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**THE ACARICIDAL EFFECT OF SULFUR ON *TETRANYCHUS URTICAE*
(ACARI: TETRANYCHIDAE) UNDER LABORATORY CONDITIONS**

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Abstract

A preliminary bibliographic study concerning the acaricide effect of sulfur reveals methodological problems and contradictory results. In order to assess the conditions which can modulate its toxic action, the acaricide effect of sulfur (micronised wettable sulfur) was studied on three developmental stages (eggs, protonymphs, females) of *Tetranychus urticae* Koch. Three parameters were tested: the temperature, the humidity and the dose of application. Whatever the conditions tested, the mortality was complete for protonymphs and high for adults.

The female fecundity and the viability of the progeny decreased when temperature and/or humidity increased. The toxic effect of sulfur on eggs (which was never proved before) depended on the combined influence of temperature and humidity, from a threshold temperature of 27°C and a threshold humidity of 75%. This study allows us to confirm that sulfur is toxic to *T. urticae*. The extension of this work will allow us to know in what proportion sulfur could be involved in controlling phytophagous mites.

INTRODUCTION

Since the second part of the XIXth century, sulfur spraying has increased in vineyards. For a long time, it was the only compound used against grape powdery mildew, *Uncinula necator* (Von Schweinitz and Burrill). With several metabolic ways of action against powdery mildew, sulfur is preventive, curative and eradivative (Bourdier and Agulhon 1987). Its side effects on phytophagous mites were first reported more than 70 years ago (Ewing 1914; Goodwin and Martin 1928, 1929). Sulfur has long been a useful fungicide-acaricide-insecticide and was the only acaricide used to control mites until about 1920 (Jeppson *et al.* 1975). Sulfur has many

advantages for use as an acaricide: low toxicity to applicators and consumers (Hoy 1987), low cost, availability in most areas, etc. Disadvantages include the fact that sulfur could be toxic to many plant species.

The mechanism of action on mites remains obscure but its efficiency is enhanced by high temperatures. A minimum of 17°C is necessary to observe the acaricidal effect (Klett 1965); at lower temperatures its efficiency is reduced (Wilcox and Howland 1956). Sulfur has been mentioned to have no effect on eggs of tetranychids, and the toxicity of dust sulfur has been mentioned to be independent of relative humidity (Grob 1951; Klett 1965).

Nevertheless, the existing literature contains conflicting statements regarding the impact of sulfur on

phytophagous mites. There is no clear pattern because of variations in species studied, sulfur formulations, dosage applied and environmental conditions (Hoy 1987; Kreiter 1987). As a consequence, sulfur is only often qualified of spider mite abundance reducer. More than 20 articles on sulfur have been published since the reviews of Hoy (1987) and Kreiter (1987), leading to contradictory conclusions. No recent study has investigated the factors affecting sulfur toxicity to spider mites.

The aim of this study is to analyse the acaricidal effect of sulfur under laboratory conditions. It was carried with different developmental stages of *Tetranychus urticae* Koch in various conditions of temperature and relative humidity, for a better understanding of variations in the miticidal effect of this fungicide.

MATERIALS AND METHODS

Mite colony

Specimens of a susceptible population of *T. urticae* were collected on *Sambucus* sp. in 1992. Those were used to start a colony which has been maintained on bean (*Phaseolus vulgaris* L. cv Contender) in climatic rooms at $25 \pm 1^\circ\text{C}$ and a photoperiod of 16:8 (L:D) h, without any exposure to pesticides.

Potter tower calibration and chemical used

A Potter spray tower (Burkard, Rickmansworth, Hertfordshire, UK) was used to apply aqueous spray suspension of sulfur in distilled water (Potter 1952). A volume of 1.5 ml of the solution was sprayed at 0.76 Mpa for 2.30 s, resulting in a wet deposit of $1.5 \pm 0.5 \text{ mg.cm}^{-2}$, determined just after spraying, using 10 plastic squares of $5 \times 5 \text{ cm}$ and a precision balance (Mettler AE 100, precision 10^{-1} mg , Mettler, Viroflay, France).

The fungicide tested is licensed and used for the control of powdery mildew on vine crop in France. The commercial formulation of sulfur Thiovit® microbilles (Syngenta, 92 845 Rueil-Malmaison, France), a wettable sulfur (micronized sulfur) 80% (AI), was used at the recommended full ($12,500 \text{ g.ha}^{-1}$) and a half ($6,000 \text{ g.ha}^{-1}$) field rates (dose per hectare in 400 liters of water). Another commercial sulfur formulation, Thiovit

jet® (Syngenta, 92 845 Rueil-Malmaison, France), was used for tests conducted after 2001.

