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Comparative Studies between the Locally Formulated Abamectin and Agremic Gold 8.4% SC Using Physical, Chemical and Biological Parameters against Two-Spotted Spider Mite, *Tetranychusurticae*

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Abstract: ***The present study was carried out to investigate the potential activity of abamactin, locally formulated as 8.4% SC.The prepared formulation's physical and chemical properties were performed with comparison to Agremic Gold 8.4% SC at different accelerated storage conditions to predict the stability of the formulations. Moreover both were evaluated their toxicity studies (lethal concentrations) against adult T. urticae under laboratory conditions. Different parameters, pH, free acidity, viscosity, density, specific gravity, surface tension and persistent foam were determined. The local formulation exhibited good stability initially and at different storage conditions. The effect of the local formulation on two-Spotted spider mite, Tetranychusurticae tested under laboratory conditions with comparison to the recommended acaricide Agremic gold 8.4% SC. The results showed that the prepared formulation had competitive acaricidal activity against two-Spotted spider mite, Tetranychusurticae. The LC_{50} of the locally prepared formulation recorded 0.0141 ppm, where it was 0.0102 ppm for Agremic gold. The toxicity index of the locally prepared formulation was 72.43% rather than Agremic gold.***

Keywords: Abamectin, Agremic gold, Physico-chemical properties, Chemical parameters, *Tetranychusurticae*, Bioassay, Leaf disk dip assay

1. Introduction

Phytophagous mites are among the most common plant pests, responsible for significant yield shortages in many economically important crops, such as fruits, cotton, vegetables and ornamentals. One of the most important species of mite is the two-spotted spider mite (TSSM), *Tetranychusurticae* Koch (Acari: Tetranychidae) which is an economically important pest of many field and greenhouse crops of the world. It is probably the most important species in the family Tetranychidae associated with 900 plant species **[1, 2]**. It affects crops by direct feeding, reducing the area of photosynthetic activity and may result in leaf abscission in severe infestation **[3].** It is reported to cause significant economic losses in tomatoes, cucumber, peppers, roses, and beans.

The avermectins are a family of macrocyclic lactones, produced by the soil organism *Streptomyces avermitilis*, which were discovered in the mid-1970's as a direct result of a screening effort for natural products with anthelmintic properties. Avermectin B_1 (abamectin), the major component of the fermentation, also showed potent activity against arthropods in preliminary laboratory evaluations and was subsequently selected for development to control phytophagous mites and insect pests on a variety of agricultural and horticultural crops worldwide. Abamectin has shown low toxicity to non-target beneficial arthropods, which has accelerated its acceptance into Integrated Pest Management (IPM) programs **[4]**.

Suspension concentrate technology has been increasingly applied to the formulation of many solid crystalline pesticides since the early 1970‟s. Pesticide particles maybe suspended in an oil phase, but it is much more usual for suspension concentrates to be dispersions in water**[5]**. Considerable attention has been given in recent years to the production of aqueous suspension concentrates by a high energy wet grinding processes such as bead milling. The use of surfactants as wetting and dispersing agents has also led to a great deal of research on the colloidal and surface chemistry aspects of dispersion and stabilization of solid/liquid dispersions **[6].** Water-based suspension concentrate formulations offer many advantages such as high concentration of insoluble active ingredients, ease of handling of application, safety to the operator and environment, relatively low cost and enable water-soluble adjuvants to be built-in for enhanced biological activity**[7].**

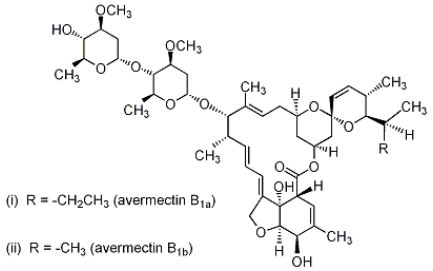
Therefore, in the present study acaricide namely abamactin, was locally formulated as 8.4% SC, the prepared formulation's physical and chemical properties were studied in comparison with Agremic Gold 8.4% SC then both were evaluated for biological studies (lethal concentrations) against *T. urticae* under laboratory conditions.

2. Materials and Methods

Materials

1. Pesticides Used

Agremic formulation (8.4% abamectin) was provided by Syngenta companyand new formulation of abamectin at the same concentration was prepared in our laboratory.The abamectin active ingredient were supplied from Kafr El-Zayat Company for Chemicals and Pesticides, Egypt.



