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Research Article

Impact of temperature on the acaricidal activity of spiromesifen on two-spotted spider mite *Tetranychus urticae*

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Abstract: The two-spotted spider mite (TSSM) *Tetranychus urticae* Koch is one of the most destructive mites in many plants due to its characteristics, such as high reproductive potential, short life cycle, and feeding method. Excessive use of chemical compounds without considering environmental factors has led to high residual toxins in food products and resistance to pesticides. Temperature is an essential non-living factor that affects various biological aspects of pests and pesticide toxicity levels. In this study, the interaction of different temperatures (15, 20, 25, and 30 °C) in the photoperiod (16L:8D h) was investigated on the toxicity of spiromesifen on the adult TSSM. Then the levels of α-esterase and glutathione S-transferase activity were measured. The highest LC_{50} was recorded at 15 °C after 24 h (LC_{50} = 21.269 mg ai/l), and the lowest value corresponds to 30 °C after 48 h (LC_{50} = 0.860 mg ai/l). The level of toxicity also increased with a temperature increase, so the toxicity was recorded 3.6 folds higher at 30 °C compared to 15 °C. The α-esterase and glutathione S-transferase activity also increased with an increase in the temperature, but this increase was significant only for esterase activity. The relationship between temperature and the power of pesticide toxicity in areas with different daily and controllable temperature changes can effectively provide a valuable proposal to reduce pesticide consumption and increase the efficiency of pest control.

Keywords: *Tetranychus urticae*, spiromesifen, temperatures, α-esterase, glutathione s-transferase

Introduction

After insects, acari are known as the most critical arthropods in human life. Spider mites are one of the most important pests of plants that have become very important in the world in recent years. Two-spotted spider mite (TSSM), *Tetranychus urticae* Koch (Acari: Tetranychidae), damages many agricultural and ornamental plants. For spider mites of the world, 1300 species have been described. TSSM is one of the most polyphagous species among the spider mite species known worldwide (Khodayari and Hamedi, 2021).

Synthetic pesticides are one of the essential tools to keep the pests population below the economic injury level (Van Leeuwen *et al*., 2015). Spiromesifen is one of the derivatives of

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the tetronic acid group that works in a new way (Acetyl-CoA-carboxylase inhibitor). This pesticide destroys the cell membrane by blocking fat production and reduces energy synthesis. Also, the main feature of this pesticide is to diminish pest resistance to other chemicals with a different mechanism (Cloyd *et al.*, 2006).

High reproduction potential, short life cycle, and excessive use of chemical pesticides to control the TSSM cause the emergence of resistance to many chemical pesticides after several applications and also the appearance of high levels of residual toxins in food products (Stumpf and Nauen, 2001; El Kady *et al.*, 2007; Hamedi, 2022).

Temperature changes can directly affect the fate of chemicals by using different mechanisms, such as increased volatility, the solubility of substances, and increasing decomposition (Noyes *et al*., 2009). Temperature is essential in pesticide resistance (Yang *et al*., 2018). In addition, the temperature can be effective in the amount of absorption and excretion of toxins by affecting the amount of nutrition, metabolism, and movement activity of the living organism (Jegede *et al.*, 2017).

The relationship between temperature and pesticide toxicity in areas with different daily and controllable temperature changes is fascinating and vital to providing valuable suggestions regarding the use of temperature in reducing the use of pesticides and increasing the efficiency of pest control.

The TSSM is a critical pest in most fields and greenhouses and deals with different temperatures during the day. Therefore, this study aims to investigate the effects of temperatures on the toxicity of spiromesifen and the changes of esterase and glutathione S-transferase enzymes on this mite.

Materials and Methods

Rearing of TSSM

The TSSM was collected from the cucumber (Tehran, Iran) and transferred to the red bean plants *Phaseolus vulgaris* L. var. Akhtar. TSSM was reared on bean leaves at 15, 20, 25, and 30 °C, photoperiod of 16L:8D h, and 60 ± 5% R. H. without using any pesticides.

