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Influence of *Melissa officinalis* Essential Oil and its Formulation on *Typhlodromips swirskii* and *Neoseiulus barkeri* (Acari: Phytoseiidae)

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The toxicity of *Melissa officinalis* L. essential oil and its formulation (Melissacide) were evaluated against eggs and females of two predatory phytoseiid mites, *Typhlodromips swirskii* (Athias Henriot) and *Neoseiulus barkeri* (Hughes), using direct spray. Results indicate that both tested materials were potent on predatory females than egg stage. *Typhlodromips swirskii* was proved to be more sensitive to the oil and formulation than *N. barkeri*.

Females mortality were (62–100%) in *T. swirskii*, and (46–69%) in *N. barkeri,* when both predatory mites were sprayed with LC50 and LC90 of the oil and Melissacide reported on *Tetranychus urticae* Koch. Females of both predators were suffered from reduction in food consumption when sprayed with two sublethal concentrations of Melissacide, while insignificant differences reported in daily number of eggs deposited by females of *T. swirskii*, when sprayed with its LC25 value of Melissacide and control.

Keywords: *Melissa officinalis*, Melissacide, *Typhlodromips swirskii, Neoseiulus barkeri*, Phytoseiidae, toxicity.

Effective control of mite pests may not be achieved by using a single control tactic (Kim and Seo, 2001; Rhodes and Liburd, 2006). Combining tactics involving reduced-risk pesticides and selective releases of predatory mites may yield more acceptable control of mite pests while maintaining predatory mite populations in the field (Hoy and Ouyang, 1986; Rhodes et al., 2006). The possibility of controlling phytophagous mites by a combination of biological and chemical methods had proved a less costly and more permanent method of control than had pesticides alone (Magouz and Saadoon, 2005). But identifying selective pesticides for Integrated Pest Management (IPM) programs is necessary to protect the natural beneficial arthropod fauna and at the same time reduce environmental pol-

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lutants. A low toxicity of these products to natural beneficial is very important. Members of the family Phytoseiidae are predatory mites and usually associated with phytophagous pest mites in fields; the extensive and long-term use of chemical pesticides has serious adverse effects on beneficial organisms, humans and the environment (Hoy and Ouyang, 1986). At present, very few synthetic pesticides meet these criteria, but pesticides of plant origin seem to be good candidates in this list (Tsolakis and Ragusa, 2008).

The use of predatory mites of the family Phytoseiidae had proved effective control method in IPM programs for controlling pest mites especially the two spotted spider mite *Tetranychus urticae* Koch (McMurtry et al., 2013).

Typhlodromips swirskii (Athias-Henriot) and *Neoseiulus barkeri* (Hughes) are two of the most important generalist predators of pest mites and are widely found on various crops. Both predators were able to control *T. urticae*, *Eutetranychus orientalis* (Klein) (both Acari: Tetranychidae) and *Aceria dioscoridis* (Soliman and Abou-Awad) (Acari: Eriophyidae), *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) (Hansen, 1988; Momen and El-Sawy, 1993; Momen, 1995; Momen and El-Borolossy, 1999).

Recently, Momen et al. (2014) showed that *Melissa officinalis* L. (family Lamiaceae) essential oil and its formulation (Melissacide) exhibited acaricidal activity against the pest *T. urtica* and the exotic predatory phytoseiid mite *Neoseiulus californicus* (McGregor).

So, the present study was aimed to examine the toxicity effect of *M. officinalis* oil and Melissacide against eggs and females of the most important endogenous predatory mites *T. swirskii* and *N. barkeri* which can be used as effective tools in IPM programs for controlling *T. urticae* in Egypt. The effect of two sublethal concentrations of Melissacide on survival, food consumption, reproduction and sex ratio of both predators were also studied.

Materials and Methods

Plant material

The aerial parts of Melissa plant which are commonly known as lemon balm *(M. officinalis)* were collected from plant originally grown in the Experimental farm of (NRC) at Giza to obtain the essential oils.

Preparation of Melissa officinalis *oil*

The air-dried plant material (aerial parts) was pulverized and the essential oils isolated after hydro distillation for 4 h in a steam distillation using a Clevenger apparatus.

Preparation of the primary emulsions

The primary emulsions of *M. officinalis* were prepared by mixing of Triton-x 100. Different concentrations of this emulsion were prepared and tested against eggs and females of the predatory mites, *T. swirskii* and *N. barkeri*.

Preparation of the tested compound as formulation

This compound was prepared as emulsifiable concentrate formulation (EC) by mixing Melissa oil in appropriate amounts of emulsifier and natural solvent (mineral and vegetable oils). This compound labeled as Melissacide.

