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2 **STORAGE OF THE OIL OF *Moringa oleifera***
3 **AFFECTS YOUR TOXICITY ABOUT *Tetranychus***
4 ***urticae*?**
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10 **ABSTRACT**
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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 $74.16 \pm 8.37\%$ 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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13 *Keywords: Keywords: mite; alternative control; storage time; Moringa.*
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16 **1. INTRODUCTION**
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18 Phytophagous mites, such as *Tetranychus urticae* Koch (Acari: Tetranychidae), are pests of
19 crops of economic importance, such as cotton, soybean, tomato, papaya, strawberry and
20 others [1 - 5]. When not controlled, these organisms can cause damage to farmers. For most
21 crops, the chemical method has been the main tool to combat this mite. The products used
22 generally have molecules of wide spectrum, eliminating even natural enemies, besides
23 possessing high residual power [5, 6].

24 Faced with this, the search for healthier products has intensified research that provides less
25 aggression to agroecosystems. Among them, studies related to predatory mites [7, 8],
26 entomopathogenic fungi [2, 9] and plants with insecticides property has gained prominence
27 [4]. Extracts, fixed and essential oils from vegetables have been studied in several insect
28 pests and vectors of diseases, providing promising results [4, 10 - 12].

29 *Moringa oleifera* Lam, Moringaceae, is a plant studied for several purposes, from biodiesel
30 production [13], to insecticidal activity on disease vectors [14 - 17] and even on *T. urticae*
31 [18]. However, the form of action and effects of the substances present in the oil are still little
32 explored on agricultural pests. In addition, information on the storage time and temperature
33 of the oil, types of storage containers of these extracts and oils [13], in order to preserve the
34 insecticidal / acaricidal characteristics, are deficient.

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

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39 **2. MATERIAL AND METHODS**

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41 **2.1 Creation and maintenance of *Tetranychus urticae***

42 The mite creation was established in the entomology sector of the Federal Institute of
43 Espírito Santo, Itapina Campus, Colatina-ES (IFES-Campus Itapina), in *Canavalia*
44 *ensiformis* plants cultivated in pots without any phytosanitary treatment. The vessels were
45 packaged in wooden cages (50 x 50 x 100 cm), coated with anti-aphid screen and with a
46 front opening closed by removable glass. Creation was maintained in air-conditioned rooms
47 regulated at $25 \pm 1^\circ\text{C}$, relative humidity $70\% \pm 10$ and 12h photophase.

48 **2.2 Extraction and storage of moringa oil**

49 Moringa seeds were collected at the IFES-Campus Itapina and subjected to the extraction of
50 the oil by cold pressing. After this procedure, the oil was filtered through a fine mesh screen
51 and stored in an amber glass container in an air-conditioned room with temperature of $25 \pm$
52 1°C , relative humidity $70\% \pm 10$ and photophase of 12h.

53 **2.3 Bioassays**

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

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

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

67 The acaricidal effect was evaluated 24, 48 and 72 hours after the application, registering the
68 mortality of the individuals. As control treatment, only the solvent was used, and these
69 mortality values were used to correct the treatments using the formula proposed by Abbott
70 [19].

71 **2.4 Statistical analysis**

72 A completely randomized design with 5 treatments (storage time) was used, with 8
73 replications, using the accumulated mortality data in 72h. Since the treatments are time-
74 repeated measures, the data were submitted to the Mauchly [20] test to verify the sphericity,
75 as recommended by Huynh & Feldt [21] for time-repeated measurements [22]. After

