

INFLUENCE OF STORAGE DURATION ON THE TOXICITY OF *Moringa oleifera* (MORINGACEAE) OIL TO *Tetranychus urticae* (ACARI: TETRANYCHIDAE)

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 $74.16 \pm 8.37\%$ at 120 days. The oil of *M. oleifera* is promising for the control of *T. urticae*, improving the acaricidal activity over time.

Keywords: mite; alternative control; storage time; Moringa.

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.

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 Rearing and maintenance of *Tetranychus urticae***

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

48 **2.2 Extraction and storage of *M. oleifera* 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% ± 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. For the 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

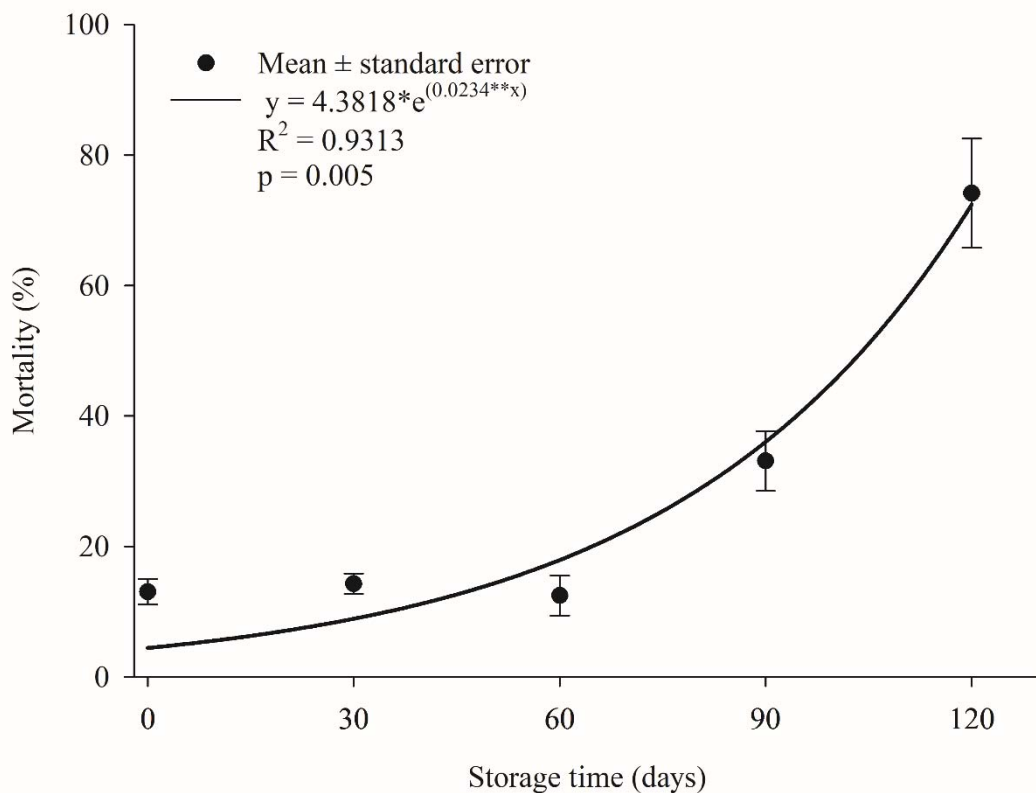
76 checking the sphericity, we performed the analysis of variance followed by non-linear
77 regression analysis in R software version 3.4 [23].

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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 result. Such a result may
91 be due to the fact that many products lose their potential/effectiveness due, perhaps, to
92 some process of deterioration and / or oxidation. On the other hand, *M. oleifera* oil can
93 maintain some properties, such as density and viscosity, and increase the percentage of free
94 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**

130
131 It is concluded that the oil of *M. oleifera* shows acaricidal activity to *T. urticae*. Storage time
132 can influence positively its toxicity to the mite. Oil stored at 120 days is most suitable for use
133 in mite control.

