



Influence of Storage Duration on The Toxicity of *Moringa oleifera* (Moringaceae) Oil to *Tetranychus urticae* (Acari: Tetranychidae)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AMH and JRC designed the study, wrote the protocol and first draft of the manuscript. Authors TPC, CMR and FAO conducted experiments and managed the literature searches and did a critical review of the manuscript. Author JRC managed the analyzes of the study. All authors read and approved the final manuscript.

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ABSTRACT

The objective of this study was to evaluate the storage time of *Moringa oleifera* oil on the acaricidal activity on *Tetranychus urticae*. Was used amber bottle for storage of oil which remained in a room at 25°C. The storage times considered in the experiment were 0, 30, 60, 90 and 120 days after extraction. At each time a suspension at the concentration of 3% (v/v) was applied on the mite. A completely randomized design with 5 treatments (storage times) was used, containing 8 replicates, composed of 12 females per replicate. The application was carried out by spraying. Mortality data were submitted to the sphericity test and then to analysis of variance, followed by non-linear regression. The analysis of variance revealed that the time factor of storage significantly affected the mortality of the mite, according to an exponential model. Mortality was increasing, reaching a mean of 74.16 ± 8.37% at 120 days. The oil of *M. oleifera* is promising for the control of *T. urticae*, improving the acaricidal activity over time.

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1. INTRODUCTION

Phytophagous mites, such as *Tetranychus urticae* Koch (Acari: Tetranychidae), are pests of crops of economic importance, such as cotton, soybean, tomato, papaya, strawberry and others [1-5]. When not controlled, these organisms can cause damage to crops. For most crops, the chemical method has been the main tool to combat this mite. The products used generally have molecules of wide spectrum, eliminating even natural enemies, besides possessing high residual power [5,6].

Faced with this, the search for healthier acaricides has intensified research that provides less aggression to agroecosystems. Among them, studies related to predatory mites [7,8], entomopathogenic fungi [2,9] and plants with insecticides property have gained prominence [4]. Extracts, fixed and essential oils from vegetables have been studied in several insect pests and vectors of diseases, providing promising results [4,10-12].

Moringa oleifera Lam, Moringaceae, is a plant studied for several purposes, from biodiesel production [13], to insecticidal activity on disease vectors [14-17] and even on *T. urticae* [18]. However, the form of action and effects of the substances present in the oil are still little explored on agricultural pests. In addition, information on the storage time and temperature of the oil, types of storage containers of these extracts and oils [13], in order to preserve the insecticidal / acaricidal characteristics, are deficient.

The objective of this study was to evaluate the action and duration of the acaricidal effect of the oil of *M. oleifera*, stored in amber glass, for the control of *T. urticae*, under laboratory conditions.

2. MATERIALS AND METHODS

2.1 Rearing and Maintenance of *Tetranychus urticae*

The mite rearing was established in the entomology sector of the Federal Institute of Espírito Santo, Itapina Campus, Colatina-ES (IFES-Campus Itapina), in *Canavalia ensiformis* plants cultivated in pots without any phytosanitary treatment. The vessels were

packaged in wooden cages (50 x 50 x 100 cm), coated with anti-aphid screen and with a front opening closed by removable glass. Rearing was carried out in air-conditioned rooms regulated at $25 \pm 1^\circ\text{C}$, relative humidity $70\% \pm 10$ and 12h photophase.

2.2 Extraction and Storage of *M. oleifera* Oil

Moringa seeds were collected at the IFES-Campus Itapina and subjected to the extraction of the oil by cold pressing. After this procedure, the oil was filtered through a fine mesh screen and stored in an amber glass container in an air-conditioned room with temperature of $25 \pm 1^\circ\text{C}$, relative humidity $70\% \pm 10$ and photophase of 12 h.

2.3 Bioassays

For the experiment, aqueous suspensions of 3% (v/v) moringa oil were used, which corresponded to the highest concentration soluble in water. As solvent, distilled water plus Tween[®] 80 adhesive spreader (0.05% v/v) was used. Thereafter, the mixture was left under stirring (magnetic stirrer) for 30 minutes at room temperature. The storage times for the oil considered in the experiment were 0, 30, 60, 90, 120 days after extraction.

Leaf discs of *C. ensiformis* ($\varnothing = 4$ cm) were introduced into Petri dishes (10 x 1 cm), containing cotton moistened around this to maintain leaf turgescence and avoid mite leakage. For each plate a leaf disc was considered, which constituted a repetition. Twelve mite females were transferred per replicate.

To perform the applications, an airbrush (Model SW-130K) was used, connected to a compressor calibrated at a constant pressure of 25 psi. The application suspension volume was 3 ml per replicate. Afterwards the plates were conditioned in an air-conditioned room (temperature of $25 \pm 1^\circ\text{C}$, RH of $70 \pm 10\%$ and photophase of 12 h).

The acaricidal effect was evaluated 24, 48 and 72 hours after the application, registering the mortality of the individuals. For the control treatment, only the solvent was used, and these mortality values were used to correct the treatments using the formula proposed by Abbott [19].

2.4 Statistical Analysis

A completely randomized design with 5 treatments (storage time) was used, with 8 replications, using the accumulated mortality data in 72h. Since the treatments are time-repeated measures, the data were submitted to the Mauchly [20] test to verify the sphericity, as recommended by Huynh & Feldt [21] for time-repeated measurements [22]. After checking the sphericity, we performed the analysis of variance followed by non-linear regression analysis in R software version 3.4 [23].