Stock culture of the predatory mites Typhlodromips swirskii *and* Neoseiulus barkeri

Both predatory mites were maintained on leaves of kidney bean plants (*Phaseolus vulgaris* L.) infested with *T. urticae*. Each leaf was placed underside up on a wet cotton wool layer in a Petri-dish (6 cm diameter), a water-saturated cotton strip was placed around the leaf margin to prevent escaping mites and to maintain the leaf fresh. The predatory mites were transferred to the leaf with a thin paint brush, and fed daily on all immature stages of *T. urticae*. Water supply was added daily and Petri-dishes were kept in an incubator at 28 ± 2 ºC, 70 ± 5% RH and L 16: D 8 h photoperiod. Predators were transferred to new and fresh infest leaf discs with *T. urticae* weekly to keep the culture healthy.

Treatment

1-Comparative toxicity (direct spray) of Melissa officinalis *oil and Melissacide to egg and female stages of the predatory mites* Typhlodromips swirskii *and* Neoseiulus barkeri

Ten fed females of both predatory mites were transferred to lower surface of *P. vulgaris* leaf discs (3 cm in diameter) and left for oviposition 24 h and removed thereafter. Eggs (0–24h old) were sprayed with different concentrations from *M. officinalis* oil / Melissacide using glass atomizer, each test contained 4 concentrations of oil / Melissacide and each concentration had 5 replicates (20 eggs / replicate). Hatchability of the predatory eggs on each concentration was recorded after 72 h. A similar number of untreated eggs were included as a control.

Newly emerged and mated females of both predatory mites (3 days old) were sprayed with different concentrations of oil / Melissacide using glass atomizer. Females were confined on the lower surfaces of kidney bean discs (as described before). Each test contained 4 concentrations of oil / Melissacide and each concentration had 5 replicates (20 females / replicate). Mortality was recorded 24–48 h after application. Mites were considered dead if they did not move their appendages when prodded with fine paintbrush. In every test, a control was included. All experiments were repeated twice.

2-Efficiency of Melissa officinalis *oil and Melissacide (*LC_{50} *and* LC_{90} *values on* Tetranychus urticae*) against eggs and females of predatory mites,* Typhlodromips swirskii *and* Neoseiulus barkeri

Ten fed females of both predatory mites were allowed to oviposit for 24 h on kidney bean leaf discs resting on wet cotton wool in a Petri-dish, surrounded with Vaseline to prevent escaping of the predators, which were then removed. Twenty eggs per disc were sprayed separately with both concentrations (LC_{50} and LC_{90} values reported on *T. urticae* by Momen et al., 2014), (5 replicates / concentration of oil / Melissacide) were tested.

Twenty mated females (3 days old) with 5 replicates in each concentration of both materials were held on similar above substrate and sprayed with both concentrations also. Mortalities were taken within 24–48 h. In every test, a control was included.

3-Indirect effect of Melissacide (two sublethal doses) on food consumption, oviposition, sex ratio and mortality of Typhlodromips swirskii *and* Neoseiulus barkeri *females*

Newly emerged and mated females of each predator were sprayed separately with two concentrations:

a) LC_{25} value which was reported on the predatory mites from their toxicity line (present study).

b) LC_{25} value which was recorded on *T. urticae* from its toxicity line of Melissacide (Momen et al., 2014).

Females of each predatory mite were transferred singly to the lower surface of *P. vulgaris* leaf discs and were provided daily with a sufficient known number of *T. urticae* nymphs for 10 days. Twenty replicates were used per treatment for each predator species. A control treatment was included in each test for each predatory mite. Observations were taken daily on food consumption, reproduction, hatchability and sex ratio of the progeny, and mortality for 10 successive days.

Statistical analysis and toxicity lines

- Corrected curves were drawn on Propit logarithmic graph paper according to the formula developed by Finney (1971).
- Mortality data of females were corrected to control mortality according to Abbott's formula, Abbott (1925).
- The toxicity index and potency levels were calculated as developed by Sun (1950).
- The percentage of reduction in food consumption was calculated according to Samsoe-Petersen (1983).
- The adverse effect of Melissacide on the predators was calculated according to Overmeer and van Zon (1982). The classification of the adverse effect by Hassan (1985) was followed in the present study.
- Sun's toxicity index $= \dfrac{LC_{50} \text{ or } LC_{90} \text{ standard material}}{LC_{50} \text{ or } LC_{90} \text{ of tested material}} \times 100$

Data on daily number of *T. urticae* nymphs consumed by each predator (food consumption) and daily number of deposited eggs by female predators, were analyzed by ANOVA, and means were compared by Duncan test using SPSS computer program. Differences were compared for the two predatory females between both concentrations used and control.

