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Chemical Analysis Of Three Acaricides And Toxicological Properties Against *Tetranychus Urticae* Koch.

Rasha. M. A. El-Saman[1] and Bassem.A.Soudy[*2]

[1] *Pesticides Analysis Researches Department, Central Agricultural Pesticides Laboratory, Agricultural Research Center, Dokki, Giza, Egypt.*

[2] *1Applied Center of Entomonematodes, Faculty of Agriculture, Cairo University, Giza, Egypt.*

Abstract

The stability of three pesticides (Pyridaben 20% WP, Spirozed 25% EC, and Actara 25% WG) was examined. For 14 days, the tested acaricides were kept at 54±2 °C. During the storage period, samples were taken after 1, 3, 7, and 14 days to deterimine the active ingredient, physical properties , fingerprint (GC/MS), and toxicological qualities of Tetranychus Urticae. According to the data, the half-lives of the three acaricides were utilized. Data showed that the half-life of in three acaricides used were 28.42, 61.46, and 662.09 respectively in Pyridaben, Spirozed, and Actara. From this data, we conclude that Actara25% WG is more stable than Spirozed 24% WP than Pyridaben 20% EC. According to FAO specifications, samples conform to the specifications in the physical properties. The effect of pyridaben for 14 days storage in 54˚ of against *T. urticae* by (1,10,25 and 50 ppm) were (27, 29,5, 2.7,5, and 26,5) effect of Actara for 14 days storage in 54C of against *T. urticae* by (1,10,25 and 50 ppm) were (80, 87, 83, and 80)% produced respectively % mortality. The estimated lethal concentration value for LT was (79 µl L-1 air) in 50 ppm Actara effect of Spirozedfor 14 days storage in 54 ± 2 °C of against *T. urticae* by (1,10,25 and 50 ppm) were (70, 70, 61, and 60) produced respectively % mortality, the effect of Actara for 14 days storage in 54 ± 2 °C of against *T. urticae* by (1,10,25 and 50 ppm) were (80, 87, 83, and 80)% produced respectively % mortality. The estimated lethal concentration value for LT was (79 µl L-1 air) in 50 ppm. This research aims to investigate the degradation of three acaricides (Pyridaben 20% WP, Spirozed 24%EC, and Actara25% WG) after storage 14 days at 54± 2°Cwe found thatActara 25%WG is more stable than Spirozed 24% WP Pyridaben 20% EC. Also, toxicity studies showed that Actara 25%WG was the most lethal effect against mites after 14 days of storage this cleared that some active ingredients are more resistant against high-temperature storage. Also clear that some pesticides such as Actara are more resistant against high-temperature storage.

Keywords *: Pyridaben, Spirodiclofen, Thiamethoxam, Stability, GC/MS, Tetranychus urticae, acaricides.*

1. Introduction

Acaricides are pesticides that kill the arachnid, which include ticks and mites **[1].** *Tetranychus urticae* attack results in brownish-grey or staining of the leaves, as well as necrotic poor skin in later stages of leaf harm[2]. When a mite attacks an open flower, it produces browning and withering of the petals, which resembles spray burn. Furthermore, when the mesophyll hankie collapses owing to the death of 18–22 cells each minute, small chlorotic poor skin can appear at feeding locations. Continuous feeding results in a speckled sun-bleached look, with the leaves becoming yellow, grey, or figure. Defoliation throughout the summer or fall might identify damage in the case of a severe infestation of trees **[3,4].** Laboratory bioassays are indispensable for many types of studies with pesticides, including investigations of structure-activity relationships, resistance mechanisms and genetic modes of inheritance, comparative toxicities, and metabolism. A chemical used in kill's mites. This class of pesticides is included antibiotic acaricides, carbamate acaricides, mite growth regulators, organophosphate acaricides, and many others acarus, a mite to kill **[5].** The tendency of spiders to develop resistance to acaricides quickly due to their high reproductive capabilities is a major issue in spider management. Acaricides' short life cycle enables host defenses. However, acaricides are

*Corresponding author e-mail: basemsoudy@gmail.com; (Bassem.A.Soudy).