Test protocols

Acaricides tend to affect more than one life stages, although some are more susceptible than others according to the acaricide (Marshall and Pree 1993). This is also true for sulfur (Abo-El-Ghar and Boudreaux 1958). On the other hand, testing only one developmental stage would negate the predictive value of the test, when considering a natural population (Robertson and Worner 1990). Consequently, the miticide effect of sulfur was evaluated on the basis of its effectiveness against eggs, protonymphs, females and their progeny. For each stage, the impact of 3 post-treatment temperatures: 20, 27.5 and $35 \pm 1^\circ\text{C}$; 4 levels of Relative Humidity: 30, 50, 75 and $90 \pm 5\%$; and 2 sulfur rates: 12 and 25 g AI ha^{-1} , were tested. A concentration-mortality experiment was also performed on sulfur treated eggs to compare the effect of both temperature and humidity. Distinct levels of relative humidity were obtained with saturated solutions in ventilated boxes (R.H.=30% with magnesium chloride $[\text{MgCl}_2 \cdot 6\text{H}_2\text{O}]$, R.H.=50% with magnesium nitrate $[\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}]$, R.H.=75% with sodium chloride $[\text{NaCl}]$ and R.H.=90% with potassium nitrate $[\text{KNO}_3]$ or in a climatic chamber Heraphyt® Phytotron HPS 500, (Heraeus Vötsch GmbH, 7460 Balingen Frommern, Germany).

Eggs

In order to obtain more than 100 eggs per bioassay, 40 *T. urticae* females were placed on 2 bean leaf discs ($\varnothing=2 \text{ cm}$) to oviposit at $25 \pm 1^\circ\text{C}$ for 24 h, after which females were removed. Silks were also gently removed with a fine camel hair brush and the discs containing the eggs were treated using a Potter spray tower. Mite eggs were counted after leaf discs were treated and dried, then held in controlled climatic conditions. The number of hatched eggs was recorded and compared to the control (sprayed with distilled water) (Abbott 1925).

The concentration-mortality experiments consisted on four climatic combinations: 27.5°C and R.H.=75%, 27.5°C and R.H.=90%, 35°C and R.H.=75% and

35°C and R.H.=90%. For each combination, 6 sulfur concentrations were tested. Each concentration consisted to treat two replicates of a minimum of 100 eggs (obtained as described above) and each treatment was replicated twice.

Other concentration-mortality experiments were conducted, with the second sulfur formulation, using *T. urticae* eggs, under the same four climatic conditions previously described. Each experimental unit consisted of 100 eggs (obtained as described above), and each treatment was replicated twice.

Larvae

In order to obtain more than 100 larvae per bioassay, 40 females *T. urticae* were placed on two bean leaf discs (Ø=2 cm) to oviposit at $25 \pm 1^\circ\text{C}$ for 24 h, after which females were removed. After one week at the same temperature, larvae hatching from those eggs were counted and treated at the full and the half recommended field rates. They were then held under the same four climatic conditions previously described.

Larvae that did not reach the protochrysalis stage were considered dead. The numbers of dead larvae were compared with the control (sprayed with distilled water).

Protonymphs

Ten newly emerged protonymphs placed on 2 cm diameter bean leaf discs were treated as previously described, then dried and stored in pre-defined climatic conditions as above. Bioassays were replicated three times. Control treatment consisted of the spray of distilled water. Evaluation consisted in determining the number of treated protonymphs that reached adulthood. Nymphs that did not reach the adult stage three days after all the nymphs of the control had reached that developmental stage were considered dead.

Adults

Mated two to three days old females were used because the precision was greater with young mites, their response to a miticide is likely to be more uniform.

Moreover, 7 days old females of *T. urticae* can be more tolerant than 1 day old females (Kabir and Chapman 1997). Three parameters were checked: mortality, fecundity and viability of the progeny of each female.

For each concentration and for the control treatment (sprayed distilled water) the following procedure was applied: 30 females and 2 batches of 30 bean leaf discs (2 cm diameter) were treated using a Potter spray tower. Leaf discs with major veins were not used to assure homogeneity of leaf surface (Warwick and Wrensch 1986). Discs were placed upper surface down on a layer of wet cotton pad in Petri dishes. In a first step, after being dried, females were transferred onto the first batch of treated discs. One female was gently placed per disc with the help of a camel hair brush and held in pre-defined controlled conditions of temperature and humidity as previously described and a photoperiod of 16:8 (L:D) h. The other 30 discs of the second batch were kept unused under the same climatic conditions. In a second step, after four days of the treatment, live females were transferred to the second batch of leaf bean discs, and maintained under the same climatic conditions for six more days.