AbamectinA mixture containing ≥80% avermectin B_{1a} (i) and ≤ 20% avermectin B_{1b}.**Mol. wt.** 873.1 (avermectin B_{1a}); 859.1 (avermectin B_{1b}) **M.f.** $C_{48}H_{72}O_{14}$ (avermectin B_{1a}); $C_{47}H_{70}O_{14}$ (avermectin B_{1b})

Methods

1) Preparation method of abamectin formulation

Approximately8.8 gram of abamact in 95% active ingredient added to 75 ml of deionized water with stirring. After 10 minutes, 10 ml of wetting agent were added drop wise. Then, the mixture was transferred to a mix miller with a glass center 0.2 cm. Polyethylene glycol as a thickener, sodium lignosulfonate as a dispersing, anticoagulant agent and silicon oxide as a filler were added respectively every 15 minutes. Finally, 1 ml of pH modifier was added to the mixture. After 1 hour, the mixture was filtered and completed to 100mlin a measuring cylinder. The obtained mixture was SC formulation of abamactin 8.4%.

2) Physical Parameters

a) Physical Properties of Formulation:

The physical properties of both Agremic gold and prepared formulation 8.4% SC were initially carried out and after storage at 0°C for 7 days followed bystorage at 54 ± 2° for 14 days to detect storage stability at elevated temperature **(CIPAC MT. 39.3 and 46.3) [8]**. In addition, the physical properties of the formulations were done as follow:

Test	Instrument	Test Method
Acidity	Automatic Titrator, (Hanna model HI 901)	CIPAC MT 191 [9]
pH	pH Meter (Jenway model pH 3510)	CIPACMT 75.3 [8]
Viscosity	Brookfield Viscometer DV-II + Pro, USA	ASTM (D 2196 – 15) [10]
Surface Tension	"Sigma 700" by du Noüy Ring, a platinum/iridium ring	ASTM (D 1331 – 14) [11]
Density & Specific Gravity	Rodulph Densitometer (DDM 2910, USA)	ASTM (D 4052 – 11) [12]

b) Physical Properties of Spray Solutions

The physical properties of 5% spray solutions of Agremic gold and prepared formulation 8.4% SC in both soft and hard water; persistent foamand theconductivity **(CIPAC MT 47.2 andMT 32) [13]**besides bothofpH **(CIPACMT 75.3) [8]**and surface tension were recorded**[11]**.

3) Chemical Parameter

Determination of the active ingredient percentages in both abamectin formulations were determined before and after both cold and hot storage conditions by liquid chromatography.

- **Reagents**

Water LC grade, (deionized water), methanol/acetonitrileLC grade and Abamectin.

- **Preparation of standard solution**

10 mg (related to purity of 100%) from abamectin standard of acaricidewere weighedinto a 10 ml volumetric flask, (hint: another weight in different volume would be used but with the same equivalence). After dilution with methanol LC grade to the mark, the resulting solution has to checked well to prepare standard solution.

- **Calibration**

Calibration for the HPLC is usually carried out at concentrations related to that of sample found in formulation sample. Inject abamectin standard solution onto HPLC column. Ensure reproducibility of injections to obtain abamectin retention time. Ensure linearity of standard injections with serial dilution. Using practice ensure baseline separation of abamectin.

- **Method of determination the thermal stability of 8.4% abamectin SC**

8.4% abamectin SC was firstly stored at (54 ± 2) °C for 14 days and at (0 ºC) for 7 days. Then, the decomposition rates of SC samples were determined by high performance liquid chromatography. All samples were measured three times in the experiment period and the mean was taken eradicate any discrepancies. Chromatographic conditions: the mobile phase: acetonitrile/water =90:10 (V/V), reagent with ultrasonic degassing filter, flow rate: 1/ml/min, column temperature: 25°C, injection volume: 5µL, the detection wavelength: 245nm, column: Luna C18 reversed-phase column. The amount of abamectin was determined by comparison to external standard solution. All reagents were HPLC grade **[14].**

- **HPLC Instrument**

The type of chromatographic HPLC system model (Agilent Technologies 1260 Infinity) with Quaternary pump, UV-detector was employed. Chromatographic C18 stainless steel

column (25 cm length, 4.6 mm inner diameter, and 4.0 µm particles).

