Degradation of Neem Oil 90% EC (AZADIRACHTIN) under Storage Conditions and its Insecticidal Activity against Cotton Leafworm *S. Littoralis*

Olfat, A. Radwan[1] and El-Shiekh, Y. W. A.[2]

Departments of [1]Pesticides Analysis and [2]Pesticides Formulation, Central Agricultural Pesticides Laboratory, Agricultural Research Center, Dokki, Egypt
yasserwahied@yahoo.com

Abstract: This study was undertaken due to our concern for determining the degradation of active ingredient of pesticides during storage condition. Neem oil 90 % EC was stored under different conditions. The results obtained during physical and chemical studies indicated that, azadirachtin active ingredient of Neem oil 90 % EC was degradated due to sunlight storage (outdoor) for 14 days by 16.6% of its values while, the accelerated hot storage at 72 ± 2ºC for 3 days gave the maximum degradation (26.6 %). The physical parameters showed a clear changing of viscosity, surface tension and acidity. Also, their spray solutions for both soft and hard water were failed because there were access foam more than 10 ml and sedimentation appeared more than 3 ml. By extension, the insecticidal activity of neem oil 90 % EC was also decreased for hot storage at 72 ± 2 ºC for 3 days and outdoor storage samples, their LC_{50} were 1521.6 and 797.7 ppm, respectively. The toxicity indexes were 19.9 % and 37.9 % with the references to the initial samples. Finally, we can presented the fact that, the outdoor (sunlight) and accelerated hot storage at 72 ± 2 ºC affect on the neem 90% EC sample so it must be protected from them which make degradation for azadirachtin active ingredient and break the formulation of emulsifiable concentrate for neem oil.
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Key words: Azadirachtin, Neem oil 90% EC, indoor (sunlight) and outdoor storage, physical or chemical properties, IR spectra, Trilogy.

1. Introduction

Azadirachtin obtained from neem tree (*Azadirachta indica*. A. Juss (family: Meliaceae)) is one of the most important biopesticide currently in use. The broad spectrum activity of azadirachtin at very low concentration coupled with the unique mode of action and non-toxicity to mammals make azadirachtin an ideal candidate for insecticidal use. Since the advent of DDT chemical pesticides have been controlling the pest problem in some of the crop system very efficiently but due to their extreme persistent, bioaccumulation, toxicity towards non-target beneficial organism, tendency to cause malignancy and increasing development of insecticidal resistance has created the serious threat to crop protection program all over the world, hence in recent years instead of the use of neurotoxic, broad spectrum, synthetic pesticides much attention is being paid towards more specific, bioactive, biodegradable environmental friendly plant based pesticide. Azadirachtin only effects the insects that consume it thus other friendly insects, predators and parasites and species which may help in pollination and other plant functions are not harmed, it quickly biodegrades by sunlight. The first commercial neem insecticide, Margosan-O was registered by the environmental protection agency (EPA) in 1985 for use on non foods crop since then various other products based on azadirachtin are being formulated and sold by a large number of companies **(Jacbson, 1988)**.

All commercial neem insecticidal formulations and other product based on azadirachtin contain azadirachtin which is extracted from the seeds of naturally grown whole plant **(Yamaski *et al.*, 1986 and Govindachari *et al.*, 1990).** This approach has the disadvantages of heterogeneity in azadirachtin content depending upon the plant genotype and environment.

Pesticides may fail to comply with the **Anonymous (2010)** meeting specifications required if is improperly stored. Chemical and physical instability usually lead to the deterioration of the active ingredient content and emulsion stability under variable climatic conditions as well as several cases. The objectives of this study aimed to, (1) study the effect of different storage conditions of temperature and sunlight on neem oil 90% EC formulation; (2) determinate the physical properties of both the formulation and the spray solutions of all samples and (3) to evaluate the insecticidal activity of all samples on the cotton leafworm.

2. Material and Methods

I- Insecticide:

Trade name: Trilogy oil 90 % EC.

Chemical Class: hydrophobic extract neem oil.

Common name: hydrophobic extract neem oil.

The sample of Trilogy oil 90 % EC was gained from the local market and divided into five parts, everyone was 25 ml. The samples were stored as follow:

Indoor: 25 ml of neem oil sample was measured in Petri dishes (15cm) and kept at room condition for 14 days.

Outdoor: 25 ml of neem oil sample was measured in Petri dishes (15cm) and kept out door to be exposed to sun light directly for 14 days.