The discs of the first batch were kept under the same environmental conditions to estimate their viability. Juvenile mortality and molting were checked daily until the protonymph stage was reached. The viability of the progeny was calculated by dividing the number of protonymphs obtained by the number of eggs laid during the first four days. Female mortality was daily checked for 10 days. Mites unable to walk but capable of some leg movement were scored as dead (Busvine 1980; Kabir *et al.* 1993). Female that had run onto the moist cotton were included in the mortality calculation.

The daily oviposition rate of each female was calculated considering only the period in which it was alive.

Similarly another experiment was conducted, consisting of the study of the mortality of females treated with another sulfur formulation. A batch of fifty females was treated (with the full and half the recommended field rates), placed on treated bean leaf discs (one female per disc) and held at one of the four climatic conditions: 27.5°C and R.H.=75%, 27.5°C and R.H.=90%, 35°C and R.H.=75% and 35°C and R.H.=90%. The mortality was checked 4 days after the beginning of the experiment, and compared with the control.

Statistical analysis

Corrected mortality of females and eggs hatching were analyzed with χ^2 tests (contingence tables $r \times k$, $\alpha = 5\%$) (Abbott 1925).

Dose-mortality data were corrected for control mortality and subjected to probit analysis (Abbott 1925; Finney 1977). LC50 and 95% confidence intervals were calculated using Probit Analysis® program (Praxeme-CNRS 1995). Mortality rates were assessed by covariance analysis. F test ($P < 0.05$) were used to test whether the variances of the slopes and intercepts were significantly different (Scherrer 1984). Fecundity and viability of the progeny of each female were analyzed with Kruskal-Wallis analysis of variance completed by Duncan Multiple Range Test ($\alpha = 5\%$).

Influence of interactions between temperature, humidity and sulfur concentrations on females fecundity and viability of their progeny were analysed with multi-way factorial analysis of variance.

RESULTS

Eggs

Sulfur toxicity to eggs was highly dependant on post-treatment climatic conditions. It increases with a rise in temperature and relative humidity (R.H.) (Fig. 1). At low R. H. levels (30 and 50%), the mortality of eggs sprayed with sulfur was very low ($< 10\%$) and not significantly different from the mortality obtained in the control ($P < 0.99$; $\chi^2 = 0.478$; $df = 10$), whatever the temperature and the sulfur rate. At the lowest temperature, mortality was also not different from the controls whatever the relative humidity and the sulfur rate sprayed ($P < 0.98$; $\chi^2 = 0.173$; $df = 6$).

For temperatures of 27.5°C and above, the ovicidal effect of sulfur was significant if $RH > 75\%$ ($P < 0.0001$; $\chi^2 = 43.661$; $df = 6$). It still remains moderate because at this temperature many larvae hatched (48% in the worst case). For R.H. level of 75% or above, and for