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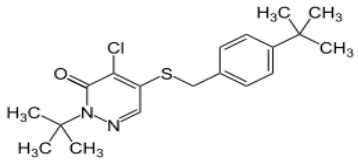
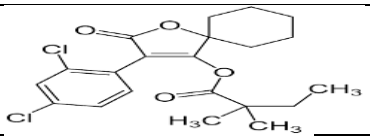
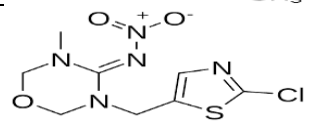
frequently necessary to keep mite populations below economic thresholds, and insecticidal chemicals are readily included in intolerance control programs. Thiamethoxam is a neonicotinoid insecticide that is used to control a variety of insects [6]. When a laboratory bioassay is meant to predict effectiveness, a function approach comparable to that employed in practice is generally chosen from among different methods of disclosing tested individuals to insecticide [7]. has been highly dissatisfied conceived and built to replicate the 2nd application of pesticides to several phytophagous organisms. Acaricides employed against T. urticae are distinguished by a wide range of substance structures and modes of action, as well as a wide range of substances poisonous to phytophagous mites. [10,11] evaluated the mechanisms of action of acaricides as well as the identification of their target locations. Spirodiclofen is an acaricidal tetronic acid. It affects mite eggs, all nymphal stages, and adult females (adult males are unaffected)[12]. Tiptronic acid compounds of spirodiclofen are non-systemic acaricides. It has been created for citrus, pome fruits, grapes, and nuts and is particularly effective over spider mites. Spirodiclofen is an acaricide with a broad spectrum of action[13]. Pyridaben is a contact miticide/insecticide that kills a variety of mites and insects. Pyridaben is now approved for use on a wide range of crops. Pyridaben is a pesticide of the pyridazinone family. Other active chemicals in this pesticide class include pyrazon and norflurazon [14]. The spider mite's primary resistance mechanism to Pyridaben may be detoxification via cytochrome P450 enzymes. butoxide (PBO), a cytochrome P450 monooxygenase inhibitor. The efficient synergist for controlling Pyridaben-resistant spider mite populations[15]. The propensity of spiders to rapidly develop resistance to acaricides due to their high reproductive capabilities and the short life cycle is a significant concern in spider management. However, the use of acaricides is frequently essential to keep mite numbers below economic thresholds.

2. Experimental

2.1.Acaricides Formulations Used:

Table 1: The structure of tested acaricides used.

Trade name	Common name	Structure
Pyridaben 20% WP*	Pyridaben	
Spirozed 24% EC**	Spirodiclofen	
Actara 25% WG***	Thiamethoam	

*WP: Wettable Powder. **EC: Emulsifable Concentrate. ***WG: Water dispersible Granules.

2.2.High-temperature storage.

Three formulations were tested and kept in an oven at 54 2 0C for 14 days. According to **[17],** samples were obtained at 1, 3, 7, and 14 days into the storage period to assess the active component level and physical qualities.2.3.Standard preparation of the three tested pesticides.

Individual standard solutions were prepared in a 25 ml volumetric flask and complete with methanol, at 10 mg for Pyridaben, Spirodiclofen, and Thiamethoxam. Next, we prepare concentrations of 400, 300, 200, 100, and 50 PPM to make the standard curve.

2.4. Sample preparation for tested pesticides:

Correctly measured enough sample composition corresponding to 10 mg of standard in a separate 25 ml volumetric flask for each sample, then gently mixed with methanol.

2.5. Determination of acaricides used by HPLC instrument.

2.5.1. Determination of Pyridaben.

An Ultraviolet has been used in HPLC (Agilent technologies 1260 Infinity II). The detector has a wavelength of 210 nm. The flow rate was 1.3 ml/min in a C18 column. The mobile phase was acetonitrile:

methanol (70:30), and the residence time (RT) of Pyridaben was 3.168 minutes.

2.5.2. Determination of Spirodiclofen.

A UV detector was used in HPLC (Agilent technologies 1260 Infinity II). The detector has a frequency of 210 nm. The flow rate was 1.3 ml/min in a C18 column. The mobile phase was acetonitrile: methanol (70:30), and the retention time (RT) of Spirodiclofen was 2.982 min under these conditions.

2.5.3.Determination of Thiamethoxam.