Chemicals

The reduced glutathione (GSH) (Sigma); α-naphthyl acetate substrate (Sigma); 1, Chloro 2,4 dinitrobenzene (CDNB) (Merck), Fast blue RR (Merck) and spiromesifen (Oberon® 240 g/l SC) (Bayer Crop Sciences) were used in this research.

Adulticide bioassays

Triton X-100 (0.1%) was used for better pesticide emulsification in water. Preliminary tests were used to determine lethal concentrations between 20 and 80% mortality. Then, concentrations were tested using logarithmic intervals between them (Scharf, 2008).

Leaf disks with a diameter of 3 cm were prepared from the bean leaves, then placed on cotton pads soaked in distilled water in a Petri dish (diam. 6 cm) so that the lower surface of the leaf was facing up. Twenty adult female mites (2-3 days old) were placed with a soft brush on each leaf disc. They were left for 30 min for the mites' settlement. Then leaf discs with mites were immersed in the desired concentrations for 5 s and placed on the cotton pads inside the Petri dish. The Petri dishes were kept at room temperature for 20 min, and after the leaves were dried, they were transferred to the experimental temperatures. The mortality rate was recorded 24 and 48 h after the experiment. Mites that did not show movement when stimulated by the brush were considered dead (Roh *et al.*, 2011). Each treatment was tested at five replications. Finally, LC_{50} values of the spiromesifen were calculated at each temperature.

After bioassays, the susceptibility of TSSM at different temperatures was compared at four constant concentrations of 2.4, 7.2, 12, and 16.8 mg ai/l 24 and 48 h after exposure.

Biochemical experiments

Adult female mites (2 to 3 days old) reared in the mentioned temperature conditions were treated with 1.75 mg ai/l spiromesifen by the leaf disc method. Distilled water and Tween X100 (0.1%) were used for the control

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treatment. After 48 h, alive mites were transferred to a microtube, and the detoxification enzymes were measured.

Esterase and GST activity was measured by Van Asperen (1962) and Habig *et al.* (1976) methods. The experiment was performed in four replication, and 100 adult female mites (2 to 3 days old) were used for each repetition. The protein concentration of each sample was measured using the Bradford method (Bradford, 1976).

Data analysis

The relative median potency test (RMP) was evaluated by comparing the significant differences between the LC_{50}s of the two treatments. LC_{50} values were used from the method of Finney (1971). In case of losses in the control treatment, they were corrected using Abbott's formula (Abbott, 1925). These calculations were done with SPSS 20 software.

The effect of concentration, temperature, and their interaction was statistically analyzed by the two-way factorial tests using the Univariate GLM method. If significant, the treatments were compared using Tukey's honestly significant difference (HSD) tests at $P \leq 0.05$. Before statistical analysis, the data's normality (mortality percentage) was checked using MINITAB14 software and normalized using the angular arcsin relationship (Arcsine) if needed. Calculations were done with SPSS 20 software. SAS software and Tukey's test method were used at the 5% level to compare the mean interaction effects. Enzyme biochemical data were analyzed using PrismDemo software.

Results

The mortality of the spiromesifen on the adult female mites was assessed 24 and 48 h after treatment. The results indicated that an increase in temperature causes an increase in the mortality rate. Based on the values of LC_{50}, the highest and lowest mortality was 1.757 mg ai/l at 30 °C and 21.269 mg ai/l at 15 °C, 24 h after exposure. The level of mortality also increased as time exposure increased. Therefore, 48 h after exposure, the LC_{50}s changed to 0.860 mg ai/l at 30°C and 10.679 mg ai/l at 15 °C (Table 1).

The relative median potency (RMP) test showed that the changes in temperature from 15 to 30 °C caused the highest sensitivity (RMP = 12.42) of the adult female mites to the pesticide (Table 2).

