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Toxicity of some plant extracts against *Tetranychus urticae* (Acari: Tetranychidae)

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Abstract

This study was conducted to evaluate the efficacy of five plant extracts namely, *Portulaca oleracea*, *Portulaca pilosa*, *Chenopodium album*, *Calotropis procera* and *Anagallis arvensis*, on *Tetranychus urticae* Koch. (Acari: Tetranychidae). Plant materials were obtained from Fayoum Governorate. Different parts of plants were extracted either by chloroform or methanol solvent to bioassay their effects by using direct spray technique. *A. arvensis* leaves extract was the most toxic, *C. album* leaves extract was the least effective one. While, methanol extract was more effective in phytochemicals including *A. arvensis* which was more than another plant after 24 hrs., the methanol extract was more effective in phytochemicals included *C. procera* after 48 hrs.

Introduction

Tetranychus urticae Koch (Acari: Tetranychidae) which is considered an agricultural pest can colonize more than 1,100 plant species (Migeon *et al*., 2010). The mite injures direct and indirect damage to plants which decreases photosynthesis and transpiration (Brandenburg and Kennedy, 1987). *T. urticae* is resistant to many selective acaricides (Van Leeuwen *et al*., 2014). Which sometimes fail to control mites below economic threshold levels (Tirello *et al.*, 2012).

Many predaceous are now used as biological control agents in various agricultural ecosystems (Ebadollahi *et al.*, 2014). In this context, the use of some plant extracts can present a realistic alternative to synthetic acaricides because of their efficiency against pests (Abo-Mousa *et al*., 2021). Plant extracts are also an environmentally interesting tool because of their biodegradability and minimal side effects on non-target organisms as well as on the environment (Cavalcanti *et al.*, 2010; Attia *et al.*, 2011, 2012a and 2012b).

Chenopodium ambrosioides L. is strongly aromatic, it is abundant in America and Africa (Rendle, 1983), and largely distributed in Egypt. It has been reported to have a wide variety of medicinal and insecticidal properties (Su, 1991 and Quarles, 1992). The solvent extraction process is relatively efficient and is usually applied to extract bioactive compounds from plants and fruits. Five plants have been used as an

alternative for the control of many pests and diseases, these plants include: *Portulaca oleracea* L. and *Portulaca pilosa*. *P. oleracea* is an annual prostrate, spreading and succulent, branched herb (Londonkar and Nayaka, 2011).

A. arvensis is a summer annual herb distributed worldwide or with a global spread abundantly found in Egypt (Yasmeen *et al*., 2020). *Calotropis procera* L. is a plant that contented important medicinal properties (Ramachandra *et al*., 2003). *Chenopodium album* L. is a strongly aromatic, perennial herb. It is largely distributed in Egypt. It has been reported to have a wide variety of medicinal and insecticidal properties (Su, 1991 and Quarles, 1992). The solvent extraction process is relatively efficient and is usually applied to extract bioactive compounds from plants and fruits.

This study aims to investigate the acaricidal effects of chloroform and methanolic extract of some wild weeds against *T. urticae* under laboratory conditions.

Materials and methods

1. Preparation of plant materials and extraction of crude bioactive compounds:

Five plants namely *Portulaca oleracea* L., *P. pilosa* L., *Chenopodium album* L., *Calotropis procera* L. and *Anagallis arvensis* L., were washed to remove dirt, mud and waste residues from the outer surface, then kept in a dark room to dryness and kept in paper pages of (20X30 cm) until ground into powder and kept in jars, the powders were extracted either by chloroform or methanol for the biological and chemical experiments.

2. Preparation of the crude extract:

Plants were extracted according to the procedure of Su and Horvat (1981) with minor modifications. 100 grams from each powder were extracted two times successively, by soaking in a brown gar for 48 hours and 72 hours (With shaking). By using two different polarity solvents, i.e., (Chloroform and methanol), were then filtered through anhydrous sodium sulphate and were combined and evaporated under reduced pressure in a rotary evaporator at temperatures not exceeding 50˚C. The crude extracts were stored in a brown glass bottle in a refrigerator until bioassay tests.

3. Rearing of mites *Tetranychus urticae*:

T. urticae was taken from the Plant Protection Research Institute in Giza, Egypt. Individuals were transferred to *Acalypha wilkesiana* leaves, carried to the laboratory, and allowed to reproduce under controlled conditions (25±5°C and 60%±5 R.H.).