UV-detector was detected at 235nm. The flow rate was 1 ml/min and the column was C18. Acetonitrile and methanol (70:30) were used as the solvent system. Thiamethoxam was has a retention time (RT) of 1.954 minutes under these conditions.

2.6. Determination of the main physical criteria corresponding to each investigated formulation.

2.6.1.Preparation and determination of physical properties of standard water used.[18], MT 18.3 Non-CIPAC Standard Waters, 18.3.1 WHO Standard Hard Water, (342 ppm hardness) were used in all tests of physical properties.

Calcium chloride $CaCl_2$ (0.304 g) and magnesium chloride $MgCl_2$. 6 H_2O (0.139 g) were dissolved in distilled water and made up to 1000 ml.

2.6.2.Emulsion stability evaluation for Spirozed formulations (20%EC).

The test was carried out according to **[19].** 5 ml of the formulation was added to 95 ml of standard water (in cylinder 100 ml), the cylinder was inverted 30 times in one min and then placed in a water bath at 30°C ± 2°C for 30 min. At the end of this time, the separated materials, if any, were measured. The volume of free oil, froth, cream, or solid matter present, if any, was recorded at the end of the 30 min period.

2.6.3.Suspensibility test of Pyridaben 20% (WP) and Actara 25% (WG) formulations.

The test was run according to **[20].** Weigh accurately sufficient sample of WP and WG (2.5 × 100/conc. of the sample) after shaking of the container. Samples were transferred quantitatively into a 250 ml Stoppard measuring cylinder containing the standard water, the volume was completed to 250 ml with the water, the cylinder was inverted 30 times in one min, and then placed in a water bath maintained at 30°C ±2°C for 30 min. At the end of this time, the separated materials or precipitation, if any, were measured.

2.7.Fingerprint characteristics for Acaricides by GC/MSanalysis.

Agilent 7890 B, 5977 A MSD gas chromatography equipped with an Agilent mass spectrometric detector, with a direct capillary interface and fused silica capillary column (30 m 0.025 mm HP-5-0.25 micron -60 to 325/325 0C) oven temperature program, 500C for 4 min, then 100C /min ramp to 2100C followed by a 100C /min ramp to 2700C followed by The split peaks were identified using the Nist 20 mass spectral database. The retention times (RT) of Pyridaben and Spirodiclofen were 25.108 and 25.323 min, respectively, under these conditions.

2.8.Kinetic study:

The equation **[21]** was used to compute the rate of degradation of the tested active component and the half-life periods (T0.5) for the tested insecticides.

$T_{0.5}$ = ln 2/ K= 0.6932K and K = $\frac{1}{TX}\ln\frac{a}{bx}$

Were K = rate of decomposition

a = initial residue

tx = Time in days of hours

bx = residue at x time

2.9.Toxicological properties against *T.urticae.*

2.9.1. Treatment of adult females *T. urticae.*

Individuals of T. urticae were collected from infested leaves and stems of both tomato plants collected. Fifty adult females *T. urticae* were transferred on each leaf of both plants placed upside down on wetted cotton pads in trays. Water was added when needed. Mites were maintained at suitable moisture and kept in an incubator at 25±2°C and 80±5% RH for two days. Thirty replicates of and achyalipha discs potted plants, ten replicates per each treatment were used. Four leaves with twenty adult females were transferred to each replicate and then kept for two days for complete transportation. Extracts of oil compounds were prepared with distilled water. Concentrations of previous prepared pesticides (1.0, 2.0, 3.0, 4.0 and 5.0%) and control were done. The mites were treated by the spraying method. The mortality rate of death mites after spraying was recorded every day.

Separate laboratory trials were set up to investigate the adverse effects of pesticides on *T.urticae*. In Petri dishes (10 cm diam.) containing wet cotton fibers, ten individuals of each predatory mite were put on *Acalypha wilkesiana* leaves. To be the same age at the start of the trial to join the adult stage. Predatory mites were released on tomato leaves one, three, and five days following treatment options in the treatment assessment. Only the prescribed doses of the pesticide were used. To keep predatory mites at bay, the Petri dishes were simply sprayed with filtered water. Both types were incubated under stable circumstances at a temperature of 25°C and a