The results of Table 3 show that the main effects of treatments (concentration and temperature) and their interaction effects on the mortality rate of spiromesifen were significant at the 1% probability level. In this regard, the mortality rate increased significantly with increasing temperature and exposure time for all tested concentrations. So the highest mortality rate was 93.62% at 30 °C with 16.8 mg ai/l after 48 h, and the lowest value was 13.2% at 15 °C with 2.4 mg ai/l and 24 h exposure time (Fig. 1).

Table 1 Toxicity of spiromesifen against the adult female of *Tetranychus urticae* after 24 and 48 h.

Temperature (°C)	Time (h)	n	P-value	$\chi 2$	Slope ± SE	LC_{50} (mg ai/l) (95% confidence limits)	LC_{90} (mg ai/l) (95% confidence limits)
30	24	524	0.903	1.589	1.047 ± 0.129	1.757 (1.353 – 2.253)	29.413 (16.566 – 73.200)
30	48	457	0.906	1.559	1.258 ± 0.152	0.860 (0.688 – 1.090)	8.978 (5.384 – 20.041)
25	24	485	0.683	2.289	0.823 ± 0.128	8.105 (5.868 – 11.689)	293.833 (115.289 – 1600.108)
25	48	502	0.978	0.781	0.877 ± 0.093	2.223 (1.616 – 3.025)	64.216 (34.450 – 159.981)
20	24	584	0.602	3.641	0.838 ± 0.113	13.002 (9.681 – 18.775)	439.541 (183.177 – 1919.703)
20	48	550	0.422	4.951	0.964 ± 0.096	3.770 (2.858 – 4.928)	80.554 (46.866 – 175.209)
15	24	492	0.751	1.918	2.143 ± 0.356	21.269 (18.541 – 26.303)	84.311 (54.837 – 193.895)
15	48	378	0.516	2.282	2.416 ± 0.417	10.679 (8.929 – 12.129)	36.218 (27.366 – 62.439)

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Table 2 Comparison of LC_{50} values of spiromesifen between different temperatures on *Tetranychus urticae* by relative median potency after 48 h.

Temperature (A/B)	RMP temperature [1]	95% confidence limits	Significance
		Lower - Upper	
15 °C/ 20 °C	2.833	1.050 - 6.329	*
15 °C/ 25 °C	4.804	1.467 - 13.910	*
15 °C/ 30 °C	12.417	4.335 - 42.330	*

* indicate significant differences between the two groups based on lower and upper 95% confidence limits.
[1] Relative Median Potency: LC_{50} (temperature A) / LC_{50} (temperature B).

Table 3 Analysis of variance of mortality of *Tetranychus urticae* by spiromesifen after 48 h.

Source of variations	df	MS	F	P-value
Concentration (C)	3	4066.934	373.455	< 0.0001
Temperature (T)	3	5664.282	520.135	< 0.0001
C × T	9	123.800	11.368	< 0.0001
Error	64	10.890		
CV = 4.685				