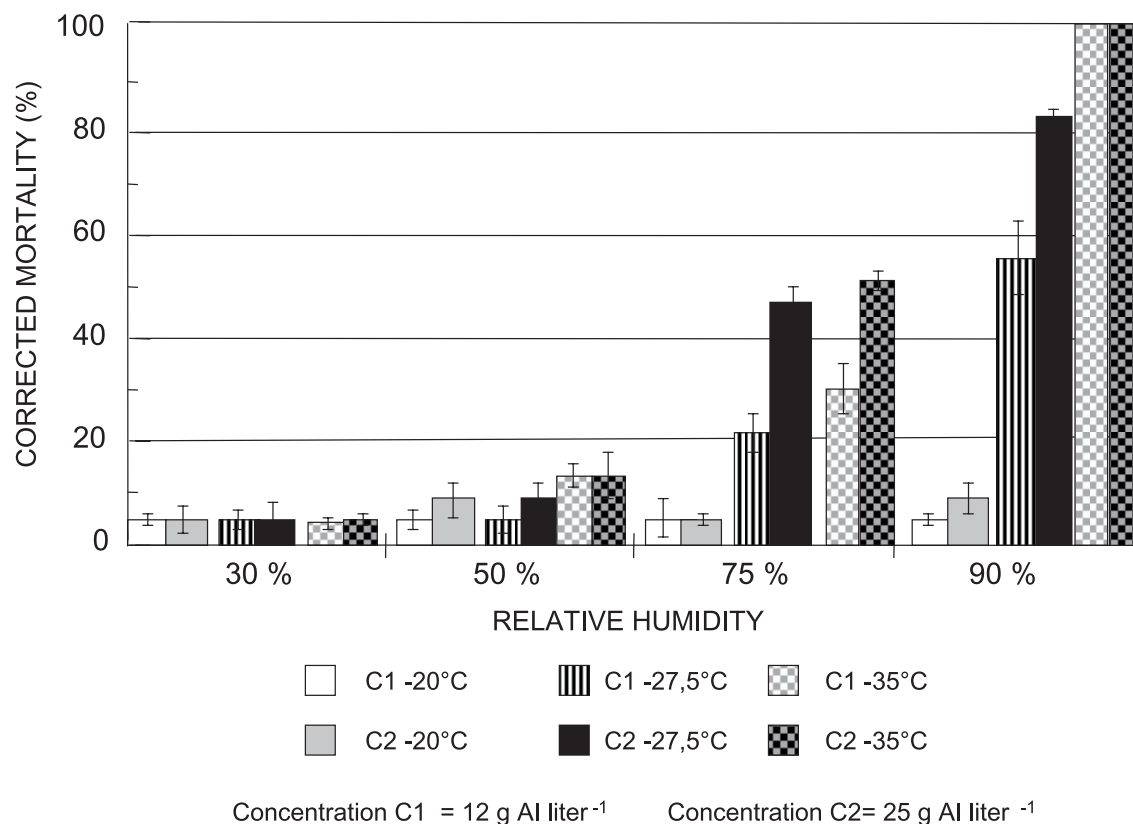


Figure 1. Influence of temperature, R.H. and sulfur concentration on *Tetranychus urticae* eggs mortality.

temperatures above 20°C, the hatching in the treated plots was significantly lower than in the control plots ($P < 0.0001$; $\chi^2 = 145.78$; $df = 10$).

Sulfur toxicity to eggs increases at temperatures and R.H., above these threshold values. The full sulfur recommended rate (10,000 AI g.ha⁻¹) was not significantly more active than half rate ($P < 0.26$; $\chi^2 = 13.529$; $df = 11$) even under favourable climatic conditions to sulfur toxicity expression ($P < 0.431$; $\chi^2 = 2.754$; $df = 3$).

The concentration-mortality experiment confirmed the cumulative effect of temperature and humidity on the acaricidal effect of sulfur. Covariance analysis revealed significant differences between responses obtained at 27.5 or 35°C and with R.H. of 75 or 90% (Table 1). Positive temperature and relative humidity coefficients were observed. Slopes of regression lines increased with rise in temperature and relative humidity, indicating positive correlation between the acaricidal potential of sulfur and the climatic parameters (Table 1). At LC₅₀, toxicity was 6-fold higher

for eggs at 27.5°C and 90% R. H. compared to eggs at 27.5°C and 75 % R. H., whereas it was 9.7-fold higher for eggs at 35°C and 90% R. H. compared to eggs at 35°C and 75% R. H. Between 27.5 and 35°C, toxicity increased by 5-fold at R. H.=75% and by 7.7 fold at R. H.=90%.

The same pattern is observed for the second formulation tested (Table 2). The increase of the toxicity was correlated with the increase in temperature and R.H.. At LC₅₀, toxicity was 3.9-fold higher for eggs at 27.5°C and 90% R.H. compared to eggs at 27.5°C and 75%, whereas it was 8.7-fold higher for eggs at 35°C and 90% R.H. compared to eggs at 35°C and 75% R. H. Between 27.5°C and 35°C, toxicity increased by 17-fold at R.H. of 75% and by 38.1-fold at R.H. of 90%.

The comparison between the two commercial formulations showed that the first was more toxic than the second for eggs. For example, at LC₅₀ corresponding to 27.5°C-90 R.H., toxicity was 16.5-fold higher for the first formulation.

Table 1. Influence of 4 climatic conditions on sulphur toxicity to *Tetranychus urticae* eggs.

Climatic conditions	No. of eggs tested (No. concentrations)	Control Mortality (%)	Sople \pm SE	LC50 (95% CI) (g AL litre ⁻¹)	Chi ²	Probability
27.5°C-75 %R.H.	1466(6)	7.62	1.235 \pm 0.098	24.280 (21.113-27.923)	5.308	0.257
27.5°C-90 %R.H.	1415(6)	8.93	2.674 \pm 0.329	3.890 (3.239-4.667)	5.191	0.268
35°C-75 %R.H.	1571(6)	9.04	2.523 \pm 0.130	4.867 (4.495-5.270)	1.972	0.741
35°C-90 %R.H.	1510(6)	5.04	3.383 \pm 0.196	0.503 (0.444-0.571)	7.439	0.059

F tests: F 0.05 (k-1, n-k-1), with k, number of curves, *Fsv*, slope variation; *fov*, ordinate variation. 27.5°C-75 % / 27.5°C-90 %: *Fsv* (6.45) $> F$ 0.05 (5.12). 27.5°C-90% / 35°C-75%: *Fsv* (0.21) $< F$ 0.05 (5.12), *Fov* (6.08) $> F$ 0.05 (5.12). 27.5°C-90% / 35°C-90%: *Fsv* (1.86) $< F$ 0.05 (5.12), *Fov* (17.32) $> F$ 0.05 (5.12).