humidity of 60%. Measurements were recorded two, five, and seven days following therapy, respectively. The abundance of predators, both living and dead, has been recorded. One such concept was a Petri plate containing tomato leaves infected with 10 predatory individuals of the same species. The treatment was repeated five times. The three pesticides were used: Pyridaben (20% WP), Spirozed (24%) EC (Spirodilofen), and Actara (25%) WG (Thiamethoxam). Apart from concentrations (10, 50, and 100) PPM. The studies were repeated for 2 weeks and an average death rate was used to calculate the optimum dosage. The results for the larvicidal activity impact on all mortalities were adjusted against the control (Abbott, 1925), and the data were analyzed with ANOVA LT50, LT90, and slope values were determined using LDP. Line assessment was conducted the following **[20,22].**

The mortality rate was calculated as the following equation:

$$\%\text{Mortality rate} = \frac{\text{The number of mites before treatment}}{\text{The number of died mites after treatment}} X\ 100$$

3.Results And Discussion

1- Effect of storage at 54±2 ^{0}C on the stability of acaricides on the tested formulations.

Table (2) shows that the active component Pyridaben was 20.00 percent at the start of the trial and 18.93, 17.6, and 15.02 percent after 3, 7, and 14 days of storage at 54 2°C, respectively. Similarly, the active component quantities of Spirozed (a.i.) were 23.92, 23.35, 22.57, and 21.18 percent after zero time, 3 and 7 days, and 14 days, respectively, at the temperatures tested. Astara's active ingredient percentage was 25.04 at the start of the trial and 24.92, 17.6, and 15.02 percent after 3, 7, and 14 days of storage at 54 2°C, respectively., 24.97, 24.92, and 24.77 after 3, 7, and 14 days, respectively.

Table 2. Effect of storage at 54±2 ^{0}C on the stability of acaricides on the tested formulations.

Storage period (days)	Pesticide used					
	Pyridaben 20% WP		Spirozed 24% EC		Actara 25% WG	
	a.i %	Loss %	a.i %	Loss %	a.i %	Loss %
Initial	20.00	0.0	23.92	0.0	25.04	0.0
3	18.93	5.35	23.35	2.375	24.97	0.28
7	17.6	12	22.57	4.42	24.92	0.48
14	15.02	24.9	21.18	11.42	24.77	1.08
$t_{0.5}$ (days)	28.42		61.46		662.09	

Initial= zero time before storage. $t_{0.5}$ =half life

According to FAO specifications, the tolerance level of active ingredient content is ± 6% of the declared content for the formulation ranging from 20% equal 6%. The limited tolerance of Pyridaben content (6×20/100) equal ± 1.2 (21.2-18.8%) this conform to the FAO specifications until 3days after this un confirm, the tolerance level of active ingredient content is ± 6% of the declared content for the formulation ranged from 20% equal 6%. The limited tolerance of Spirozed content (6×24/100) equal ± 1.44 (25.44-23.56%) this conform to the FAO specifications at zero time in 3days and after this un confirm, the tolerance level of active ingredient content is ± 6% of the declared content for the formulation ranged from 20% equal 6%. The limited tolerance of Actara content (6×25/100) equal ± 1.5 (26.5-23.5%) this conforms to the FAO specifications all results.

Also, data showed that the half-life of in three acaricides used were 28.42, 61.46, and 662.09 respectively in Pyridaben, Spirozed, and Actara. From these results, we conclude that Actara 25%WG is more stable than Spirozed 24% EC than Pyridaben20% EC.

The results are in agreement with **[23],** which found that the rate of degradation of thiamethoxam (half-life 29.23 days) is similar to our obtained results in this paper. Also, these results were matched with**[24],** they found that the rate of degradation of Pyridaben is similar to our obtained results in our study.