4. Bioassay tests:

T. urticae was the target of a biocide, which was evaluated in a lab using either chloroform or methanol. Each extract was tested at five different concentrations.

5. Treatment of adult stages:

Ricinus communis leaf discs were arranged in Petri dishes on moist cotton pads. Ten adult females of *T. urticae* (Similar in size and age) were placed on each disc, then treated with one of the different treatments, sprayed by glass atomizer with different concentrations of the chloroform and methanol extracts.

Five concentrations (1250, 2500, 5000, 10000, and 20000 ppm) of the chloroform and methanol extracts of each plant were used to draw the dosage mortality regression line. Four replicates were used for

each concentration. Control discs were sprayed with a similar solvent was used in the extract. All treated discs were kept at 25±.5 ℃ and 55%±5 RH. Mortality was estimated for adult females after 24 hrs. and 48 hrs. from spraying. Mortality percentage was determined and corrected by Abbott's formula (Abbott, 1925) as follows:

Percentage of mortality = (% tested mortality-% control mortality)/"100-% control mortality " × 100

LC_{50}, LC_{90} and slope values were calculated according to Finney (1971), and using (Ldp line) software by Bakr (2000).

Results and discussion

1. Miticidal activity of chloroform crude extract:

1.1. After 24 hours of treatment:

The concentration mortality regression lines obtained from the tested 5 plants (with 7 crude extracts) after 24 hrs. are given in Figure (1). The LC_{50} and LC_{90} values were plotted and tabulated in Table (1) with their corresponding slopes, toxicity index and relative potency to each other's. The toxicity index was obtained by comparing the efficiency of the tested extracts, at the fixed levels, (LC_{50} and LC_{90}) at their most effective one. *Calotropis procera* was the most toxic crude extract; it was used as a baseline for comparison in calculation.

Toxicity of used standard material is always 100%. From Table (1) *C. procera* has the highest potency levels that were 6.29 times as toxic as the corresponding level of *C. album"*leaves" (Which is the least effective one) followed by *P. pilosa, A. arvensis* "leaves", *A. arvensis* "flowers" *P. oleracea and C. album* "flowers" by 4.63, 4.54, 4.41, 2.53 and 2.12 times as toxic as the corresponding level of *C. album* "leaves" respectively . Data of LC_{50} and LC_{90}, indicated that *C. procera* proved to be highly toxic, with the LC_{50} (10112.47 ppm) followed ascendingly by *P. pilosa* (13733.16 ppm) and *A. arvensis* leaves (14005.85 ppm), *A. arvensis* flowers (14447.75 ppm), *P. oleraceae* (25158.97 ppm), and *C. album* flowers (30091.52 ppm) then *C.album* leaves (63653.18 ppm).

Based on the LC_{90} value, the order of efficiency was the same, *i. e.*, *C. procera* leaves (40060.61 ppm), *A.arvensis* leaves (74294.46 ppm), *P.pilosa* (98981.85 ppm), *A. arvensis* flowers (217065.30 ppm), *C.album* flowers *(*517570.40 ppm), *P.oleraceae (*541473.20 ppm) and *C.album* leaves *(*838376.30 ppm).Comparing the slope value of the toxicity lines (Table 1 and Figure 1), *C. procera* showed the steepest lines (Slope = 2.14) whereas *P. oleraceae* showed the flattest one (0.96). The slope value of the other toxicity lines was (1.77) for *A. arvensis* leaves, (1.49) for *P. pilosa*, (1.14) *C. album* leaves, (1.09) *A. arvensis* flowers and (1.04) *C. album* flowers. *P. oleraceae* exhibits a "flat" dose-response line.

This indicated that a large change in dosage is required before a significant change in response will be observed. However, *C. procera* exhibited a "steep" dose-response line where a relatively small change will cause a large change in response.

1.2. After 48 hours of treatment:

The concentration mortality regression lines obtained are given in Figure (2). The toxicity index was obtained by comparing the efficiency of the tested extracts, at the fixed levels, (LC_{50} and LC_{90}) at their most

effective one. *A. arvensis* leaves extract was the most toxic crude extract. The toxicity index was calculated at LC_{50} and LC_{90} levels. Toxicity of used standard material is always 100%. From Table (2) *A. arvensis* leaves have the highest potency levels. The potency levels of *A. arvensis* for *T. urticae* were 6.57 times as toxic as the corresponding level of *C. album* leaves extract (1.00) followed by *C. procera, P. pilosa , A. arvensis* "flowers", *C. album* flowers extract then *P. oleracea* by 5.06, 2.88, 2.59, 2.53and 1.21times as toxic as the corresponding level of *A. arvensis* "leaves" respectively .