Table 2. Influence of 4 climatic conditions on sulphur toxicity to *Tetranychus urticae* eggs.

Climatic conditions	No. of eggs tested (No. concentrations)	Control Mortality (%)	Sople \pm SE	LC50 (95% CI) (g AL litre ⁻¹)	Chi ²	Probability
27.5°C-75 %R.H.	944(6)	1.35	1983 \pm 0.0222	249.452(204.744-329.440)	2.169	0.705
27.5°C-90%R.H.	942(6)	0.68	1.617 \pm 0.107	64.102(55.21-74.61)	5.569	0.234
35°C-75%R.H.	1045(5)	1.11	1.568 \pm 0.084	14.664(12.514-17.198)	6.319	0.229
35°C-90%R.H.	1063(5)	2.18	3.548 \pm 0.868	1682(1.156-2.452)	24.485	0.000

F tests: F 0.05 (k-1, n-k-1), with k, number of curves, *Fsv*, slope variation; *fov*, ordinate variation. 35°C-90% / 27.5°C-90 %: *Fsv* (5.71) $> F$ 0.05 (5.12). 27.5°C-90% / 35°C-75%: *Fsv* (0.16) $< F$ 0.05 (5.12), *Fov* (37.92) $> F$ 0.05 (5.12). 27.5°C-75% / 27.5°C-90%: *Fsv* (1.69) $< F$ 0.05 (5.12), *Fov* (193.43) $> F$ 0.05 (5.12).

Larvae

At all tested environmental conditions and whatever the sulfur rate sprayed, mortality was total, while in the control mortality was only of 3%. It seems that the larval stage is about as susceptible to sulfur as the protonymphal stage.

Protonymphs

No matter what the temperature, humidity conditions or sulfur rate, the mortality was always total. Part of treated protonymphs developed into the deutochrysalis or teleiochrysalis stages but were unable to proceed development to the following stages while in the control plots the mean mortality was only of 4.4%. Results indicated that the protonymphal stage of *T. urticae* was one of the most susceptible to sulfur.

Adults

The mortality was total for nearly every plot of treated females except two assays performed at 20°C with R.

H.levels of 30% and 50% (Fig. 2-B). Because in most cases as the female mortality was total at the end of the experiment, a comparison of the pattern of sulfur toxicity expression at different temperatures and R. H. levels was done by considering observed mortalities changed, four days after treatment (Fig. 2-A). At this time, mortality in the treated plots was already significantly higher than the mortality observed in the control plot, for which the mean value was $9.6\% \pm 2.2$ (SE) ($P < 0.0001$; $\chi^2 = 127.62$; $df = 22$).

When using another sulfur formulation, the mortality was complete at all tested climatic conditions tested, whatever the sulfur rate, while mortality observed in the control never exceeded 5%.

As observed for egg experiments, the acaricidal effect of sulfur on females increased with temperature ($P < 0.0001$; $\chi^2 = 71.3$; $df = 14$) or R.H. ($P < 0.0001$; $\chi^2 = 67.25$; $df = 15$).

Mortality observed at 20°C was significantly lower than at observed at 27.5°C ($P < 0.0001$; $\chi^2 = 67.566$; $df = 7$), which is itself lower than the mortality obtained for a temperature of 35°C ($P < 0.001$; $\chi^2 = 25.795$; $df = 7$). Full rate of sulfur was significantly more effective than half rate at 20 and 27.5°C ($P < 0.003$; $\chi^2 = 15.244$; $df = 7$).