Results and Discussion

1-*Comparative toxicity (direct spray) of* Melissa officinalis *oil and Melissacide to egg and female stages of the predatory mites,* Typhlodromips swirskii *and* Neoseiulus barkeri

Fig. 1 (a–d) shows the Ldp-lines of toxicity effects of Melissa oil and Melissacide on females and eggs of both predatory phytoseiid mites *T. swirskii* and *N. barkeri.*

Table 1 shows that in general, Melissacide was more potent to egg and female stages of *T. swirskii* and *N. barkeri* than that of the Melissa oil. *Mentha piperita* L. and *Majorana hortensis* Moench, both essential oils of the family Lamiaceae were less toxic to females of *T. swirskii* ($LC_{50} = 2.41$ and 3.07%) than *M. officinalis* oil in the present study, respectively (Amer and Momen, 2002).

Melissa oil was toxic to females of *N. barkeri* ($LC_{50} = 0.37$ and $LC_{90} = 7.31$) while it has less activity against the egg stage ($LC_{50} = 40.11$ and $LC_{90} = 168.89$) (Table 1). Research has been done by Momen and Amer (1994) established that Lupin and Canna foliar extracts were more toxic to females than eggs of *N. barkeri* ($LC_{50} = 1.53$ and 2.23) ($LC_{50} = 10.03$ and 11.86), respectively. In 1999, the above authors indicated that *Rosmarinus officinalis* L. essential oil was toxic to females of *N. barkeri* while *M. hortensis* was slightly toxic to the predator. The essential oil of *M. piperita* had more toxic effect on *N. barkeri* females than *Mentha virdis* essential oil ($LC_{50} = 3.72$ and 8.8%) respectively, but both oils were less toxic to the predator compare to Melissa oil in the present study (Momen et al., 2001). *Ocimum basilicum* L. essential oil had less toxic activity on *N. barkeri* females ($LC_{50} = 4.18\%$) than Melissa oil here (Momen and Amer, 2003).

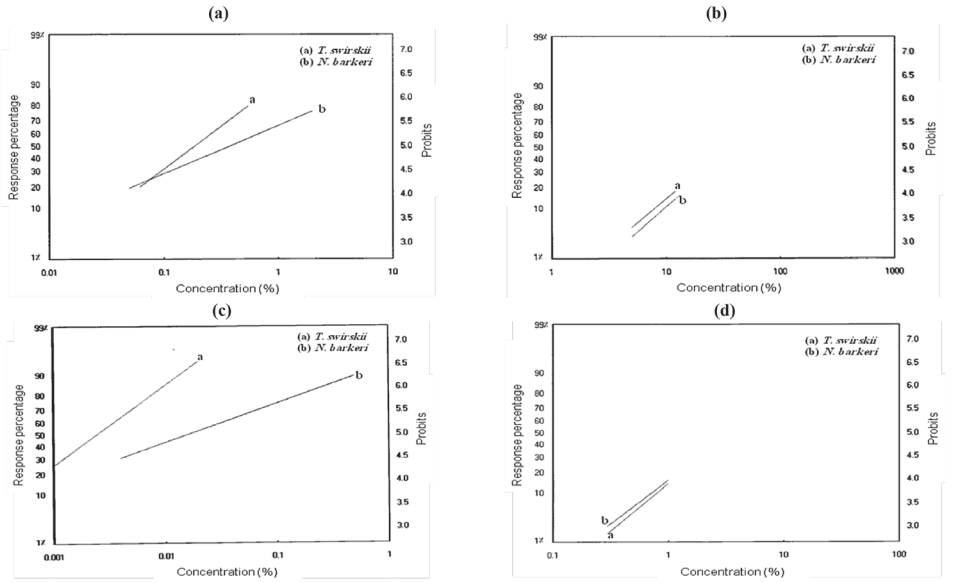

Fig. 1. (a–d) Ldp-lines of toxicity effect of *Melissa officinalis* essential oil on females **(a)** and eggs **(b)** of *Typhlodromips swirskii* and *Neoseiulus barkeri*. Melissacide on females **(c)** and eggs **(d)** of *Typhlodromips swirskii* and *Neoseiulus barkeri*

Table 1
Comparative toxicity (direct spray) of *Melissa officinalis* oil and Melissacide on females and eggs of the predatory phytoseiid mites, *Typhlodromips swirskii* and *Neoseiulus barkeri*