For leaves of *A. arvensis* LC_{50} was (3787.54 ppm) followed by *C. procera* (4916.25 ppm) and *P. pilosa* (8644.21 ppm), *A. arvensis* flowers (9597.21 ppm), *C. album* flowers (9819.86 ppm), and *P. oleracea* (20507.56 ppm) then *C. album* leaves (24889.60 ppm). Based on the LC_{90} value, the order of efficiency was *A. arvensis* leaves extract (17057.67 ppm), *C. procera* (26419.39 ppm), *A. arvensis* flowers (43398.88 ppm), *P. pilosa* (49675.65 ppm), *C. album* flowers *(*56947.73 ppm), *C. album* leaves *(*227777.30 ppm) and *P. oleraceae* leaves *(*430187.60 ppm).

Comparing the slope value of the toxicity lines (Table 2 and Figure 2), *A. arvensis* leaves and *A. arvensis* flowers extracts showed the same steepest lines (Slope = 1.96) whereas *P. oleracea* showed the flattest one (0.97). The slope value of the other toxicity lines was (1.76) for *C. procera*, (1.69) for *P. pilosa*, (1.68) *C. album* flowers and (1.33) for *C. album* leaves. *P. oleracea* exhibits a "flat" dose-response line. *A. arvensis* leaves and *A. arvensis* flower extracts exhibited a "steep" dose-response line where a relatively small change will cause a large change in response.

This result agrees with Shobowale *et al*. (2013) who indicated that the plant leaves and latex of *C. procera* contain bioactive constituents which can effectively inhibit the growth of some microorganisms. Hence; *C. procera* crude extract could be controlled *T. urticae*.

2. Miticidal activity of methanol crude extract:

2.1. After 24 hours of treatment:

The concentration mortality regression lines obtained are given in Figure (3). The toxicity index was obtained by comparing the efficiency of the tested extracts, at the LC_{50} levels at their most effective one. *A. arvensis* leaf extract was the most toxic crude extract; it was used as a baseline for comparison in the calculation.

The toxicity index was calculated at LC_{50}. Toxicity of used standard material (crude leaves extract of *A. arvensis*) is always 100%. From Table (3) *A. arvensis* leaves extract has the highest potency levels. The potency levels of *A. arvensis* leaves extract for *T. urticae* were 13.82 times as toxic as the corresponding level of *C. album* flowers extract (which is the least effective one) (1.00) followed by *C. procera*, *A. arvensis* flowers, *C. album* leaves, *P. oleracea and P. pilosa* by 3.14, 2.81, 2.18, 1.63 and 1.12 times as toxic as the corresponding level of *C. album* flowers extracts, respectively.

Data in Table (3) and Figure (3) indicated that *A. arvensis* leaves extract proved to be highly toxic, with the LC_{50} (15944.57 ppm) followed ascendingly by *C. procera* (70136.01 ppm) and *A. arvensis* flowers

(78321.95 ppm), *C. album* leaves (100995.00 ppm), *P. oleracea* (135284.70 ppm) and *P. pilosa* (196985.40 ppm) then *C. album* flowers (220346.30 ppm). Comparing the slope value of the toxicity lines (Table 3 and Figure 3), *A. arvensis* leaves extract showed the steepest lines (slope = 1.62) whereas *C. album* flowers extract showed the flattest one (0.64).

The slope value of the other toxicity lines was (0.94) for both *C. procera* and *C. album* leaves, (0.86) for *A. arvensis* flowers, (0.68) *P. oleracea* and (0.66) for *P. pilosa*. *C. album* flowers extract exhibits a "flat" dose-response line. This indicated that a large change in dosage is required before a significant change in response will be observed. However, *A. arvensis* leaves extract exhibited a "steep" dose-response line where a relatively small change will cause a large change in response.