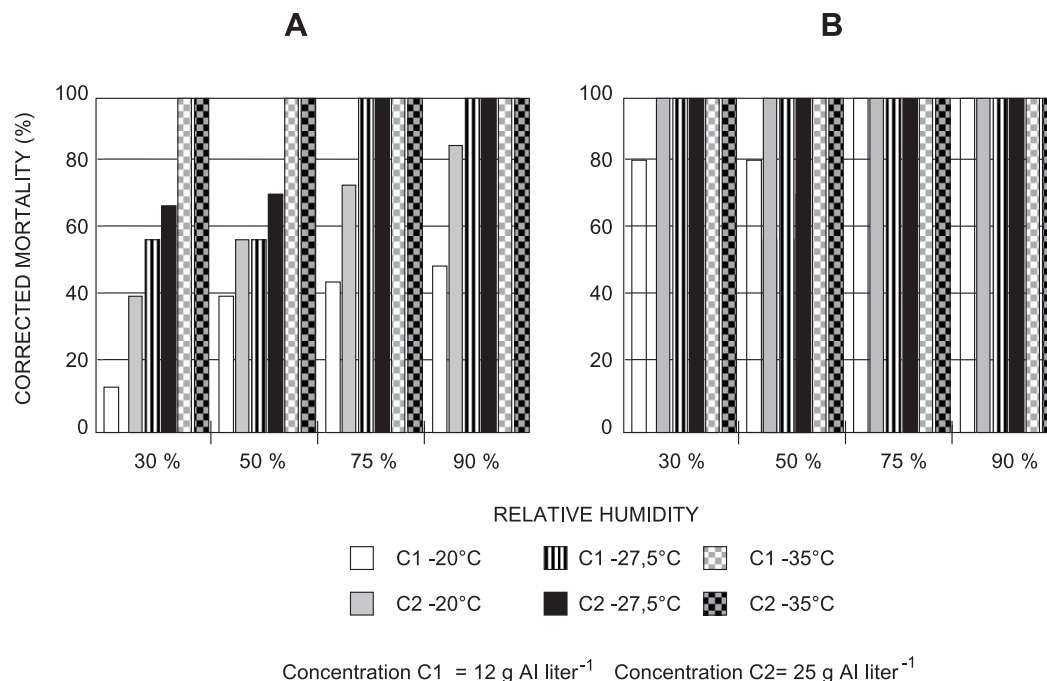


Figure 2. Influence of temperature, R.H. and sulfur concentration on *Tetranychus urticae* females mortality at D+4 (full lines) and D+10 (dotted lines).

Post-treatment temperature, R.H. and sulfur concentration were also key factors in sulfur activity on female fecundity. Treated female fecundities were always significantly lower than the one obtained in the control plots ($P < 0.0001$; $F = 21.91$), and reduced by half in the best case (Fig. 3). This acaricidal potential on female fecundity was significantly influenced by temperature ($P < 0.0001$; $F = 114.75$), R. H. ($P < 0.0001$; $F = 51.75$) and sulfur concentration ($P < 0.0001$; $F = 26.72$). The acaricidal potential of this fungicide was more pronounced at progressively higher post-treatment temperatures and R.H. levels and concentrations. Significant temperature x R. H. interaction ($P < 0.0001$; $F = 10.12$) as well as high significant temperature x sulfur concentration interaction ($P < 0.0009$; $F = 7.19$) but not a R. H. x sulfur concentration interaction ($P < 0.2066$; $F = 1.53$) on female fecundity were also put into evidence by three-way factorial ANOVA (Table 3). Second order interaction was also significant ($P < 0.0008$; $F = 3.93$).

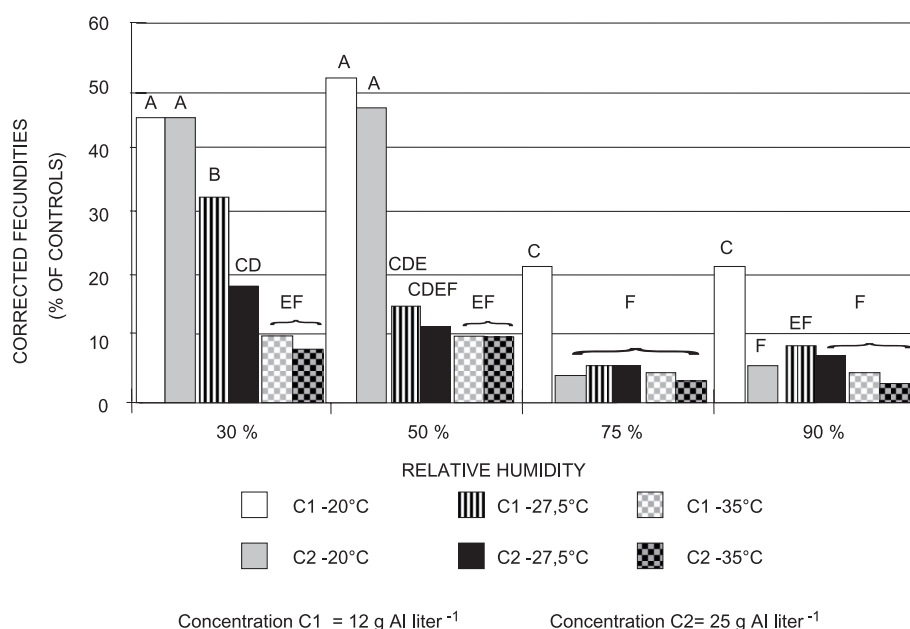
Sulfur strongly affected the viability of the progeny of treated females ($P < 0.0001$; $F = 6.63$) (Fig. 4). Viability of the progeny was always reduced by more than half in comparison with the control for the lowest

temperature and R.H. tested (20°C, R. H.=30 and 50%), with half the rate applied.