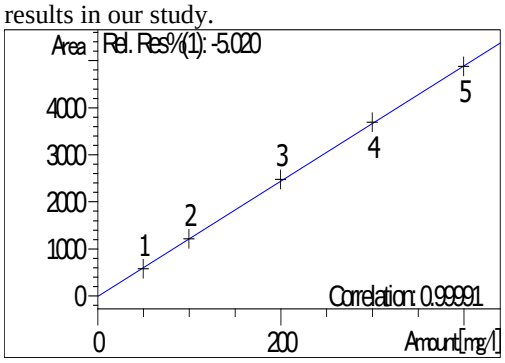


Figure 1: Calibration curve for Pyridaben

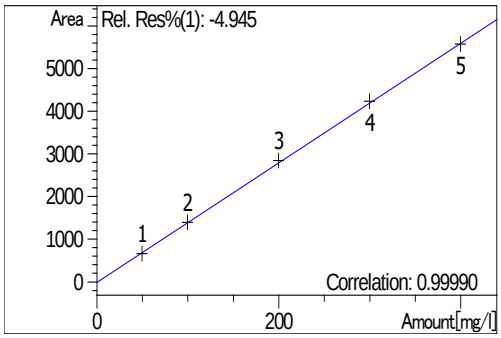


Figure 2: Calibration curve for Spirodiclofen (concentration from 50-400 mg/L).(concentration from 50-400 mg/L).

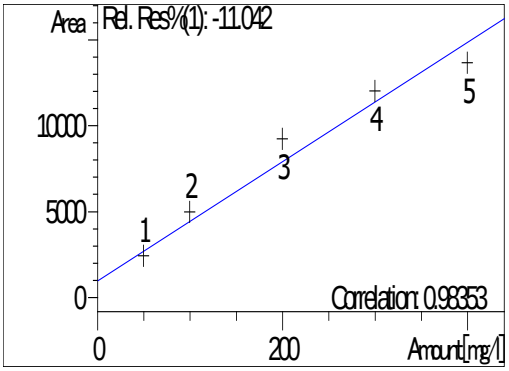


Figure 3: Calibration curve for Thiamethoxam (concentration from 50-400 mg/L).

Fig.1, 2, and 3. Calibration curve for acaricides used (concentration from 50-400 mg/L).

2-Effect of storage at 54 ± 2 ^{0}C on suspensibility ofPyridaben20%WPand Actara 25% WG.

Table 3. Effect of storage at 54 ± 2 ^{0}C on suspensibility of Pyridaben 20% WP and Actara 25% WG.

Storage period (days)	Pesticide used	
	Pyridaben 20% WP	Actara 25% WG
Initial	-	-
3	-	-
7	-	-
14	*	*

(-means No Sediment) (* means a little Sediment)

The data presented in Table (3) showed a suspensibility test for Pyridaben20% WPand Actara 25% WGstored at 54 ± 2°C. Data revealed that no sediment was formed during the storage period in initial, 3 and 7for Pyridaben20% WP and Actara 25% WG but a little of sediment appeared in for Pyridaben 20% WP and Actara 25% WG in 14 days from storage. According to FAO specifications data showed that the samples conform to these specifications.

3-Effect of storage at 54 ± 2 ^{0}Con the emulsion stability ofSpirozed 24% EC formulation.

The emulsion test was 54 ± 2°C for Spirozed 24% EC, data revealed no sediment showed during the storage period.

According to FAO specifications data showed that the samples confirm the specifications. In terms of the proportion of the physical properties.

4-Identification of Pyridaben 20% WP and Spirozed 24% EC by chemical ionization by GC/MS spectroscopy.

Data in Table (4) showed that Rt of Pyridaben and spirodiclofen was 25.109 and 25.321 min before and after storage and no shift happened during the storage period at 54±2°C.

Table 4. Identification of Pyridaben 20% WP and Spirozed 24% EC by chemical ionization by GC/MS spectroscopy.

Storage periods per (days)	Pyridaben 20% WP			Spirozed 24%EC		
	Pyridaben degradation	Retention time(RT)	Formula	Spirodiclofen degradation	Retention time(RT)	Formula
Initial	Pyridabenm/z (364.93)	25.109	$C_{19}H_{25}CIN_2OS$	Spirodiclofen m/z (411.11)	25.321	$C_{21}H_{24}Cl_2O_4$
After 14 days of storage at 54°C ±2	Pyridaben m/z (364.93)	25.109	$C_{19}H_{25}CIN_2OS$	Spirodiclofen m/z (411.11)	25.321	$C_{21}H_{24}Cl_2O_4$

5. Effect of storage at 54±2 ^{0}C on toxicological properties against *T.urtica*on the testedAcaricides.

5.1.Effect of storage at 54 ± 2 ^{0}C on pyridaben 20% WP against *T.urticae*on*achalipha* leaves.