2.2. After 48 hours of treatment:

The concentration mortality regression lines obtained are given in Figure (4). The LC_{50} values were plotted and tabulated in Table (4) with their corresponding slopes, toxicity index and relative potency to each other's. The toxicity index was obtained by comparing the efficiency of the tested extracts, at the LC_{50} levels at their most effective one. *C. procera* leaves extract was the most toxic crude extract, it was used as a baseline for comparison in the calculation. Toxicity of used standard material, crude leaves extract of *C. procera* is always 100%. From Table (4) *C. procera* leaves extract has the highest potency levels.

The potency levels of *C. procera* for *T. urticae* were 27.79 times as toxic as the corresponding level of *C. album* leaves extract (which is the least effective one) followed by *A. arvensis* leaves, *A. arvensis* flowers, *P. pilosa*, *P. oleracea* then *C. album* flowers extract by 8.57, 3.45, 2.34, 1.80 and 1.71 times as toxic as the corresponding level of *C. album"* leaves" respectively.

Data in Table (4) and Figure (4) based on LC_{50} indicated that *C. procera* leaves extract proved to be the highly toxic one, with the LC_{50} (3060.98 ppm) followed ascendingly by *A. arvensis* leaves extract (9921.48 ppm) and *A. arvensis* flowers extract (24628.32 ppm), *P. pilosa* (36425.56 ppm), *P. oleracea* (47158.62 ppm), and *C. album* flowers extract (49598.56 ppm) then *C. album* leaves (85060.18 ppm).

Comparing the slope value of the toxicity lines of these plant extracts (Table 4 and Figure 4), *C. procera* leaves extract showed the steepest lines (Slope = 2.11) whereas *A. arvensis* flowers extract showed the flattest one (0.76). The slope value of the other toxicity lines was (1.51) for *A. arvensis* leaves extract, (1.06) for *P. pilosa*, (0.93) *C. album* flowers, (0.86) *C. album* leaves extract and (0.78) for *P. oleracea* extract.

Table (1): LC_{50} and LC_{90}, slope, toxicity index and relative potency values of chloroform crude extracts of different plants against *Tetranychus urticae* after 24 hours from treatment.

Plant	Extracted Part	LC_{50}	95% confidence limits		LC_{90}	Slope	Toxicity index at		Relative potency at	
		Ppm	Lower	Upper	ppm		LC_{50}	LC_{90}	LC_{50}	LC_{90}
Chenopodium album	Leaves	63653.18	27726.76	775260.10	838376.30	1.14	15.89	4.78	1.00	1.00
Ch.album	Flowers	30091.52	16116.70	138938.70	517570.40	1.04	33.61	7.74	2.12	1.62
Calotropis procera	Leaves	10112.47	8061.15	13410.54	40060.61	2.14	100.00	100.00	6.29	20.93
Anagallis arvensis	Flowers	14447.75	9286.84	33145.57	217065.30	1.09	69.99	18.46	4.41	3.86
A.arvensis	Leaves	14005.85	10448.38	21675.99	74294.46	1.77	72.20	53.92	4.54	11.28
Portulaca oleraceae	All plant	25158.97	13675.16	113077.00	541473.20	0.96	40.19	7.40	2.53	1.55
P.pilosa	All plant	13733.16	9830.37	23214.90	98981.85	1.49	73.64	40.47	4.63	8.47