Temperature ($P < 0.0001$; $F = 25.79$), R. H. ($P < 0.0001$; $F = 44.23$) and sulfur concentration ($P < 0.0001$; $F = 63.47$) significantly affect sulfur acaricidal effect. As with other previous parameters, sulfur toxicity increased with rise in temperature and R.H. (Table 3). Mortality of progeny was significantly influenced by interactions between temperature and R. H. ($P < 0.0001$; $F = 6.58$), temperature and sulfur concentration ($P < 0.0002$; $F = 9.02$) and R. H. and sulfur concentration ($P < 0.0001$; $F = 15.56$) (Table 3). Second order interaction was not significant ($P < 0.0826$; $F = 1.88$).

DISCUSSION

Our results confirm that larvae and protonymphs are the most susceptible stages to sulfur. However, unlike results obtained by Klett (1965), we have demonstrated a clear effect of sulfur on eggs of *T. urticae*. Under our experimental conditions, the combined action of temperature and humidity significantly affects the results,

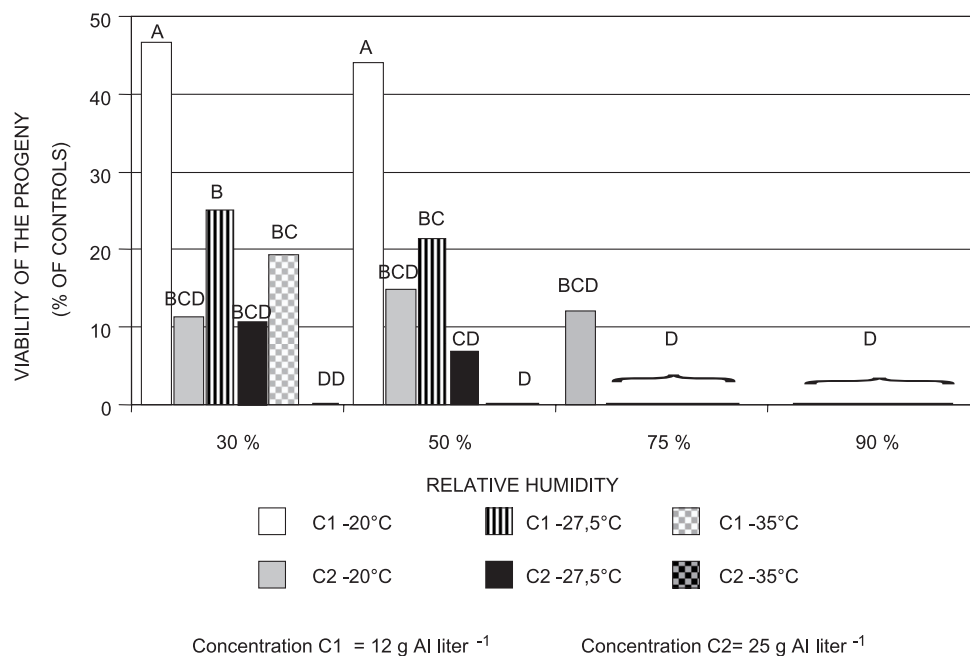


Means sharing a common letter are not significantly different ($P < 0.05$) Duncan's Multiple Range Test

Figure 3. Influence of temperature, R.H. and sulfur concentration on *Tetranychus urticae* females fecundity.

Table 3. Three-way analysis of fecundity and viability of the progeny of sulfur treated *Tetranychus urticae* females.

Source of variation	DF	Fecundity		Viability of the progeny	
		F-value	Probability	F-value	Probability
Temperature (A)	2	114.749	0.00001	25.798	0.00001
R.H. (B)	3	51.705	0.00001	44.229	0.00001
Sulphur concentration (C)	1	26.722	0.00001	63.466	0.00001
A x B	6	10.12	0.00001	6.587	0.00001
A x C	2	7.197	0.00086	9.024	0.00015
B x C	3	1.529	0.20660	15.56	0.00001
A x B x C	6	3.934	0.00079	1.884	0.08261



Means sharing a common letter are not significantly different ($P < 0.05$) Duncan's Multiple Range Test

Figure 4. Influence of temperature, R.H. and sulfur concentration on the viability of the progeny of treated females of *Tetranychus urticae*.

and mortality of eggs is observed above 27.5°C for a R. H. of at least 75%.