The effect of storage at 54 ± 2 ^{0}C for 14 days on pyridaben against. *urticae* by (1, 10, 25, and 50 ppm) was (27, 29.5, 2.7, 5, and 26.5) produced respectively % mortality. The estimated lethal concentration value for LT was (22.565 µl L^{-1} air) in 50 ppm Pyridaben. For zero the effect of Pyridaben against *T. urticae* were 30, 33, 34, and 30) produced respectively % mortality. The estimated lethal concentration value for LT was (43.231 µl L^{-1} air) in 50 ppm Pyridaben for zero (Table 5) and Fig 4.

Table 5.Effect of storage at 54±2 ^{0}C on mortality rate of adult *T. urticae*on the tested formulations.

Conc. %	1ppm	10ppm	25ppm	50ppm
Pyridaben**	27±1.71	29.5±1.22	27.5±1.71	26.5±1.61
Pyridaben*	30±0.82	33±1.87	34±0.92	30±0.82
Spirozed**	70±2.24	70±2.24	61±2.24	60±2.24
Spirozed*	77.5±1.71	79±2.64	71±2.24	70±2.24
Actara**	80±0.82	87±2.24	83±2.44	80±2.44
Actara*	95±0.82	92±0.92	88±2.94	98±2.94
Control	10±1.29			

(* means initial of experimental) (** means after 14 days from storage at 54±2°C)

5.2. The effect of storage at 54 ± 2 ^{0}C on Spirozed 24%ECagainst *T.urticae* on *Achalipha* leaves.

The effect of storage at 54 ± 2 ^{0}C for 14 days on Spirozedagainst *T. urticae* by (1,10,25 and 50 ppm) was (70, 70, 61, and 60) produced respectively % mortality. The estimated lethal concentration value for LT was (65 µl L^{-1} air) in 50 ppm Spirozed. For zero the effect of Spirozed against *T. urticae* were 77, 79, 71, and 70) produced respectively % mortality. The estimated lethal concentration value for LT was (58.231 µl L^{-1} air) in 50 ppm Spirozed for zero (Table 5) and Fig 5.

5.3.The effect of storage at 54 ± 2 ^{0}C on Actara 25%WGagainst *T.urticae* on *achalipha* leaves.

The effect of storage at 54 ± 2 ^{0}C for 14 days on Actaraagainst *T. urticae* by (1,10,25 and 50 ppm) was (80, 87, 83, and 80)% produced respectively % mortality. The estimated lethal concentration value for LT was (79 µl L^{-1} air) in 50 ppm Actara. For zero the effect of Actara against *T. urticae* was 95, 92, 88, and 98)% produced respectively % mortality. The estimated lethal concentration value for LT was (93.231 µl L^{-1} air) in 50 ppm Actara for zero (Table 5) and Fig 6.

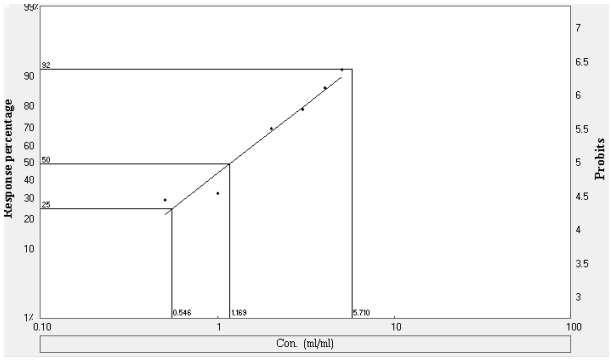


Fig.4. Regression lineforPyridabenagainst *T.urticae* on *Achalipha* leaves

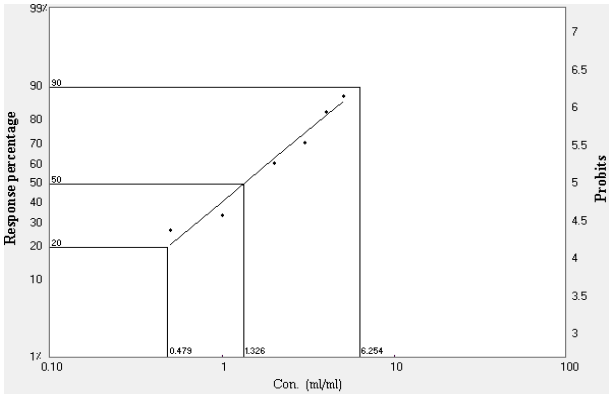


Fig.5. Regression line forSpirozed against *T.urticae*on*Achalipha* leaves.