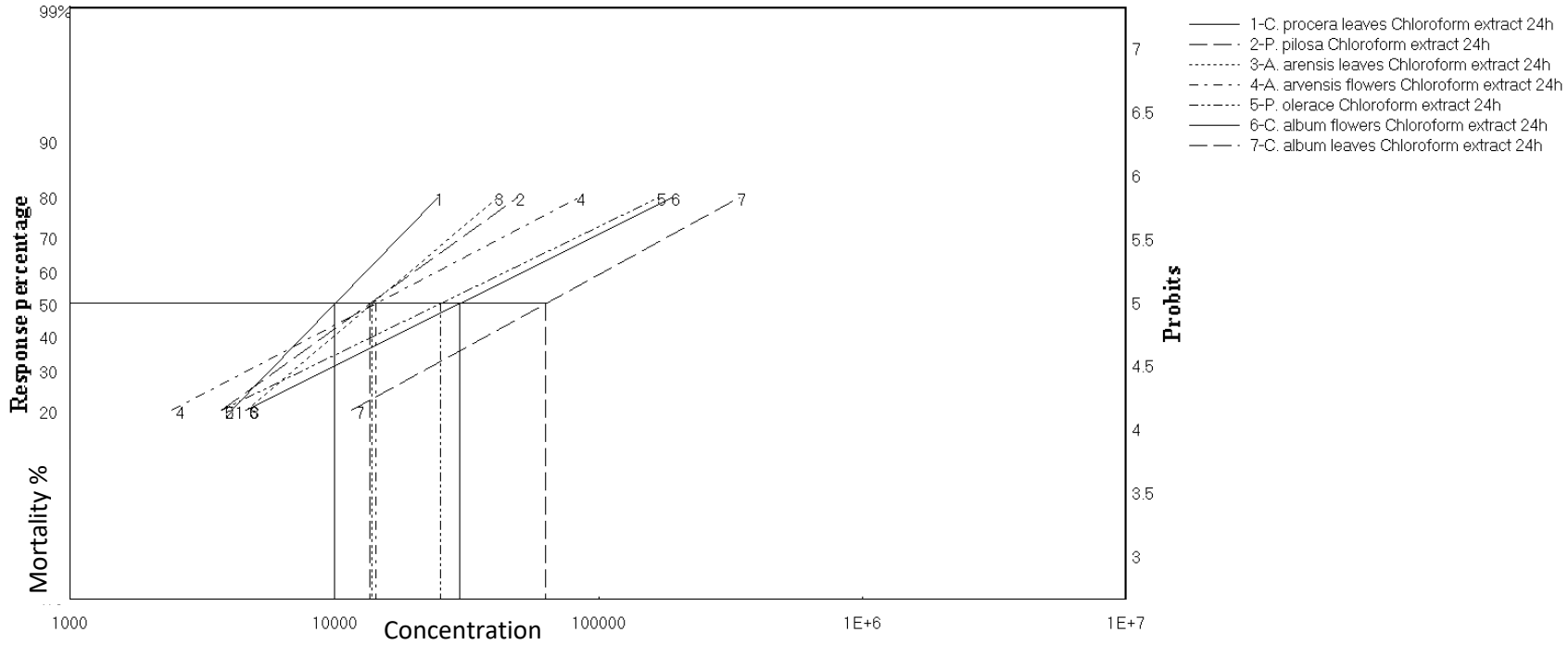

Figure (1): Toxicity lines of chloroform crude extracts of different plants against *Tetranychus urticae* after 24 hours from treatment.

Table (2): LC_{50} and LC_{90}, slope, toxicity index and relative potency values of chloroform crude extracts of different plants against *Tetranychus urticae* after 48 hours from treatment.

Plant	Extracted Part	LC_{50}	95% confidence limits		LC_{90}	Slope	Toxicity index at		Relative potency at	
		Ppm	Lower	Upper	Ppm		LC_{50}	LC_{90}	LC_{50}	LC_{90}
Chenopodium album	Leaves	24889.60	15432.60	65396.09	227777.30	1.33	15.22	7.49	1.00	1.89
Ch.album	Flowers	9819.86	7468.13	14108.93	56947.73	1.68	38.57	29.95	2.53	7.55
Calotropis procera	Leaves	4916.25	3782.76	6378.06	26419.39	1.75	77.04	64.56	5.06	16.28
Anagallis arvensis	Flowers	9597.21	7542.27	12981.20	43398.88	1.96	39.46	39.30	2.59	9.91
A.arvensis	Leaves	3787.54	2935.44	4776.48	17057.67	1.96	100.00	100.00	6.57	25.22
Portulaca oleraceae	All plant	20507.56	11760.93	73811.27	430187.60	0.97	18.47	3.97	1.21	1.00
P.pilosa	All plant	8644.21	6624.25	6624.25	49675.65	1.69	43.82	34.34	2.88	8.66