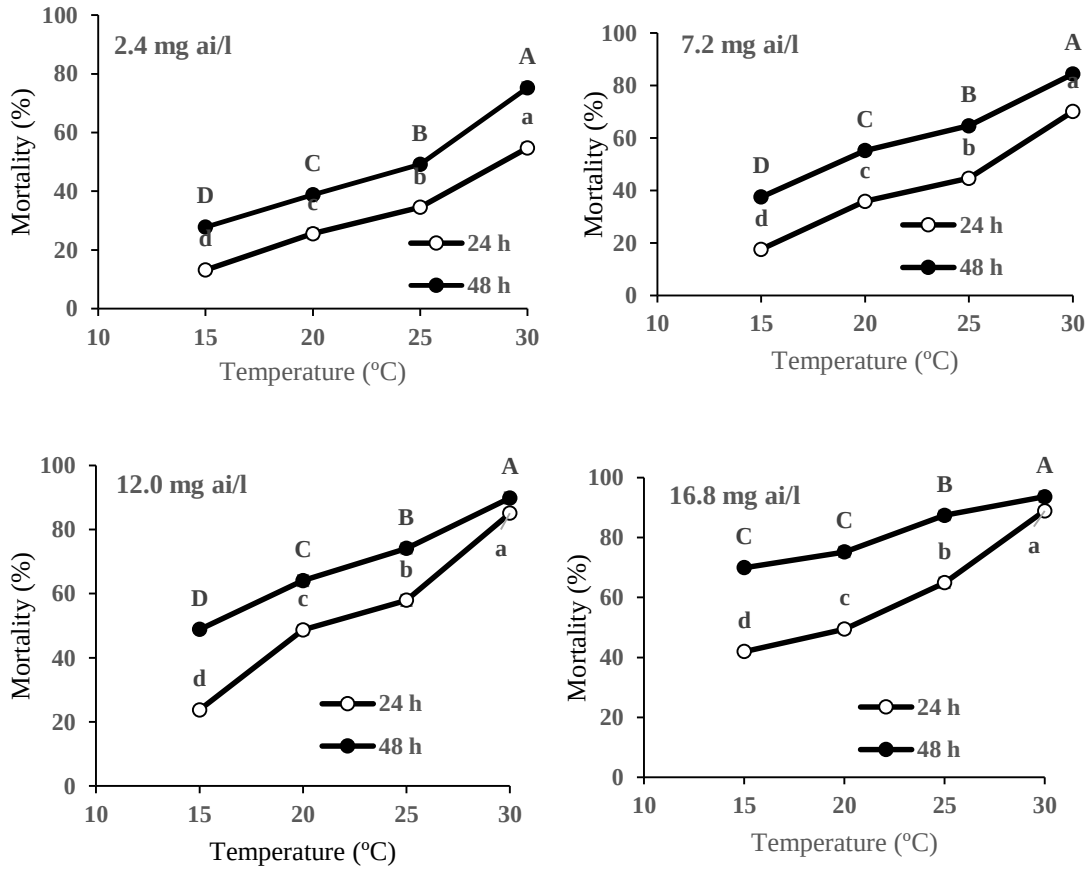

Figure 1 Mean (± SE) mortality of different concentrations of spiromesifen on *Tetranychus urticae* at different temperatures (15, 20, 25, and 30 °C) after 24 and 48 h. Means followed by the same letters in each line are not significantly different (Tukey's test, $P \leq 0.05$).

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Esterase and GST Activity

Esterase and GST activity of adult female mites at 1.75 mg ai/l of spiromesifen are shown in Fig. 2. The results showed that an increase in temperature causes a significant increase ($F=12.13$; $df = 3,7$; $p < 0.01$) in the α-esterase activity so that this activity increased 2.4 folds when the temperature increased from 15 to 30 °C. There were no significant differences in GST activity with increasing temperature (Fig. 2).