4) Bioassay techniques

a) Rearing of two-spotted spider mite:
The mites, *T. urticae* used in the study were obtained from our laboratory where it was reared for more than 40 generations on the *Acalyphamarginata* plant at 25±2°C and 60 ± 10% RH and 14:10 hr photoperiod (Light:Dark). Fresh plants were supplemented at regular intervals to maintain the culture for experimentation**[15].**

b) Leaf disk dip assay
The leaf disk assay was conducted using method described **[16]**. The *Acalyphamarginata* leaf disks (2.5 cm diameter) were dipped in series concentrations of tested formulations (0.0195, 0.0391, 0.1563, 0.3125, 0.625 and 1.25 µg/ml) for 20 seconds. Concentration mortality relation were based on tests with six concentrations of each formulation, and were expressed as parts per million of active ingredient. The discs were put on wet cotton wool in petri-dish and kept under constant conditions ((25 ± 2 ºC, 65 ± 5% R.H. and 16:8 photoperiod). Ten adult females (one day old) of *T. urticae* were transferred on each disk with a fine brush. Control disks were dipped in distilled water. Tests were repeated 3 replicates for each concentration of each formulation. Mortality of mites treated with formulations in all tests was assessed after 24 hr. Mortality data was corrected according to **Abbott formula [17]** and the corrected mortality percentage of each acaricide was statistically computed **[18].** The corresponding concentration probit lines were also estimated to determine the 50 and 90% mortalities. Slope values of the tested acaricides were recorded. The efficiency of tested formulations was measured by a comparison method and the toxicity was calculated according to the equation **[19],**

$$\text{Toxicity Index} = \frac{\text{LC50 of the most effective } compound}{\text{LC50 of the tested compound}} x100$$

3. Results and Discussions

3.1 Physical Properties

3.1.1 Physical Properties of the formulations

The most important parts of chemical stability are performances on accelerated storage and kinetics of pH profiles **[20]**. The prepared formulation exhibited acidic pH value. The pH values of the prepared formulation were in range (5.34–5.95) and the free acidity values were in range of (0.017–0.251 % as H_2SO_4), where the pH's of Agremic gold were in range (5.34 – 5.43) and the free acidity values were in range of (0.119 – 0.252 % as H_2SO_4). Both formulations indicated that they have acidic character implying that it will have good biological activity**[21].**Both of the prepared formulation have the surface tension range (32.815 – 34.97mN/m) and the Agremic Gold surface tension range (36.22–39.21 mN/m). Lower surface tension is a desirable character for most agricultural sprays because it facilitates the spreading of droplets upon impaction on leaves or other target surfaces in order to increase the surface-active area and to improve penetration and up taking of the product into the plant **[22]**. It was mentioned that the deposits on treated plant leaves were increased by decreasing the surface tension and decreasing the pH values **[23]**. A change in the pH on storage can give an indication of instability of the active substance or product **[24].**

The densities of the formulation were ranged from 0.9778 to 0.9834gm/cm^3 and specific gravities range were 0.9807–0.9864.Whereas, the densities of Agremic gold range were 1.0222–1.0367 gm/cm^3 and specific gravities range were 1.0253–1.0398. The data obtained from densities and specific gravities of both prepared formulation and Agremic gold showed no valuable change according to different storage conditions. Viscosities of prepared formulation were in range (706.56–1175.51cP.), whereas the viscosities of the Agremic gold were in range (855–971.79 cP.). Increasing viscosity of spray solution caused reduction drift and an increase in retention sticking and pesticidal efficiency **[25]**.

Table 1: Physical Properties of Agremic Gold and Local Formulation 8.4% SC at initial, after storage at 0 °C for 7 days and after accelerated hot storage at 54 ± 2 °C for 14 days