Hot storage: Samples of neem oil formulation were stored in glass package, in the oven at 54 ± 2ºC for 14 days according to **Anonymous (2010)** and at 72 ± 2ºC for 3 days according to **WHO specifications (1979).**

II- Chemical parameters

a. The effect of temperature on the variation of the active ingredient percentage:

The percentage of azadirachtin as active ingredient of neem oil 90% EC formulation was determined before and after storage by high performance liquid chromatography (HPLC) instrument according to **Dorbat and Martijn (2005).**

b. Infrared absorbance of neem oil 90% EC.

The Fourier transform infrared (Avtar 330 Thermo Nicolet) was used to study the effect of storage on the absorbance of function groups and finger print of neem oil formulation according to the method of **Barbara (1985)** with some modification. Samples were prepared by homogenized 0.01g of sample with 0.1 g of dry (KBr) by agatemortar and pests to a clean stainless steel slide and placed in piston to make a clear and thin film of desk sample.

III- Physical parameters

The physical properties of the neem oil 90% EC samples and its spray solutions in soft and hard water were carried out according to CIPAC handbook methods. The methods were acidity/ or alkalinity (MT 31), pH (MT 73), conductivity (MT 32), density & specific gravity (MT 3.1), persistent foam (MT 47.2) and emulsion stability (MT 36.3) (**Dobrat and Martijn, 1995)**. Also surface tension, refractive index, viscosity and flash point were carried out according to American Society of Testing and Materials **(ASTM 2001, 2002, 2005 and 2007).**

IV- Bioassays

Newly moulted 4th instars of cotton leaf worm larvae (*S. littoralis*) were exposed to Neem oil 90% EC formulation using the leaf dipping technique (**Ahmed, 2009)**. Concentrations of 100, 200, 400, 800 and 1600 ppm from the tested insecticides were prepared using distilled water. Castor leaves were thoroughly washed and dried under laminar flow. 5 cm^2 of castor leave discs were cut and dipped into each insecticide concentration for 10 s with gentle agitation and then allowed to dry on towel on both sides. After drying, one leaf disc was placed in a sterile 9-cm-diameter Petri dish. Ten larvae were released on to each leaf disc. Three replicates of 10 larvae were used for each concentration. The same number of leaf discs per treatment was dipped into distilled water as an untreated check. All Petri dishes were kept in a sterile culture room with environmental simulating of 25 ± 2 ºC, relative humidity of 60 ± 5 %.

V- Statistical analysis:

Morality was scored 48 h after the larvae were placed on treated leaf discs. Larvae were considered dead if they showed no sign of the movement. Data were corrected for control morality **(Abbott, 1925.)**. The corrected percentage of growth inhibition was used to calculate the LC_{50} values according to **Finny (1971)**. Toxicity index was calculated according to **Sun (1950).**

$$Toxicity \quad \text{Index} = \frac{LC_{50} \text{ of the most effective sample}}{LC_{50} \text{ of the sample}} \times 100$$

3. Results

I- Effect of storage temperatures on chemical properties:

(A)Effect of storage temperatures on percentage of azadirachtin as active ingredient of neem oil 90% EC formulation :

The data summarized in table (1) showed that persistence of active ingredient % of azadirachtin in neem oil formulation was affected by storage condition and exposure periods. The data indicated that azadirachtin stored inside door at room temperature, out door in sunny place and in the oven at 54ºC ± 2ºC for 14 days was stable while storage at 72± 2ºC accelerated the chemical decomposition whereas the azadirachtin active ingredient percentage was represent a 0.30% of the zero time sample, our result in agreement with **Carter *et al.*, (1991)** who investigated the storage stability of azadirachtin in formulations and found that degradation in neem formulations depends greatly on the ration of protic to aprotic solvents. In particular, the presence of water in the formulation speeds up the decomposition of the azadirachtin, for that the azadirachtin in the oil formulation is more stable and **Oscar *et al.*, (2010)** indicated that, the UV radiation may also affect the % of azadirachtin present in neem oil.

Table (1): Effect of storage conditions on azadirachtin percentage as active ingredient of Neem oil 90% EC formulation

	Active ingredient %	% degradation
Zero time	0.3	----
In door at 25ºC for 14days	0.28	6.67
Outdoor for sunlight for 14 days	0.25	16.67
54 ± 2ºC for 14 days	0.27	10
72 ± 2ºC for 3 days	0.22	26.67

All values are a mean of three replicates of samples.