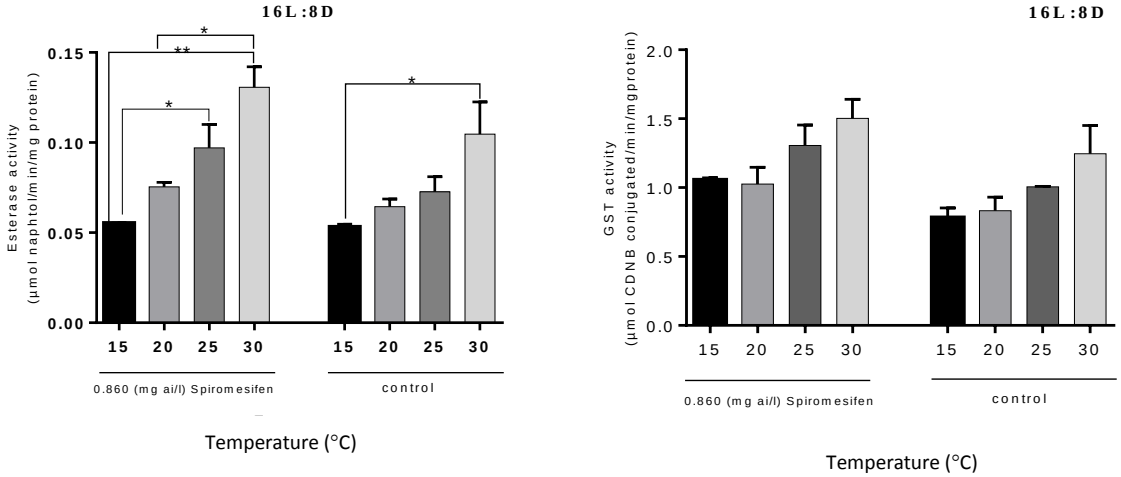

Figure 2 Esterase and GST activity of *Tetranychus urticae* at different temperatures with a concentration of 1.75 mg ai/l spiromesifen 48 h after exposure. * $P < 0.05$, ** $P < 0.01$.

Discussion

The toxicity of pesticides depends on the chemical formula, dosage, growth stage of the pest, and microclimate (Auger *et al.*, 2003). Increases in temperature and changes in climate conditions can directly affect the fate of chemical pesticides through mechanisms such as increased volatility, solubility, and degradation (Noyes *et al.*, 2009).

Temperature changes strongly affect the effectiveness of spiromesifen in this study. At each concentration, the toxicity increased significantly with temperature changes. For example, by changing the temperature from 15 to 30 °C at a high concentration (16.8 mg ai/l), the toxicity increased about 1.3 folds, and at a low concentration (2.4 mg ai/l), it was increased by 2.7 to 4.1 folds. In some cases, the temperature increase from 15 to 30 °C can increase mortality by more than 12 folds (Table 2).

Exposure of living organisms to chemicals and an increase in temperature may increase sensitivity to chemicals and decrease heat tolerance, which may be due to the increased metabolic activity of chemicals under increased temperature (Slotsbo *et al.*, 2009). In the reports of Heugens *et al.* (2001) and Sokolova and Lannig (2008), it was mentioned that when living organisms are exposed to high temperatures and chemicals, their sensitivity to chemicals increases. Studies showed that the resistance levels of diamondback moths in spring and autumn are much higher than in summer (Wu and Jiang, 2004). In the research of Askari Saryazdi *et al.* (2013) and Sarbaz *et al.* (2017). The value of LC_{50} of spiromesifen on the TSSM was recorded as 26.39 and 5.95 mg ai/l, respectively. The difference in bioassay compared to our results may be due to the difference in pesticide application and duration of exposure. Our studies show the very functional role of temperature on the effect of spiromesifen on the TSSM. From the results, it can be concluded that by increasing the temperature to a particular value, if the environment and the host plant have the conditions of increasing temperature, the effectiveness of the pesticide can be significantly

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increased. It could be concluded that the role of temperature is more visible at low concentrations than in high concentrations (Cho *et al.*, 1999).

The mechanism of the effect of temperature on the rate of pesticide penetration and excretion, as well as pesticide activity, is very complex. The reduction in toxicity at low temperatures may be due to several reasons, including the degradation of the pesticide (Elshazly, 2015) as well as less activity of the pest at low temperatures. Other factors, including the slower penetration of the pesticide through the cuticle and the slower transfer to the target site inside the pest's body, are effective in slowing down the activity at low temperatures (Cagan, 1998; Garcia *et al.*, 2011; Ismail *et al.*, 2015; Jegede *et al.*, 2017). Relatively low or high temperatures are responsible for various physiological stress responses in mites. Thermal stress is caused by increased active oxygen, which causes oxidative damage (Stamou *et al*., 1995). Mites at high temperatures have more dynamic movement and are exposed to a higher dose of acaricide, which is detected by higher oxygen consumption.