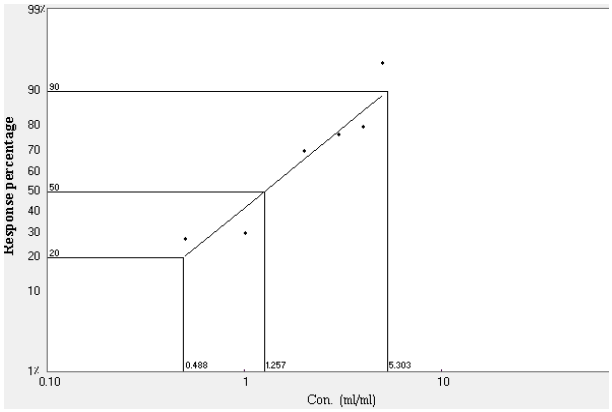


Fig.6. The regression line for Actara against *T.urticae* on *Achalipha* leaves.

T. urticae acaricides have a variety of chemical structures and modes of action that may be identified among the numerous types of phytophagous mite toxic chemicals. The mechanisms of action of acaricides were explored, as well as the identification of their target locations. **[25,26].** Based on **[27],** we provide a summary of acaricides classified by group, a database of chemical compounds (subchem), their primary site of action, and resistance development. Even though the number of agents available for mite control appears to be enormous. The use of a few common acaricides, such as organotin compounds and mitochondrial electron transport inhibitor-acaricides, is mostly responsible for population control of *T. urticae*. (fenazaquin, fenpyroximate, pyridaben, chlorpyrifos, and tebufenpyrad), as well as pyrethroids and tebufenpyradas However, due to its short life cycle and reproducing capabilities, it is unlikely to create a conflict with this drug **[28].** Repeated treatment can cause resistance and impede the spider mites' natural biological control mechanisms **[29,30].** Because of its resistance to a wide spectrum of pesticides, the two-spotted spider mite is known as the "most resistant species" in many parts of the world **[31].** The cost of utilizing them to combat spider mite damage in the European Union is presently exceeding more money each year **[32]** Because of the widespread use of pesticides, as well as the attendant issues regarding resistance and pollution, there is a growing need for ecologically acceptable pest management alternatives. Furthermore, management strategies that rely on synthetic acaricides may not consistently control spider mite populations below commercially feasible levels **[33].** At the moment, acaricides are only approved if they are safer or "greener" pesticides and fulfill specific eco-toxicological conditions set by the environmental protection agency **[34].** Repeated treatment can cause resistance and disrupt the spider mites' normal biocontrol systems. not immediate repercussions Changes in the physiology and biology of live creatures, as well as changes in fertility and attitude **[35,36],** and also pesticides' considerable influence on mite mortal enemies **[37],** are also troublesome. The absence of natural enemies as a consequence of pesticide use can lead to a large increase in *T. urticae* populations **[38].** Pesticide usage has been linked to an increase in spider mite populations, according to many studies **[39].** There is an increasing need for environmentally sound, long-term management techniques. Furthermore, management strategies that rely on synthetic acaricides may not consistently control spider mite populations below commercially feasible levels **[40].** Currently, acaricides are approved for use provided they are safer or "greener" pesticides and fulfill specific eco-toxicological conditions imposed by the Environmental Protection Agency **[41]**. Repeated therapy can cause resistance and impede the spider mites' natural biological control mechanisms **[42,46].**

4.Conclusion

We conclude that Actara 25%WG is more stable than Spirozed 24% ECthen Pyridaben 20% WPalso toxicity studies showed that Actara 25%WG was the most lethal effect against mites after 14 days of storage this cleared that some active ingredients are was more resistant against high-temperature storage like Actara. Also clear that some pesticides are more resistant against high-temperature storage, Actara 25% WG, Spirozed 24% WP then Pyridaben 20% EC.

5.References

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