate and Tween 40 respectively, from the above results it can be concluded that, all tested surface active agents were suitable to prepare potassium salicylate as soluble concentrate formulation.

3.3. Preparation of potassium salicylate as 20 % soluble liquid formulation (SL):

To determine the suitable wetting and spreading agent to prepare this formulation, the active ingredient was mixed with Tween 40, Triton X-100 and sodium lauryl sulfate with a concentration of 5 % and the surface tension was measured for each formulation diluted with water. Table (3) showed that, the mixture of active ingredient under study and Triton X-100 gave low surface tension when diluted at 0.5 % with water, than when it was mixed with Tween 40. On the other hand sodium lauryl sulfate was insoluble when mixed with the active ingredient in the suggested formula. The mixture of the active ingredient and Triton X-100 should be used since it decreased the surface tension compared with other mixtures and water.

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

Data in Table (4) showed that, there were no observable changes for the soluble concentrate local formulation before and after accelerated storage, as it showed nearly the same values for free alkalinity, surface tension and solubility with no sedimentation in both cases indicating the ability of the formulation to keep its properties in either normal or accelerated storage conditions.

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

Table (5) showed physico-chemical properties of spray solution at field dilution rate. It showed low surface tension, high viscosity, high electrical conductivity, no sedimentation and little alkaline pH value compared with water and the active ingredient. The increase or decrease in each physical property mentioned above determines one of the parameters related to the efficiency of spray solution. **Ryckaert *et al.*, 2007** stated that, the decrease in surface tension of pesticide spray solution gives a prediction of increasing spreading on the treated surface with a consequence increase in pesticidal efficacy. Also the increase in electrical conductivity of spray solution would led to deionization of insecticide, increase its deposits and penetration in the treated surface with a consequence increase in its insecticidal efficacy as stated by **Twifik and El-Sisi (1987).** It was also reported by **Spanoghe *et al.* (2007)** that, increasing viscosity of spray solution causes reduction drift, retention sticking with an increase of pesticidal efficiency.

Table (5): Physico-chemical properties of spray solution at field dilution rate.

Property	Surface tension dyne/cm	Viscosity cm/poise	Conductivity µ mhos	PH
Water	72	0.89	380	9.14
Potassium salicylate	67.5	5.4	50	14.12
Local formulation	31.76	9.27	900	8.15

Data in Table (6) showed the effect of potassium salicylate on the three parameters under study of wheat as monocotyledon under laboratory conditions, it showed direct proportion between concentration and percentage of inhibition on root and shoot growth, but the effect was greater in case of root than in case of shoot. The EC_{50}

Table (6): Effect of potassium salicylate on germination, root and shoot growth of wheat as pattern for monocotyledonous plants under laboratory conditions.

LCP line parameter	Root	Shoot	Germination
Slope	0.275	0.3	0.0
EC_{50}	30.96	46415	0.0
EC_{90}	1397830	85769589	0.0

Table (3) :Trials for the preparation of potassium salicylate as 20 % soluble liquid formulation.

Wetting and spreading agent (WSA)	WSA %	Solubility	Sedimentation	Surface tension 0.5 % dil. In water
Tween 40	5.0	soluble	Nil	36.8
Triton X-100	29	soluble	Nil	35.8
Sodium lauryl sulfate	27.8	N.S*	N.C*	N.C

N.S* :means insoluble N.C* :means uncalculated.

Table (4): Physico-chemical properties of local 20 % soluble liquid formulation before and after accelerated storage.

Before storage				After storage			
Surface tension dyne/cm	Free alkalinity as % NaOH	Solubility	Sedimentation	Surface tension dyne/cm	Free alkalinity as % NaOH	Solubility	Sedimentation
31.75	0.4	soluble	Nil	28	0.32	soluble	Nil

values for root and shoot were 30.96 and 46415 ppm respectively. But it showed no inhibition effect on germination.

The previously shown results from Tables (6) and (7) declared clearly that potassium salicylate affects markedly root and shoot growth of monocotyledonous plants and root growth of dicotyledonous plants, which supports considering potassium salt as active ingredient and could be formulated to apply in further studies. Data presented in table (7) showed the effect of potassium salicylate on radish as dicotyledon under laboratory conditions. The EC_{50} value for root growth was 1618 ppm, but it showed no inhibition neither on germination nor on shoot growth.

Table (7): Effect of potassium salicylate on germination, root and shoot growth of radish as pattern for dicotyledonous plants.

LCP line parameter	Root	Shoot	Germination
Slope	4.4	0.0	0.0
EC_{50}	1618	0.0	0.0
EC_{90}	3001	0.0	0.0

Table (8) showed the effect of the 20 % soluble concentrate formulation of potassium salicylate on the three parameters under study of wheat under laboratory conditions .It inhibited markedly the growth of root, shoot and germination as their EC_{50} values were 251, 562 and 727 ppm respectively.

Table (8): Effect of 20 % soluble concentrate formula- tion of potassium salicylate on germina-tion, root and shoot growth of wheat as pattern for monocotyledonous plants under laboratory conditions.

LCP line parameter	Root	Shoot	Germination
Slope	3	2.8	2.4
EC_{50}	251	562	727
EC_{90}	670	1611	1918

Table (9) showed the effect of potassium salicylate formulation on germination, root and shoot growth of radish under laboratory conditions. It inhibited markedly root and shoot growth which appeared clear from their EC_{50} Values as it were 158.4 and 368 ppm for root and shoot respectively, but it showed no inhibition effect on germination.