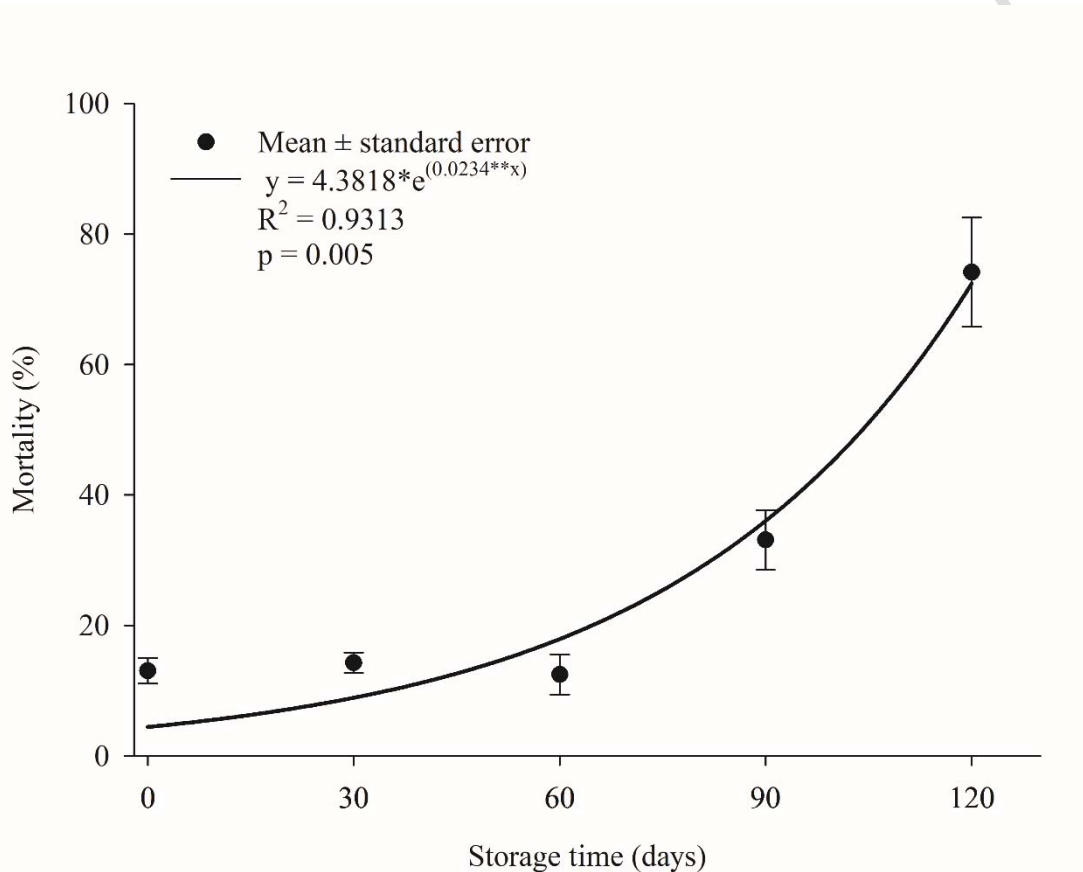
76 checking the sphericity, we performed the analysis of variance followed by non-linear
77 regression analysis in R software version 3.4 [23].

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79 3. RESULTS AND DISCUSSION

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



86

87 Figure 1. Mortality of *Tetranychus urticae* treated with *Moringa oleifera* oil with different
88 storage times. ** and * significant regression coefficient at the 1 and 5% level, respectively.

89 The oil from moringa seeds is a potential for the management of *T. urticae*. The increase in
90 mortality with the advancement of storage time was an interesting and even unexpected
91 result. Such a surprise is due to the fact that many products lose their potential/effectiveness
92 due, perhaps, to some process of deterioration and / or oxidation. On the other hand, *M.*
93 *oleifera* oil can maintain some properties, such as density and viscosity, and increase the
94 percentage of free fatty acid for up to 24 months [13].

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

97 On *Aedes aegypti* (L.) (Diptera: Culicidae) the aqueous extract of the seed presented
98 larvicidal action and ovicidal, being able to cause 100% mortality after 24 h of exposure [16].
99 For the malaria vector, *Anopheles stephensi* Liston (Diptera: Culicidae), the use of methane
100 extract from moringa seeds, provided larval and pupal mortality [15]. In *Sitophilus oryzae* (L.)
101 (Coleoptera: Curculionidae), *Oryzaephilus mercator* (Faur) (Coleoptera: Cucujidae) and
102 *Ryzopertha dominica* (Fabr.) (Coleoptera: Bostrichidae) methanoic extract caused mortality
103 of more than 90% of insects [26]. Aqueous extracts of moringa seeds presented high toxicity
104 to *T. urticae*, resulting in a lethal concentration for 50% of the population around 12.39%
105 [18]. These results demonstrate that, independent of the solvent used, *M. oleifera* oil is toxic
106 to different groups of insects and to the mite.

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

113 The moringa studies show that plants of this genus are rich in lectin [32], α - and γ -
114 tocopherols, glycosylates, nitriles, glycosides, quercetin, canferol, rhamnosides,
115 isothiocyanates and steroids [33]. In addition, the oil has as main component oleic acid (\approx
116 78.0%) [34].

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

123 In the case of fatty acids, larvicidal and anti-nutritive activity of oleic acid have been reported
124 on *A. aegyptii*, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), *Lymantria dispar* L.
125 (Lepidoptera: Lymantriidae), *Culex quinquefasciatus* Say (Diptera: Culicidae) [36, 37]. Faced
126 with this, it can be seen that *M. oleifera* presents lethal and anti-nutritive activity, as well as
127 sublethal effect.
128

129 **4. CONCLUSION**

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131 It is concluded that the oil of *M. oleifera* shows acaricidal activity to *T. urticae* and that the
132 storage time made possible its toxicity to the mite. Oil stored at 120 days is most suitable for
133 use in mite control.
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135 **COMPETING INTERESTS**

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138 Authors have declared that no competing interests exist.
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