Tested materials	Predator stage	Predator	LC_{50}	Lower limit	Upper limit	LC_{90}	Slop
Melissa officinalis oil	Females	*T. swirskii*	0.19	0.15	0.22	0.97	1.78 ± 0.20
		N. barkeri	0.37	0.26	0.50	7.31	0.99 ± 0.12
	Eggs	*T. swirskii*	35.12	20.15	458.31	155.79	1.98 ± 0.66
		N. barkeri	40.11	21.45	1259.24	168.89	2.05 ± 0.73
Melissacide	Females	*T. swirskii*	0.0024	0.0005	0.0042	0.0133	1.73 ± 0.14
		N. barkeri	0.0159	0.0098	0.0234	0.5550	0.83 ± 0.10
	Eggs	*T. swirskii*	3.36	1.88	24.55	14.28	2.04 ± 0.58
		N. barkeri	3.44	1.90	26.12	16.60	1.88 ± 0.53

Results indicated also that Melissacide has a higher toxicity effect against females than eggs of *T. swirskii* and *N. barkeri.* Similarly, Melissacide was found to be more toxic to females and eggs of the predatory phytoseiid mite *N. californicus* (LC_{50} = 0.29 and 3.93%) than the *M. officinalis* oil (LC_{50} = 3.91 and 44.48%) (Momen et al., 2014).

2-*Efficiency of* Melissa officinalis *oil and Melissacide (*LC_{50} *and* LC_{90} *values of* T. urticae*) against eggs and females of the predatory mites,* Typhlodromips swirskii *and* Neoseiulus barkeri

The acaricidal activity of LC_{50} and LC_{90} values of oil and formulation reported on *T. urticae* (Momen et al., 2014) against eggs and females of *T. swirskii* and *N. barkeri* was tested by direct application (Table 2). The results show that LC_{50} value of *M. officinalis* oil on *T. urticae* cause 62.0% mortality to females of *T. swirskii* and increased to 96.5% in case of using Melissacide. On the other hand, LC_{90} values of Melissa oil and Melissacide of *T. urticae* cause 98.5 and 100% mortality of the female predator, respectively.

In case of *N. barkeri,* the LC_{50} and LC_{90} values of *M. officinalis* oil recorded on *T. urticae* were causes 46 and 64% mortality of females and 0.0 and 1.5% mortality of eggs, respectively. Spraying with Melissacide (LC_{50} and LC_{90}) values was caused 56 and 69% mortality of *N. barkeri* females which less than that reported in *T. swirskii.* When *N. californicus* sprayed with the above two concentrations of Melissa oil and formulation, female mortalities were shown a very low percentage of (8.5–13%), compared to the predatory phytoseiids tested here, respectively (Momen et al., 2014).

3-Indirect effect of Melissacide (two sublethal doses) on food consumption, oviposition, sex ratio and mortality of Typhlodromips swirskii *and* Neoseiulus barkeri *females*

Results in Table 3 indicated that a significant reduction in food consumption of *T. swirskii* and *N. barkeri* on *T. urticae* nymphs was recorded, when predatory females sprayed with two sublethal concentrations of Melissacide (ANOVA: F = 125.96, 8.92), respectively. Similarly, a reduction in food consumption of above two predators at conc. 2% of *O. basilicum* oil was recorded by Momen and Amer (2003).

Table 2
Efficiency of *Melissa officinalis* oil and Melissacide (LC_{50} and LC_{90} values of *T. urticae**) on females and eggs of the predatory phytoseiid mites, *Typhlodromips swirskii* and *Neoseiulus barkeri*

Tested materials	Concentration*	% Mortality			
		T. swirskii		*N. barkeri*	
		Females	Eggs	Females	Eggs
M. officinalis oil	LC_{50}	62.00	1.50	46.00	0.00
	LC_{90}	98.50	3.50	64.00	1.50
	Control	0.00	1.00	1.00	0.00
Melissacide	LC_{50}	96.50	1.50	56.50	1.50
	LC_{90}	100.00	2.50	69.00	2.50
	Control	1.00	0.50	1.00	1.00

* LC_{50} and LC_{90} values recorded on *T. urticae* from its toxicity line (Momen et al., 2014)

According to Hassan (1985), the adverse effect values show that the LC_{25} value of *T. swirskii* was slightly harmless on *T. swirskii* while the LC_{25} value of *N. barkeri* was moderately harmful on *N. barkeri* which causing 45% mortality and 22.86% reduction in food consumption (Table 3). Amer and Momen (2005) found that French lavender oil, at concentration of LC_{25} was considered to be safe for *N. barkeri* since no mortalities had been recorded. A harmful adverse effect of *O. basilicum* oil to *T. swirskii* and *N. barkeri* was reported by Momen and Amer (2003).