134 135 136 **COMPETING INTERESTS**

137
138 Authors have declared that no competing interests exist.

139 140 141 **REFERENCES**

142
143 01. El-Moneim AMA, Fatma SA, Turkey A. Control of *Tetranychus urticae* Koch by extracts of
144 three essential oils of chamomile, marjoram and Eucalyptus. Asian Pac J Trop Biomed.
145 2012; 2(1):24-30.

- 146 02. Moro LB, Polanczyk RA, Pratisoli D, Carvalho JR, Franco CR. Potencial do uso de
147 fungos entomopatogênicos no controle de *Tetranychus urticae* Kock (Acari: Tetranychidae)
148 em mamoeiro: efeito de cultivares sobre a patogenicidade. Arq Inst Biol. 2011; 78:267-272.
149 Portuguese.
- 150 03. Cazaux M, Navarro M, Bruinsma KA, Zhurov V, Negrave T, van Leeuwen T, Grbic V,
151 Grbic M. Application of Two-spotted Spider Mite *Tetranychus urticae* for Plant-pest
152 Interaction Studies. J Vis Exp. 2014; 89: e51738.
- 153 04. Paes JPP, Rondelli VM, Costa AV, Vianna UR, Queiroz VT. Caracterização química e
154 efeito do óleo essencial de erva-de-santa-maria sobre o ácaro-rajado de morangueiro. Rev
155 Bras Frutic. 2015; 37(2):346-354. Portuguese.
- 156 05. Pokle PP, Shukla A. Chemical control of two spotted spider mite, *Tetranychus urticae*
157 Koch (Acari: Tetranychidae) on tomato under polyhouse conditions. Pest Manag Hort
158 Ecosyst. 2015; 21(2):145-153.
- 159 6. Sato ME, Silva M, Gonçalves LR. Differential toxicity of pesticides to *Neoseiulus*
160 *californicus* (McGregor) (Acari: Phytoseiidae) and *Tetranychus urticae* Koch (Acari:
161 Tetranychidae) on strawberry. Neotrop Entomol. 2002; 31(3):449-456.
- 162 7. Wekesa VW, Vital S, Silva RA, Ortega EMM, Klingen I, Delalibera Júnior I. The effect of
163 host plants on *Tetranychus evansi*, *Tetranychus urticae* (Acari: Tetranychidae) and on their
164 fungal pathogen *Neozygites floridana* (Entomophthorales: Neozygitaceae). J Invertebr
165 Pathol. 2011; 107(2):139-145.
- 166 8. Schlesener DCH, Duarte AF, Guerrero MFC, Cunha US, Nava DE. Efeitos do nim sobre
167 *Tetranychus urticae* Koch (Acari: Tetranychidae) e os predadores *Phytoseiulus macropilis*
168 (Banks) e *Neoseiulus californicus* (Mcgregor) (Acari: Phytoseiidae). Rev Bras Frut. 2013;
169 35(1):059-066. Portuguese.
- 170 9. Santos MD, Duarte ME, Valente ECN, Barbosa TJA, Silva ES. Eficiência de isolados
171 comerciais de *Beauveria bassiana* (Bals.) Vuillemin e *Metarhizium anisopliae* (Metsch.)
172 Sorok (Hypocreales: Cordycipitaceae, Clavicipitaceae) sobre o ácaro rajado *Tetranychus*
173 *urticae* Koch (Acari: Tetranychidae). Rev Bras Biocienc. 2016; 14:76-79. Portuguese.
- 174 10. Bestete LR, Pratisoli D, Queiroz VT, Celestino FN, Machado LC. Toxicidade de óleo de
175 mamona a *Helicoverpa zea* e a *Trichogramma pretiosum*. Pesqui Agropecu Bras. 2011;
176 46:791-797. Portuguese.
- 177 11. Celestino FN, Pratisoli D, Machado LC, Costa AV, Santos Junior HJG, Zinger FD.
178 Toxicidade do óleo de mamona à broca-do-café [*Hypothenemus hampei* (Ferrari)
179 (Coleoptera: Curculionidae: Scolytinae)]. Coffee Sci. 2015; 10:329-336. Portuguese.
- 180 12. Regnault-Roger C, Vincent C, Arnason JT. Essential Oils in Insect Control: Low-Risk
181 Products in a High-Stakes World. Annu Rev Entomol. 2012; 57:405-424.
- 182 13. Fotouo-M H, Du Toit ES, Robbertse PJ. Effect of storage conditions on *Moringa oleifera*
183 Lam. seed oil: Biodiesel feedstock quality. Ind Crops Prod. 2016; 84:80-86.
- 184 14. Ferreira PMP, Carvalho AFU, Farias DF, Cariolano NG, Melo VMM, Queiroz MGR,
185 Martins AMC, Machado-Neto JG. Larvicidal activity of the water extract of *Moringa oleifera*