Data in Table (10) clearly showed the role of formulation in increasing or decreasing the effectiveness of the active ingredient (potassium salicylate) on mono and dicotyledonous plants. The formulation increased the effectiveness of its active ingredient except for root growth of wheat, according to EC_{50} values; the formulation was more effective than its active ingredient by 98.7 and 100 % for shoot growth and germination respectively. On the other hand, it showed greater effect on comparison to its active ingredient, which clearly appeared from the EC_{50} values, the increase in effectiveness was 90.2 and 100 % for root and shoot growth respectively in case of radish.

The LCP line of the soluble concentrate formulation against germination, shoot and root growth was sharp, slope values were 2.4, 2.8, and 3 respective to germination, root and shoot of wheat. Similarly, in case of radish, the slope values for shoot and root growth were 3 and 1.5 respectively.

On comparing potassium salicylate as active ingredient and its 20 % soluble concentrate formulation. In case of monocotyledonous plants, the formulation showed inhibition effect on germination while the active ingredient itself did not show any inhibition on germination. On the other hand, the formulation inhibited markedly shoot growth, even although the effect of formulation on root growth was lower than its active ingredient, it was considered as a good result as the EC_{50} value of formulation was 251 ppm. Taking the same idea into consideration, the formulation inhibited markedly both root and shoots growth, the results that appeared clear from EC_{50} and slope values of dicotyledonous plants. The previously reported results may be explained on the basis of the role of surfactants. Surfactants (derived from surface active agent) may affect all components of the spray system, from solubilization of the a.i of the spray solution, to penetrate and transport of the a.i via the plant however, from the growers perspective, the primary use are to improve spray wetting and a.i penetration **(Bukovac *et al.*, 2003).**

Under greenhouse conditions wheat and radish plants were sprayed by two concentrations 10000 and 20000 ppm from the spray solution. Both plants died

Table (9): Effect of 20 % soluble concentrate formulation of potassium salicylate on germination, root and shoot growth of radish as pattern for dicotyledonous plants under laboratory conditions.

LCP line parameter	Root	Shoot	Germination
Slope	1.5	3	0.0
EC_{50}	158.4	368	0.0
EC_{90}	1130	984	0.0

Fig.2: Effect of spray solution on radish after three days from treatment by 10000 and 20000 ppm under greenhouse conditions.

Table (10): EC_{50} and slope values for potassium salicylate (active ingredient), its 20 % soluble concentrate formulation and the percentage of increase or decrease in efficiency for both plants under study.

Plant Kind	Monocotyledonous plant						Dicotyledonous plant					
Compound / Parameter	Germination		Shoot growth		Root growth		Germination		Shoot growth		Root growth	
	EC_{50}	Slope	EC_{50}	Slope	EC_{50}	Slope	EC_{50}	Slope	EC_{50}	Slope	EC_{50}	Slope
Active ingredient	-	-	46415	0.3	30.96	0.275	-	-	-	-	1618	4.4
SL formulation	727	2.4	562	2.8	251	3	-	-	368	3	158.4	1.5
Increase or decrease in effectiveness	100 %		98.7 %		*		-		100 %		90.2%	

- : means uncalculated. *: means decrease the effectiveness

Fig.3: Effect of spray solution on wheat after seven days from treatment by 10000 and 20000 ppm under greenhouse conditions.

completely, after three days in case of radish (dicotyledonous plant) and seven days in case of wheat (monocotyledonous plant). These results were consistent with that previously obtained under laboratory conditions.

Conclusion and Recommendation

It could be concluded that, the preparation of potassium salicylate as soluble liquid formulation showed high herbicidal effect against both mono and dicotyledonous plants when sprayed at 10000 and 20000 ppm in greenhouse experiment, Moreover it inhibited germination, root and shoot growth of monocotyledonous seeds, and root and shoot growth of dicotyledonous seeds, Therefore it could be used for controlling monocotyledonous weed seeds as pre-emergence treatment and dicotyledonous plants as post - emergence treatment. Since formulated potassium salicylate showed this effect in laboratory and greenhouse experiments, therefore semi-field and field experiments should be carried out in field in different crops for controlling different weeds.

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الكفاءة الإبادية للمادة الدوائية سلسلات البوتاسيوم بمفردها ومستحضرها المركز القابل للذوبان في الماء بتركيز 20 % على النباتات ذوات الفلقة الواحدة والنباتات ذوات الفلقتين

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

الملخص العربى:

تم تجريب سلسلات البوتاسيوم على ثلاثة عوامل نباتية (الإنبات ونمو الجذرونمو الساق) فى نباتى القمح والفجل كأمثلة للنباتات ذوات الفلقة الواحدة والنباتات ذوات الفلقتين تحت ظروف المعمل. أَظهرت سلسلات البوتاسيوم تأثيرا مثبطا ملحوظا على نمو الجذر والساق فى حالة القمح ونمو الجذر فى حالة الفجل تحت ظروف المعمل ، لذلك أُعتبرت مادة فعالة وجُهزت فى صورة سائل قابل للذوبان فى الماء بتركيز 20 % وإجتاز المستحضر الجديد الإختبارات الفيزيائية والكيميائية التى حددتها المنظمات الدولية المتخصصة فى مجال المبيدات ثم جُرب المستحضر الجديد على النباتين تحت الدراسة تحت ظروف المعمل فزادت الفعالية بنسبة 98.7 و 100 % للساق و الإنبات على الترتيب فى حالة النباتات ذوات الفلقة الواحدة كما زادت أيضا الفعالية على النباتات ذوات الفلقتين بنسبة 90.2 و 100 % للجذر والساق على الترتيب مقارنة بالمادة الفعالة. كما تمت معاملة النباتين بمحلول الرش تحت ظروف الصوبة الزجاجية فماتت النباتات ذوات الفلقتين كليا بعد ثلاثة أيام من المعاملة وذوات الفلقة الواحدة بعد اسبوع من المعاملة.