The influence of humidity was never fully tested before, what could have been the reason for the action of the normal sulfur rate on eggs have never been demonstrated before. Klett's experiments were conducted only with dust sulfur, at 27.5°C and 70% R.H. The

ineffective effect of sulfur on eggs determined by that author could be related to the formulation he used or to the low level of R.H. in his experiments. The variable effect of different sulfur formulations on the control of tetranychid mites has already been verified on *T. urticae* and *Tetranychus turkestani* Ugarov and Nikolski (Jackson and Leigh 1967; Basu and Pramanik 1968). We have

also demonstrated that the formulation plays a role in the toxicity of sulfur when applied on eggs.

Concerning the acaricidal effect of sulfur on *T. urticae* adults, as Klett (1965) had demonstrated, temperature influences sulfur toxicity. The effect of temperature on the toxicity of some acaricides on *T. urticae* is well known (Boller *et al.* 1984; Kreiter and Le Menn 1993). Nevertheless, we observed that the humidity could also increase the toxicity of sulfur to females. Sulfur toxicity in females appears to be the result of a cumulative effect of temperature and humidity and not a combined effect, as it is for eggs.

As female mortality was complete since the fifth day for the two higher humidities, numerous females were moribund the days following treatment. As a consequence, a significant reduction the fecundity of female treated with sulfur was observed.

Results on juveniles have been reinforced and completed by those obtained on female viability of progeny. It appears that sulfur had an interesting residual effect because under certain climatic conditions, those corresponding to a low egg hatching (for high temperatures and R.H.), the viability of the progeny tended to be nil.

Nevertheless, at low temperatures and humidity levels, we have sometimes obtained protonymphs in significant number but always less than half the number obtained in the control. These results would undoubtedly differ a bit, reducing the viability of the progeny, if this index was calculated on the basis of the number of adults obtained instead of the number of protonymphs. The residual effect may also affect deutonymphs and teleiochrysalis.

As climatic factors influence acaricidal potential of sulfur, this perhaps partly explains the inconsistency of the results obtained in field experiments. According to our results, sulfur could be used as other biologically pest control materials for spider mites, but the result of the application may not always be satisfactory, because of the significant influence of temperature and humidity on its effect on the mites. In tropical countries this interesting side effect would be of a great interest.

Furthermore, like with other agrochemicals, herbicides (Boller *et al.* 1984; Kreiter and Le Menn 1993), insecticides and fungicides (Grout *et al.* 1997; Sterk *et al.* 1999), according to the Integrated Pest Management concept, specific attention must be paid to side effects on beneficials, since outbreaks of tetranychid

mites often occur after the application of non-selective products (Trichilo and Wilson 1993). Mites of the Phytoseiidae family have a great potential as biological control agents of phytophagous mites (McMurtry and Croft 1997). The susceptibility of the phytoseiids to sulfur has long been known; some cases of resistance of phytoseiids to sulfur have also been demonstrated (Cutright 1944; Hoy and Standow 1982). Nevertheless, as in the case of phytophagous mites, there are conflicting results. There are an equal number of citations showing sulfur toxicity and sulfur innocuity to phytoseiids. Concerning other beneficial arthropods, sulfur effect on an important species of the International Organisation for Biological Control, *Trichogramma cacoeciae* Marchal (Hassan 1998), depending on life stage, could vary from harmless to moderately harmful (Hassan 1994).

Studying the influence of climatic factors such as temperature and humidity with certain key factors on sulfur acaricidal activity on beneficials would enable us to know more about the side effects of this inorganic fungicide.

As some mite species seem to be more susceptible to sulfur than others (Mistic and Rainwater 1952; Abo-El-Ghar and Boudreaux 1958; Jackson and Leigh 1967), it would be interesting to test the effect of sulfur on other Tetranychidae or Eriophyidae to find out whether it could play a role in controlling other phytophagous mite species.

CONCLUSION

This study contribute to the knowledge of the acaricidal effect of wettable sulfur. It confirms that sulfur is a product intrinsically active against *T. urticae*. Sulfur is adulticide, nymphicide and, under specific conditions of temperature and humidity, wettable sulfur is also ovicide. Even if temperature dependent efficiency of acaricides is a well known phenomenon (Fisher and Hansel 1964; Everson and Tonks 1981; James *et al.* 1988; Marshall and Pree 1993; Kabir and Chapman 1997), this is the first study of the combined effect of temperature and humidity on the acaricidal activity of a fungicide. Our study demonstrates that there is either a combined or cumulative effect of temperature and humidity and interactions between these factors that influence the acaricidal potential of sulfur.

To date, the mode of action of sulfur on mites remains poorly understood. As the interactions between temperature and dose of pesticide are complex, involving penetration, distribution, excretion, metabolism and action of the pesticide, getting information on the mode of action of sulfur in relation to climatic conditions would be of great interest.

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