The amount of α-esterase activity for spiromesifen increased significantly with increasing temperature. Assessment of tetronic acids on *T. urticae* and *Panonychus ulmi* indicated the induction of metabolic resistance by monooxygenases P450s and esterase as general detoxifying enzymes (Demaeght *et al.*, 2013). Our findings showed that the α-esterase in control increased with increasing temperature. Due to feeding on leaves and having special chemicals (allelochemicals) in the control mites, esterase enzymes for neutralization have probably increased (Durak *et al.*, 2021; Hung *et al.*, 1990; Mullin *et al.*, 1982). In our research, the α-esterase was more affected by the spiromesifen than the glutathione S-transferase. The esterase may affect the resistance of this pesticide in the future.

Conclusion

Many types of research have been done on the impact of temperature on the power of pesticide toxicity (Auger *et al.*, 2003; Elshazly, 2015; Everson and Tonks, 1981; Ismail *et al.*, 2015; Jegede *et al.*, 2017; Subramanyam and Cutkomp, 1987); however, there is a lack of data on TSSM in response to spiromesifen in the changing temperature. The study showed that temperature changes could affect pesticide toxicity and detoxification enzyme activity. Therefore, by adjusting the temperature and determining the appropriate spraying time in protected areas with controllable temperatures, higher pest control efficiency can be achieved by reducing pesticide use.

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تأثیر دما بر فعالیت کنه‌کشی اسپیرومسیفن روی کنه تارتن دولکه ای *Tetranychus urticae*

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چکیده: کنه تارتن دولکه ای *Tetranychus urticae* Koch به‌دلیل ویژگی‌هایی مانند پتانسیل تولیدمثلی بالا، چرخه زندگی کوتاه و روش تغذیه یکی از مخربترین کنه‌ها در بسیاری از گیاهان به‌شمار می‌رود. استفاده بیش‌از حد از ترکیبات شیمیایی بدون درنظر گرفتن عوامل محیطی منجر به افزایش باقی‌مانده سموم در محصولات غذایی و مقاومت در برابر آفتکش‌ها شده است. دما یک عامل غیرزنده ضروری است که بر جنبه‌های مختلف بیولوژیکی آفات و سطوح سمّیت آفتکش‌ها تأثیر می‌گذارد. در این مطالعه، اثر متقابل دماهای ۱۵، ۲۰، ۲۵ و ۳۰ درجه سلسیوس در دوره نوری ۱۶ ساعت روشنایی و ۸ ساعت تاریکی روی سمّیت اسپیرومسیفن روی کنه‌های بالغ بررسی شد. سپس سطح فعالیت آلفا استراز و گلوتاتیون S-ترانسفراز اندازه‌گیری شد. بالاترین LC_{50} در دمای ۱۵ درجه سلسیوس پس از ۲۴ ساعت (LC_{50} = 21.269 mg ai/l) و کم‌ترین مقدار در دمای ۳۰ درجه سلسیوس پس از ۴۸ ساعت (LC_{50} = 0.860 mg ai/l) مشاهده شد. سطح سمّیت نیز با افزایش دما افزایش یافت، بنابراین سمّیت در دمای ۳۰ درجه سلسیوس ۳/۶ برابر بیشتر از ۱۵ درجه سلسیوس ثبت شد. فعالیت α-استراز و گلوتاتیون S-ترانسفراز نیز با افزایش دما افزایش یافت، اما این افزایش تنها برای فعالیت استراز معنی‌دار بود. به‌طور کلی قدرت سمّیت آفتکش‌ها در مناطقی با تغییرات دمایی قابل کنترل می‌تواند به‌طور مؤثر تأثیر ارزشمندی برای کاهش مصرف آفتکش‌ها و افزایش کارایی کنترل آفات داشته باشد.

واژگان کلیدی: *Tetranychus urticae*، اسپیرومسیفن، دما، آلفا-استراز، گلوتاتیون‌اس ترانسفراز

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