(B) Effect of storage temperatures on the absorbance of neem oil 90% EC formulation by infrared:

The infrared spectrum of azadirachtin analysis and effect of different type of storage on the absorbance is presented in table (2). After storage at room temperature, in sunny place, at 54± 2ºC and 72 ± 2ºC and results showed that the percentage of match were 100, 100 and 98 and 98 %, respectively.

Table (2): Effect of storage conditions on IR absorbance spectrum of Neem oil 90% EC formulation

Initial time	Room temp.	Sunny place	54 ± 2ºC	72± 2ºC
722.10	722.10	722.10	722.11	722.10
1115.89	1115.89	1115.89	1115.89	1115.89
1167.07	1167.07	1167.07	1167.07	1167.07
1239.92	1239.92	1239.92	1239.92	1239.92
1377.68	1377.68	1377.68	1377.68	1377.68
1465.28	1465.28	1465.28	1465.27	1465.28
1743.56	1743.56	1743.56	1743.56	1743.56
2853.99	2853.99	2853.99	2853.97	2853.99
2924.90	2924.90	2924.90	2924.90	2924.89
3442.54	3442.54	3442.54	3442.56	3442.54
Match %	**100**	**100**	**98**	**96**

II- Effect of storage conditions on physical properties:

1.1. Physical parameters of neem oil 90% EC formulation Samples:

Data in table (3) illustrate the physical properties of neem oil 90% EC samples at 0 time before storage, indoor, outdoor for 14 days and accelerated hot storage for (54 ± 2 ºC for 14 days and 72 ± 2 ºC 3 days), respectively.

1.1.1 Density, Specific Gravity and Refractive Index:

The physical properties density, specific gravity and refractive index of samples initial, indoor and 54 ± 2 °C not varied during the conditions of storage while outdoor storage sample gave a moderate variation and the last sample (storage at 72 ± 2 °C) gave the maximum variation.

1.1.2 Acidity (%):

Acidity of neem oil 90% EC had the minimum value 0.52% for 72 ± 2 °C after 3 days samples followed by outdoor sun light storage for 14 days samples 0.73% expressed as % of H_2SO_4 while there was no significant change for the other 3 samples.

1.1.3. Viscosity (cp):

The viscosity of the five samples had a significant change, the maximum decrease of viscosity was 420.1 cp of samples stored at 72 ± 2 °C for 3 days followed by outdoor storage at the sun light samples 425.6 cp, respectively where no significant change in viscosity of the initial, indoor samples and hot storage samples at 54 ± 2 °C for 14 days.

1.1.4. Surface Tension (dyne/cm):

The surface tension of samples hot storage at 72 ± 2 °C for 3 days and outdoor (sunlight) was decreased and their values were 30.9 and 31.3 dyne/cm, respectively. While the surface tension of the other samples didn't changes with the reference to the initial samples.

1.1.5. Flash point (°C):

All the samples make a flash point over 75 °C during different storage conditions.

Table (3): Physical parameters of neem oil 90% EC samples at different storage conditions

	Acidity (% as H_2SO_4)	Density (gm/cm^3)	Specific Gravity	Viscosity (cp.)	Surface Tension (dyne/cm)	Refractive Index	Flash Point
Initial	0.98	0.93	0.85	431.2	32	1.5327	> 75
Indoor for 14 days	0.98	0.93	0.85	431.5	32.1	1.5329	> 75
54 ± 2 °C for 14 days	0.90	0.95	0.84	433	33	1.5330	> 75
Outdoor (sunlight) for 14 days	0.73	0.88	0.79	425.6	31.3	1.5399	> 75
72 ± 2 °C for 3 days	0.52	0.83	0.77	420.1	30.9	1.5422	> 75

1.2 Physico-chemical properties of neem oil 90% EC samples spray solution:

The data presented in table (4) illustrated the physico-chemical properties of neem oil 90%EC samples spray solution (soft and hard) water samples at 0 time before storage, indoor, outdoor for 14 days and accelerated hot storage for (54 ± 2 °C for 14 days and 72 ± 2 °C 3 days), respectively. We concluded that, the spray solutions (soft and hard water) for samples initial, indoor and 54 ± 2 °C for 14 days were acceptable due to their values where no significant change between each other, no sedimentation or excess foam more than 10 ml were appeared. pH values varied in rang 6.5 – 6.7 for soft water and 7.3 – 7.6 for hard water. By extension, conductivity values varied in range 185 – 195 µs for soft water and 380 – 400 µs for hard water samples. Also, the salinity of soft water samples was 0.1 ‰ where it was 0.3 ‰ for hard water samples. The surface tension of soft water samples was changed in range 0.3 dyne/cm and 0.4 dyne/cm for hard water samples. The spray solutions of initial, indoor and hot storage at 54 ± 2 °C for 14 days samples were stable and didn't affected by storage conditions. Whereas, the spray solution of samples outdoor and hot storage at 72 ± 2 °C for 3 days were failed, where there were excess foam appeared more than 10 ml and also failed in the emulsion stability where there were about 3ml sedimentation in all samples. The surface tension of these samples was changed strongly which reflected that the break of the formulation of the samples due to the effect of heat and sunlight of the samples.