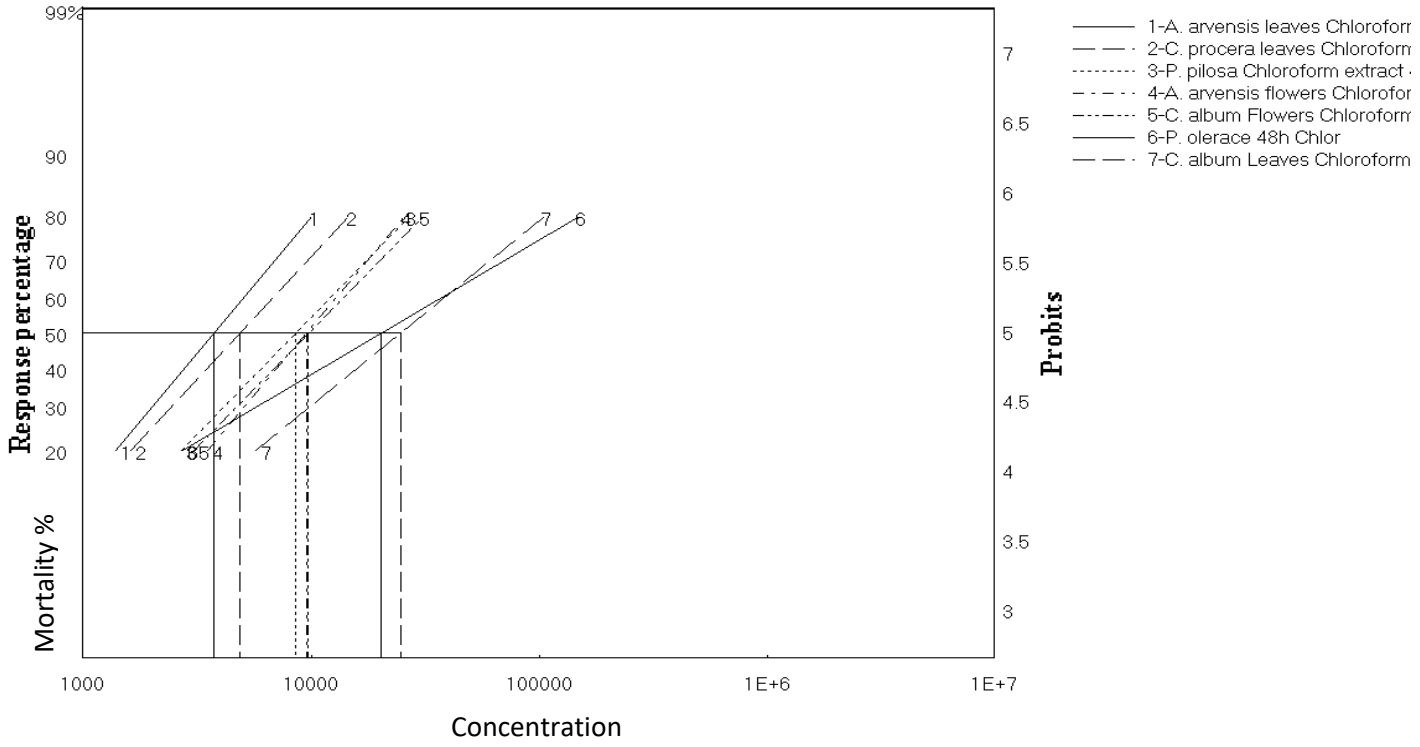

Figure (2): Toxicity lines of chloroform crude extracts of different plants against *Tetranychus urticae* after 48 hours from treatment.

Table (3): LC_{50} and LC_{90}, slope, toxicity index and relative potency values of methanol crude extracts of different plants against *Tetranychus urticae* after 24 hours from treatment.

Plant	Extracted Part	LC_{50}	95% confidence limits		LC_{90}	Slope	Toxicity index at	Relative potency at
		Ppm	Lower	Upper	Ppm		LC_{50}	LC_{50}
Chenopodium album	Leaves	100995.00	33289.45	N.A.	N.A.	0.94	15.79	2.18
C. album	Flowers	220346.30	N.A.	N.A.	N.A.	0.64	7.24	1.00
Calotropis procera	Leaves	70136.01	26967.47	N.A.	N.A.	0.94	22.73	3.14
Anagallis arvensis	Flowers	78321.95	27491.16	N.A.	N.A.	0.86	20.36	2.81
A. arvensis	Leaves	15944.57	11427.52	27271.54	98981.52	1.62	100.00	13.82
Portulaca oleracea	All plant	135284.70	N.A.	N.A.	N.A.	0.68	11.79	1.63
P. pilosa	All plant	196985.40	N.A.	N.A.	N.A.	0.66	8.09	1.12