	Initial		Cold Storage at 0°C for 7 days		Accelerated hot storage at 54°C for 14 days	
	Local Formulation*	Agremic Gold*	Local Formulation*	Agremic Gold*	Local Formulation*	Agremic Gold*
pH	5.95 ± 0.35	5.43 ± 0.28	5.34 ± 0.37	5.53 ± 0.29	5.34 ± 0.37	5.59 ± 0.31
Acidity (% as H_2SO_4)	0.251 ± 0.011	0.252 ± 0.011	0.021 ± 0.002	0.119 ± 0.005	0.017 ± 0.003	0.129 ± 0.006
Surface Tension (mN/m)	33.65 ± 1.3	36.74 ± 1.06	34.97 ± 0.98	39.21 ± 1.07	32.815 ± 0.91	36.22 ± 0.99
Density (gm/cm^3)	0.9778 ± 0.0013	1.0346 ± 0.0014	0.9824 ± 0.0013	1.0222 ± 0.0014	0.9834 ± 0.0013	1.0367 ± 0.0014
Specific Gravity	0.9807 ± 0.0013	1.0377 ± 0.0014	0.9854 ± 0.0013	1.0253 ± 0.0014	0.9864 ± 0.0013	1.0398 ± 0.0014
Viscosity (cP.)	1088.4 ± 70.6	855.08 ± 54.5	1175.51 ± 75	971.79 ± 61.9	706.56 ± 45.1	965.19 ± 61.6

*** Results ± uncertainty**

3.1.2 Physical Properties of Spray Solutions

Table (2) showed the physico-chemical properties of spray solutions of the prepared formulation in comparison with Agremic gold 8.4% SC in both soft and hard water at different storage conditions. The conductivity of spray solution of the prepared formulation, showed the highest value 585 µMHOS in hard water after cold storage while the highest conductivity value of spray solution of Agremic gold was 559 µMHOS in

hard water after hot storage. The highest value of surface tension of the prepared formulation were 29.52 mN/m in soft water of the initial sample, while the highest value of the surface tension of the Agremic gold was 33.82 in soft water of hot stored sample. The highest acidic value of the prepared formulation was recorded pH 4.48 in hard water of the initial sample, whereas, the highest acidic value of Agremic gold was recorded pH 4.73 in hard water of initial sample also. Increased electric conductivity of the spray solution coupled with increased mortality rateare referred to the increasing in the deposition and penetration of the formulated particles **[26].** It was also shown that the decrease in surface tension of pesticidal spray solution gives a prediction of increasing wettability and spreading over tested surface with increasing pesticidal efficiency **[27].**It is interesting to note that; pH can affect a pesticides chemical breakdown (hydrolysis) in spray solutions. It has been documented that certain insecticides degrade or undergo hydrolysis faster in water with a high pH. For example, if the water supply is alkaline, especially at pH ≥ 8 and the applied pesticide is sensitive to hydrolysis. Therefore, the pH of the water should be lowered in the spray tank **[28].** On the other hand, it is well-documented that decreasing in the pH value of spray solution would lead to the deionization of the content which increase in its deposit's and penetration in the tested surface with a consequence increase in their pesticidal efficiency **[29]**. It is also worthy to mention that cold and hot storage has no valuable effect on all tested physical properties of the prepared formulation.

Table 2: Physical Properties of spray solutions (soft and hard water) of Agremic Gold and Formulation 8.4% SC at initial, after storage at 0°C for 7 days and after accelerated hot storage at 54 ± 2°C for 14 days.

	Agremic Gold 8.4% SC						Local Formulation 8.4% SC					
	Initial		Cold storage		Hot storage		Initial		Cold storage		Hot storage	
	SW	HW	SW	HW	SW	HW	SW	HW	SW	HW	SW	HW
Foam (ml)	3	3	2	2	1.5	1.5	3	3	4	4	4	4
Surface Tension (mN/m)	29.0	29.43	32.49	32.51	33.82	33.26	29.52	29.42	29.15	28.98	29.00	29.04
pH	4.94	4.73	5.16	5.14	5.25	5.18	4.54	4.48	5.61	5.87	5.09	5.51
Conductivity (µMHOS)	167.7	555	153.1	552	162	559	182	583	193.2	585	203	579
Salinity (‰)	0.1	0.3	0.1	0.3	0.1	0.3	0.1	0.3	0.1	0.3	0.1	0.3

3.2 Chemical parameters

The stability of heat storage of 8.4% abamectin SC was characterized with decomposition rate of abamectin in both Syngenta formulation (Agremic gold 8.4% SC) and local formulation at the same concentration. The decomposition rate of abamectin was determined by high performance liquid chromatography. The decomposition rate of abamectin in Agremic gold formulation was higher than the decomposition rate of abamectin in local formulation under hot and cold storage conditions. The decomposition rate was recorded at (13.29%-10.79%) for both formulations under hot storage conditions, while of the two formulations was recorded at (7.53% - 4.64%) for Agremic gold and local formulation under cold storage conditions, respectively **(Table 3)**.