Table 3
Effect of Melissacide (two sublethal concentrations) on food consumption and mortality of the predatory phytoseiid mites, *Typhlodromips swirskii* and *Neoseiulus barkeri*

Predators	Concentrations	Number of *T. urticae* (nymphs) consumed by female predators / day (mean ± S.E)	(%) Reduction in food consumption	(%) Adverse effect	(%) Mortality during experiment
T. swirskii	LC_{25} value of *T. swirskii****	15.02 ± 0.54 b	15.71	31.15	25.00
	LC_{25} value of *T. urticae**	00.00 ± 00.00 c	100.00	100.00	100.00
	Control	17.82 ± 0.31 a			10.00
	F	125.96**			
N. barkeri	LC_{25} value of *N. barkeri****	14.44 ± 1.20 b	22.86	64.00	45.00
	LC_{25} value of *T. urticae**	13.08 ± 0.57 b	30.13	83.90	70.00
	Control	18.72 ± 0.77 a			5.00
	F	8.92**			

Means values within a column followed by the same letter are not significantly different at $P = 0.05$
* LC_{25} value of *T. urticae* recorded from its toxicity line (Momen et al., 2014)
** Highly significant
*** LC_{25} values recorded on *T. swirskii* and *N. barkeri* from their toxicity lines (present study)

Table 4
Effect of Melissacide (two sublethal concentrations) on reproduction, hatchability and sex ratio of the progeny of the predatory phytoseiid mites, *Typhlodromips swirskii* and *Neoseiulus barkeri*

Predators	Concentrations	Number of eggs deposited by female predators (mean ± S.E)	(%) Hatchability	Sex ratio (♂:♀)
T. swirskii	LC_{25} value of *T. swirskii***	1.43 ± 0.10 a	94.84	1:1.8
	LC_{25} value of *T. urticae**	00.00 ± 00.00 b	–	–
	Control	1.77 ± 0.06 a	100.00	1:2.2
	F	34.17**		
N. barkeri	LC_{25} value of *N. barkeri****	1.13 ± 0.16 b	93.64	1:1.3
	LC_{25} value of *T. urticae**	0.87 ± 0.19 b	91.43	1:1.1
	Control	1.89 ± 0.12 a	100.00	1:2.7
	F	13.10**		

Means values within a column followed by the same letter are not significantly different at $P = 0.05$
*LC_{25} value of *T. urticae* recorded from its toxicity line (Momen et al., 2014)
** Highly significant
*** LC_{25} values which recorded on *T. swirskii* and *N. barkeri* from their toxicity lines (present study)

Results from Table 4 indicated that there was insignificant differences in the number of eggs laid by female *T. swirskii* / day between the control and (LC_{25} value of *T. swirskii*) of Melissacide, while significant differences were recorded between both sublethal doses of formulation (ANOVA: F = 34.17). Insignificant differences were recorded also in the daily number of eggs deposited by *N. barkeri* females sprayed by both sublethal concentrations of Melissacide (ANOVA: F = 13.10). Research has been carried out by Momen and Amer (1999) revealed that *N. barkeri* was suffered from a reduction in food consumption and depression in reproduction when females treated with 1% of *R. officinalis* and *M. hortensis*. A similar reduction in food consumption and depression in reproduction of females *T. swirskii* was known, when sprayed with two sublethal concentrations of *M. hortensis*, *R. officinalis*, *M. piperita* and *Lavandula officinalis* Chaix, oils (Amer and Momen, 2002). With the exception of (LC_{25} value of *T. urticae*) on *T. swirskii*, hatchability of eggs deposited by treated two predatory females was 91.4–94.8%, respectively (Table 4). Most of the sex ratio of the progeny was in favour to females.

Conclusions

Present results indicated also that the LC_{25} value of *T. urticae* of Melissacide was harmful on both predators since causes 100% and 70% mortality and 100% and 30.13% reduction in food consumption on *T. swirskii* and *N. barkeri*, respectively (Table 3).

In integrated control programme, a careful choice of essential oil should be made to harm predatory phytoseiids population as little as possible. Our present results and also of Momen et al. (2014) suggest that, more toxicological and biological studies of more pred-

atory phytoseiid mites with *M. officinalis* oil and its formulation is essential to select the best one which can be effective to *T. urticae* and also safe when select the oil / formulation in IPM programme. This work presented here is based on laboratory data; care should be taken in translating results of laboratory to the field. Such information is of critical importance in the search for new methods of pest management based on natural products.

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