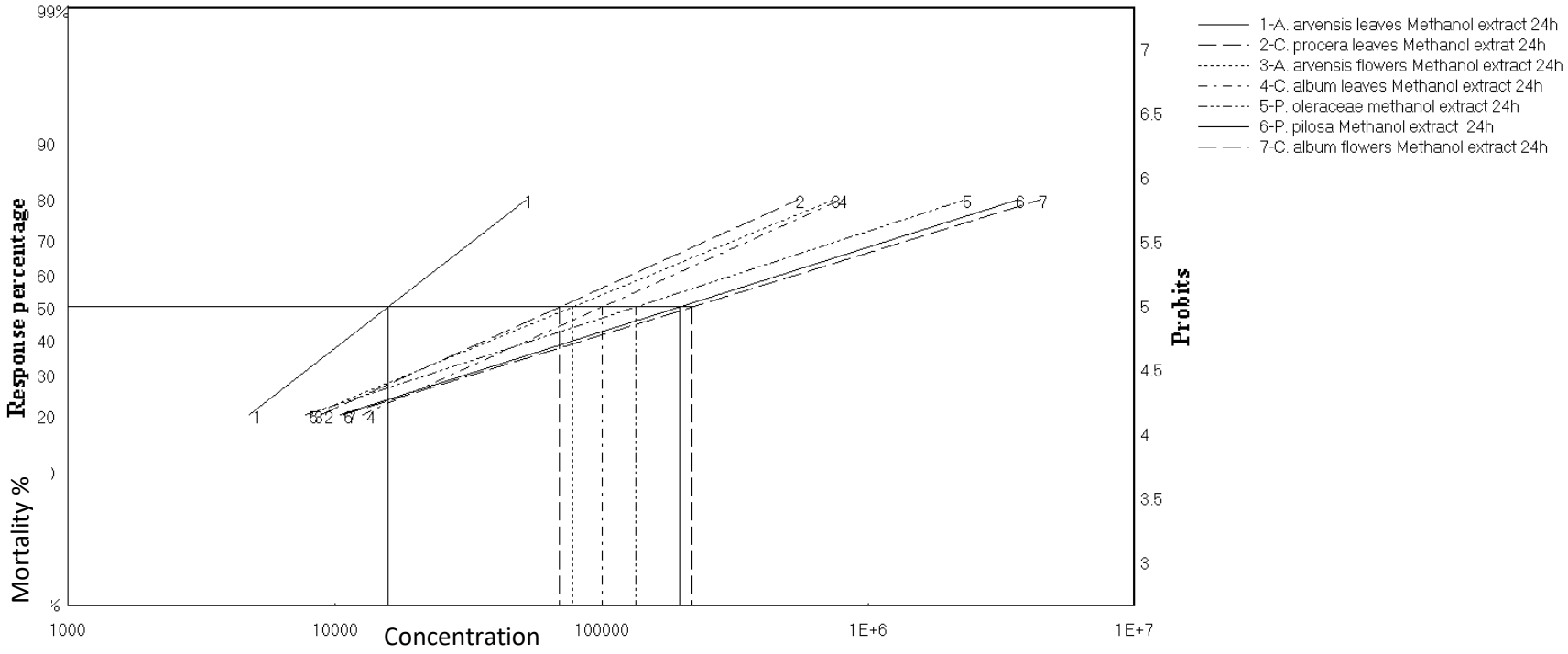

Figure (3): Toxicity lines of methanol crude extracts of different plants against *Tetranychus urticae* after 24 hours from treatment.

Table (4): LC_{50} and LC_{90}, slope, toxicity index and relative potency values of methanol crude extracts of different plants against *Tetranychus urticae* after 48 hours from treatment.

Plant	Extracted Part	LC_{50}	95% confidence limits		LC_{90}	Slope	Toxicity index at	Relative potency at
		ppm	Lower	Upper	Ppm		LC_{50}	LC_{50}
Chenopodium album	Leaves	85060.18	30844.70	N.A.	N.A.	0.86	3.60	1.00
C. album	Flowers	49598.56	21600.02	636659.40	N.A.	0.93	6.17	1.71
Calotropis procera	Leaves	3060.98	2363.99	3822.60	12436.48	2.11	100.00	27.79
Anagallis arvensis	Flowers	24628.32	12036.57	232860.50	N.A.	0.76	12.43	3.45
A.arvensis	Leaves	9921.48	7351.75	14998.31	70129.92	1.51	30.85	8.57
Portulaca oleracea	All plant	47158.62	18957.74	N.A.	N.A.	0.78	6.49	1.80
P.pilosa	All plant	36425.56	18593.53	204122.00	597274.30	1.06	8.40	2.34