Table 3: Active ingredient and decomposition percent of Agremic gold and locally prepared formulation 8.4% SC at different storage times

	The content of abamectin % Initial	Decom %	The content of abamectin % at hot storage	Decom. %	The content of abamectin % at cold Storage	Decom. %
Agremic Gold 8.4% SC	9.03	0	7.83	13.29	8.35	7.53
Local Formulation 8.4% SC	8.62	0	7.69	10.79	8.22	4.64

3.3 Biological Studies

The toxicity of the locally formulated 8.4% SC abamactin and Agrmic gold 8.4% SC to *T. urticae*using leaf disk dip assay represented in **(table 4)**. Based on LC_{50} values, results in the table indicated that Agremic gold was the most potent formulation, followed by the locally prepared formulation to adult females of *T. urticae* with LC_{50}values 0.0102 and 0.0141 ppm, respectively. Slope values of the log concentration-probitlines in (**table 4**) and **figure (1)** indicated that both Agremic gold and the locally prepared formulation are having close values. The toxicity index of the locally prepared formulation was 72.73% of the Agremic gold. Results indicated that abamectin was highly toxic to adult mites. Toxicities of abamectin to mites have been reported by various researchers; LC_{50} of abamectin was calculated as 0.34 ppm **[30]** which was higher than the LC_{50} (0.0135 ppm)**[31].** LC_{50} values abamectin against *T. urticae* calculated as 1.50 ppm **[32].**It was reported LC_{50} of abamectin as 0.17 ppm against susceptible strains of *T. urticae***[33]**.

Table 4: Toxicities of locally prepared formulation and Agremic gold 8.4% SC against *T. urticae*

	LC_{50} (ppm)	LC_{50} (fiducial limits, ppm)		LC_{90} (ppm)	LC_{90} (fiducial limits, ppm)		Slope	Toxicity Index
		Lower	Upper		Lower	Upper		
Agremic Gold 8.4% SC	0.0102	0.0005	0.0282	0.742	0.2803	11.9561	0.6885	100
Local Formulation 8.4% SC	0.0141	0.0053	0.0251	1.0526	0.5224	3.6438	0.6839	72.43

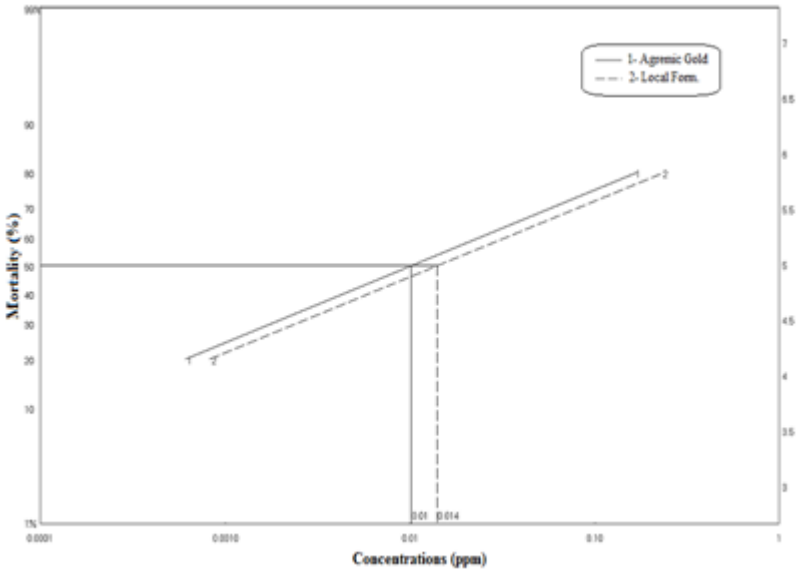


Figure 1: Ldp lines of Agremic gold and local formulation 8.4% SC against *Tetranychusurticae*

4. Conclusion

Herein, a comparative study between the standard Agremic gold and Abamectinsuspension concentrate formulation was done by implying different parameters such as pH, free acidity, viscosity, density, specific gravity and surface tension under different storage conditions. Remarkably, the prepared formulation showed astonishing and promising potency. Also, it is anticipated that for future assessments of that formulation as an acaricide would exhibit better efficiency in the greenhouse conditions.

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