Table (4): Physico-chemical properties of spray solutions of neem oil 90% EC samples at different storage conditions.

			Foaming (mm)	Emulsion Stability	Viscosity (cp.)	Surface Tension (dyne/cm)	pH	Conductivity (µs)	Salinity (‰)
Pass	**Initial**	**S.W**	4	✓	≃ 2.1	35	6.5	185	0.1
		H.W	6	✓	≃ 2.1	32.4	7.3	380	0.3
	Indoor for 14 days	**S.W**	4	✓	≃ 2.1	34.8	6.6	190	0.1
		H.W	7	✓	≃ 2.1	32.3	7.4	390	0.3
	54 ± 2 °C for 14 days	**S.W**	7	✓	≃ 2.1	34.7	6.7	195	0.1
		H.W	8	✓	≃ 2.1	32.1	7.6	400	0.3
Failed	**Outdoor for 14 days**	**S.W**	10	×	≃ 2.1	29.2	7.5	300	0.2
		H.W	12	×	≃ 2.1	27.1	8.1	630	0.5
	72 ± 2 °C for 3 days	**S.W**	14	×	≃ 2.1	26.3	7.8	410	0.3
		H.W	15	×	≃ 2.1	22.2	8.9	650	0.5

✓= Pass (no sedimentation) ×= failed (sedimentation over 3ml)

III- Bioassays:

Estimation of LC_{50} of neem oil 90% EC samples upon *Spidoptora litorallis* 4th instar larvae:

The insecticidal activities of neem oil 90% EC samples prepared at different storage conditions upon the 4th instar larvae of *S. littoralis* was given in table (5). It was recorded that, the most effective neem oil sample was the initial sample; the LC_{50} of it was 301.77 µg/ml and the toxicity index was 100%. Then the lowest effective one was the neem oil sample stored at 72 ± 2°C which recorded LC_{50} 1521.63 µg/ml and the lowest toxicity index (19.83 %). Finally the remained tested samples namely; indoor, 54 ± 2 °C sample and outdoor (sunlight sample) had LC_{50}'s 363.1, 546.84 and 797.68 µg/ml and showed toxicity indexes 83.11, 55.19 and 37.83%, respectively.

The Ldp-lines of different tested neem oil samples at different storage conditions were plotted on a logarithmic paper (Log concentration) against the percentage of the larval mortality and presented in figure (1). The highest slope was observed with initial sample (1.98) and the lowest one observed with the sample stored at 72 ± 2°C (1.14). The slopes of the outdoor sample and the stored sample at 54 ± 2 °C were approximately the same (1.42 and 1.47), respectively. At the end, the slope of the indoor stored sample was (1.72).

Generally, the neem oil 90 % EC samples prepared at different storage conditions can be arranged increasingly according to their LC_{50}'s and toxicity indexes as follows:

Initial < Indoor < 54 ± 2°C stored sample < outdoor (sunlight) < 72 ± 2°C stored sample

Table (5): Insecticidal activity of neem oil 90% EC samples at different storage conditions on cotton leaf worm *S. littoralis*

	Concentration in ppm					LC_{10}	LC_{50}	LC_{90}	Slope	Toxicity index
	100	200	400	800	1600					
Initial	17.17	36.21	59.56	79.86	92.37	67.77	301.77	1343.82	1.98	100
Indoor	16.7	32.75	52.89	72.30	86.67	65.65	363.1	2008.43	1.72	83.11
54 ± 2 °C	13.98	26.11	42.12	59.57	75.27	72.97	546.84	4098.2	1.47	55.19
Outdoor	10.1	19.76	33.57	50.07	66.56	99.13	797.68	6418.48	1.42	37.83
72 ± 2 °C	8.99	15.87	25.52	37.57	50.98	112.88	1521.63	20511.7	1.14	19.83