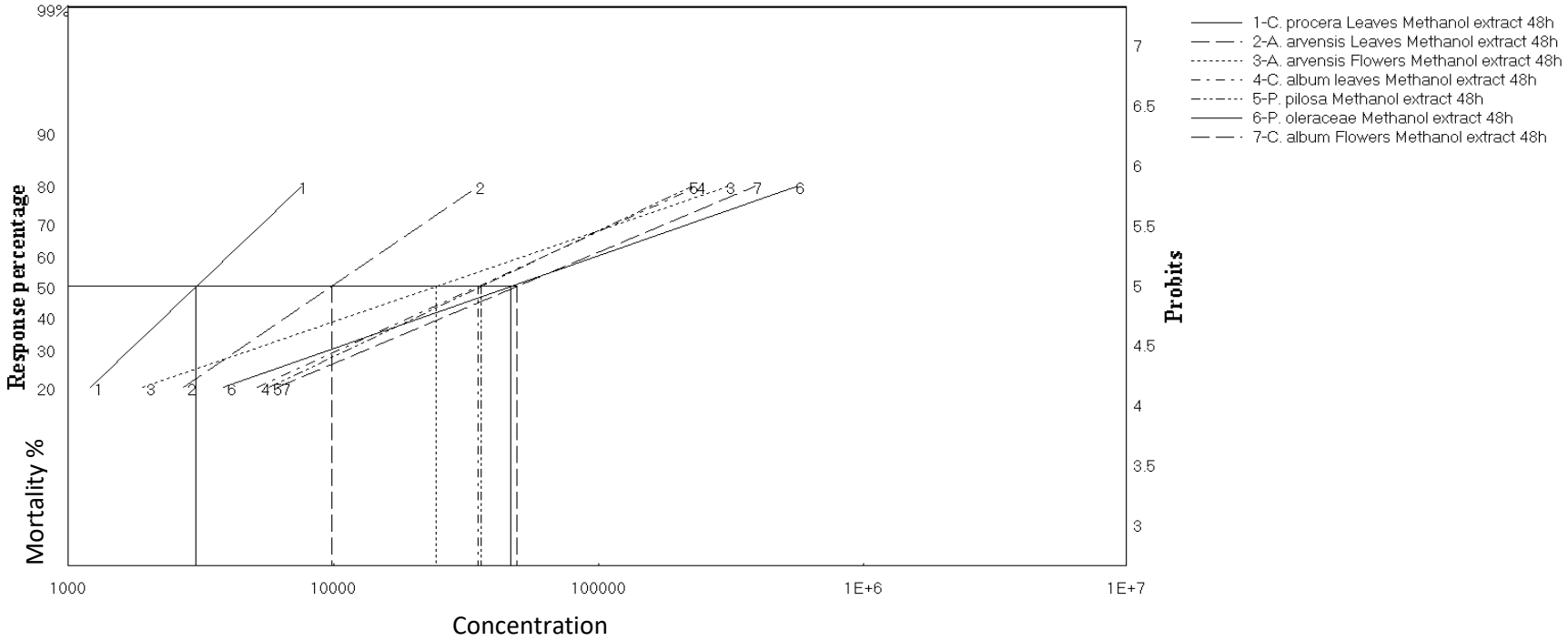

Figure (4): Toxicity lines of methanol crude extracts of different plants against *Tetranychus urticae* after 48 hours from treatment.

A. arvensis flowers leaves extract exhibit a "flat" dose-response line. This indicated that a large change in dosage is required before a significant change in response will be observed. However, *C. procera* leaves extract exhibited a "Steep" dose-response line where relatively small change will cause a large change in response. Our results are supported by (Numa *et al*., 2015 and Idrees *et al*.,2016). They reported that the mortality of individuals was recorded at 24,48 and 72 hrs.

Begum *et al*., (2010) found that ethanol extracts of leaves of *C. procera* was quite effective against the housefly larvae. These extracts drastically affected the pupation and emergence of the adults from pupae in dose dependent manner. This result indicated by Emam and Ibrahim (2020) they concluded that, after 72 hrs., the highest mortality 93.33% was recorded by treatment with seeds of *C.procer*.

The results of the present study indicated that the methanol crude extracts and chloroform crude extracts of *Anagallis arvensis* and *Calotropis procera* leaves inhibit the growth of *T. urticae*. We conclude that compounds in *A. arvensis* leaves and *C. procera* have great acaricidal potential.

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