3. RESULTS AND DISCUSSION

The covariance matrix showed sphericity ($W = 0.61$, $p = .38$). Based on this principle, the analysis of variance was performed. It was verified that the time factor was adjusted to the exponential model ($F = 55.22$; $p < .01$) (Fig. 1). There was an increase in the mortality of the mite with the increase of the storage time, reaching $74.16 \pm 8.37\%$ of mortality with 120 days of storage.

The oil from moringa seeds is a potential for the management of *T. urticae*. The increase in mortality with the advancement of storage time was an interesting result. Such a result may be due to the fact that many products lose their

potential/effectiveness due, perhaps, to some process of deterioration and / or oxidation. On the other hand, *M. oleifera* oil can maintain some properties, such as density and viscosity, and increase the percentage of free fatty acid for up to 24 months [13].

Toxicity activity of *M. oleifera* in arthropods was reported by other researchers in coleopterans [24-27], dipterans [14-17], lepidopterans [28-30] and mites [18].

On *Aedes aegypti* (L.) (Diptera: Culicidae) the aqueous extract of the seed presented larvicidal action and ovicidal, being able to cause 100% mortality after 24 h of exposure [16]. For the malaria vector, *Anopheles stephensi* Liston (Diptera: Culicidae), the use of methane extract from moringa seeds, provided larval and pupal mortality [15]. In *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *Oryzaephilus mercator* (Faur) (Coleoptera: Cucujidae) and *Ryzopertha dominica* (Fabr.) (Coleoptera: Bostrichidae) methanoic extract caused mortality of more than 90% of insects [26]. Aqueous extracts of moringa seeds presented high toxicity to *T. urticae*, resulting in a lethal concentration for 50% of the population around 12.39% [18]. These results demonstrate that, independent of the solvent used, *M. oleifera* oil is toxic to different groups of insects and to the mite.

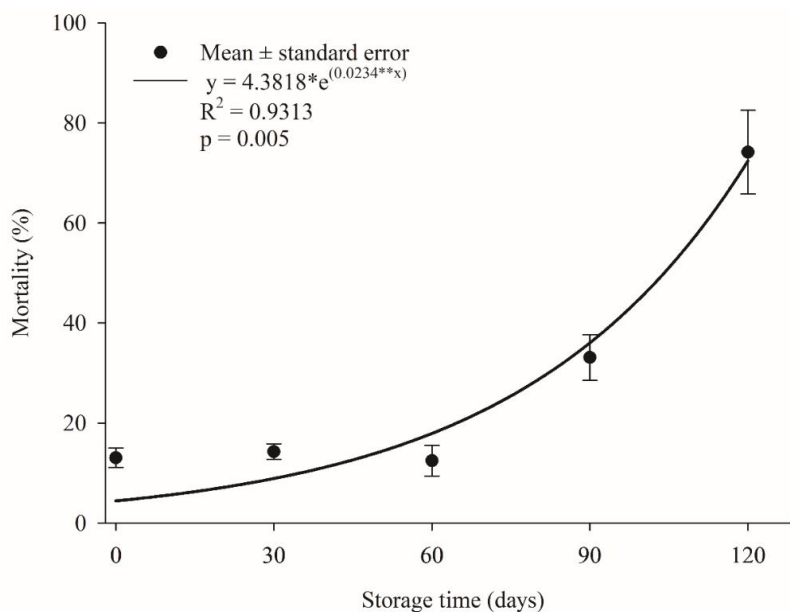


Fig. 1. Mortality of *Tetranychus urticae* treated with *Moringa oleifera* oil with different storage times

** and * significant regression coefficient at the 1 and 5% level, respectively

Moringa oil is a fixed type, as are soybean oils (*Glycine max* - Fabaceae), canola (*Brassica napus* - Brassicaceae) and castor bean (*Ricinus communis* - Euphorbiaceae). Thus, the choice of the solvent is crucial for the solubilization and homogenization of the suspension so that it does not alter its properties [31]. Therefore, the use of the Tween[®] adhesive spreader provides these characteristics without affecting the potentiality of the oil, as well as reported in papers used with *R. communis* oil [10,11].

The moringa studies show that plants of this genus are rich in lectin [32], α - and γ -tocopherols, glycosylates, nitriles, glycosides, quercetin, canferol, rhamnosides, isothiocyanates and steroids [33]. In addition, the oil has as main component oleic acid (\approx 78.0%) [34].

Lectin is a type of protein, which prevents the process of digestion and absorption of nutrients in the insects, causing death by malnutrition [30, 32]. Seeds of *M. oleifera* contain cMoL (coagulant *M. oleifera* Lectin) and WSMoL (Water-Soluble *M. oleifera* lectin) lectins that promoted mortality in *A. aegypti* larvae [30,35]. At the concentration of 1% (m/m), lectin cMoL caused mortality of pupae of *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) and delayed the total development cycle of surviving insects [28].

In the case of fatty acids, larvicidal and anti-nutritive activity of oleic acid have been reported on *A. aegyptii*, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), *Lymantria dispar* L. (Lepidoptera: Lymantriidae), *Culex quinquefasciatus* Say (Diptera: Culicidae) [36, 37]. Faced with this, it can be seen that *M. oleifera* presents lethal and anti-nutritive activity, as well as sublethal effect.

4. CONCLUSION

It is concluded that the oil of *M. oleifera* shows acaricidal activity to *T. urticae*. Storage time can influence positively its toxicity to the mite. Oil stored at 120 days is most suitable for use in mite control.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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