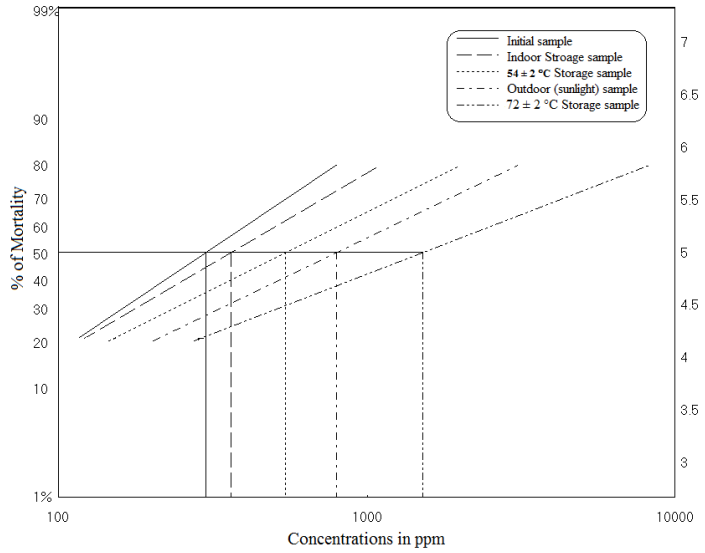

Fig.(1): Ldp lines of neem oil 90% EC samples at different storage conditions against cotton leaf worm *S. littoralis*

4. Discussion

Azadirachtin (tetranortriterpenoids) is the predominant active insecticidal component found in

neem seeds and leaves **(Butterworth and Morgan, 1968)**. It is the best known derivative which has been effectively used against more than 400 species of insects **(Schmutterer and Rembold, 1980; Schmutterer, 1990; Isman, 1999; Walter, 1999; Hasan and Ansari, 2011)**. This compound displays an array of effects on insects acting as a phago and oviposition deterrent, repellent, antifeedant, growth retardant, molting inhibitor, sterilant, and preventing insect larvae from developing into adults **(Schmutterer, 1990, 1995; Mordue and Blackwell, 1993)**. Insects from different orders differ markedly in their behavioural responses to azadirachtin. Lepidopterans are sensitive to azadirachtin and show effective antifeeding agent.

From our present study, we found that, the outdoor (sunlight) and accelerated hot storage at 72 ± 2 ºC affect on azadirachtin as the active ingredient of neem oil 90% EC so it must be protected from them which make degradation for azadirachtin active ingredient. This was confirmed by **Sundaram *et al.*, 1995; Szeto and Wan 1996 and Jarvis *et al.*, 1998** whom found that crystalline azadirachtin is a relatively stable substance if stored in the dark. Its laboratory half-life in mildly acidic solutions (pH 4 and 5) is about 50 days at room temperature, but rapid decomposition occurs at higher temperatures and in alkaline and strongly acidic media. Azadirachtin is light sensitive **(Ermel *et al.*, 1987)**. Neem formulations retain their azadirachtin content for at least a year when stored at 25 ºC. Studies on the behavior of various azadirachtin formulations in the environment were recently reviewed **(Sundaram, 1996)**.

Also, we studied the physical properties of different azadirachtin neem oil 90% EC before and after storage conditions (indoor, outdoor at sunlight, at 54 ºC for 14 days and hot storage at 72º C for 3 days). By monitoring the succeeded samples initial, indoor and storage at 54 ºC for 14 days had not any serious changes affect on the physical behavior of the formulation. Samples stored outdoors and at 72 ºC found to be changed in their physical properties when compared to the initial sample. These were in agreement with **El-Sheikh *et al.*, (2010) and El-Sheikh and Radwan (2011).**

The neem oil 90% EC showed insecticidal activities at different storage conditions upon the 4th instar larvae of *S. littoralis*. Neem preparations have deterrent or antifeedant activities against many insect species. More important, azadirachtin markedly affects insect metamorphosis and reproduction, including fecundity, but these effects manifest slowly. Depending on the dose, azadirachtin causes growth inhibition, malformation, and mortality in insect larvae. The steroid-like compound disturbs insect development, apparently by interfering with the release or action of ecdysteroids and/or other hormonal regulators of insect molt **(Marco *et al.*, 1990)**. The insect toxicity of azadirachtin, however, cannot be entirely explained by its effect on the endocrine system alone. Because commercial neem formulations contain not only azadirachtin but also other minor, but potentially bioactive limonoid components, the insecticidal effect of the preparation is more complex than that observed for pure azadirachtin.

Corresponding author
El-Shiekh, Y. W. A.
Pesticides Formulation, Central Agricultural Pesticides Laboratory, Agricultural Research Center, Dokki, Egypt
yasserwahied@yahoo.com

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