- 186 seeds against *Aedes aegypti* and its toxicity upon laboratory animals. An Acad Bras Cienc.
187 2009; 81(2):207-216.
- 188 15. Prabhu K, Murugan, K, Nareshkumar A, Ramasubramanian N, Bragadeeswaran S.
189 Larvicidal and repellent potential of *Moringa oleifera* against malarial vector, *Anopheles*
190 *stephensi* Liston (Insecta: Diptera: Culicidae). Asian Pac J Trop Biomed. 2011; 1(2):124-9.
- 191 16. Santos NDL, Moura KS, Napoleão TH, Santos GKN, Coelho LCBB, Navarro DMAF,
192 Paiva PMG. Oviposition-stimulant and ovicidal activities of *Moringa oleifera* lectin on *Aedes*
193 *aegypti*. PLoS One, 2012; 7(9): e44840.
- 194 17. Nwankwo EM, Okonkwo NJ, Ogbonna CU, Akpom CJO, Egbuche CM, Ukonze BC.
195 *Moringa oleifera* and *Annona muricata* seed oil extracts as biopesticides against the second
196 and fourth larval instar of *Aedes aegypti* L. (Diptera: Culicidae). JBiopest. 2015; 8(1):56-61.
- 197 18. Holtz AM, Carvalho JR, Franzin ML, Pires AA, Coffler T, Marchiori JJP. Toxicity aqueous
198 extracts of *Moringa oleifera* for *Tetranychus urticae*. Rev Iles Cien. 2016; 1:4-13.
199 Portuguese.
- 200 19. Abbott WS. A method of computing the effectiveness of an insecticide. J Econ Entomol.
201 1925; 18:265-267.
- 202 20. Mauchly JW. Significance Test for Sphericity of a Normal n-Variate Distribution. Ann
203 Math Stat. 1940; 11(2):204-209.
- 204 21. Huynh H, Feldt LS. Conditions under which mean square ratios in repeated
205 measurements designs have exact F-distributions. J Am Stat Assoc. 1970; 65:1582-1589.
- 206 22. Cruz CD, Carneiro PCS, Regazzi AJ. Análise de dados experimentais com medidas
207 repetidas. In: Cruz CD, Carneiro PCS, Regazzi AJ, Editors. Modelos Biométricos Aplicados
208 ao Melhoramento Genético. Viçosa, Editora UFV, 2014; 616-635. Portuguese.
- 209 23. R development core team. R: A language and environment for statistical computing. R
210 Foundation for Statistical Computing, Vienna, Austria, 2017. Accessed:10 jun 2017.
- 211 24. Mbaiguinam M, Maoura N, Bianpambe A, Borro G, Alladoubaye E. Effects of Six
212 Common Plant Seed Oils on Survival, Eggs Lying and Development of the Cowpea Weevil,
213 *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). J Biol Sci. 2006; 6(2):420-425. DOI:
214 10.3923 / jbs.2006.420.425
- 215 25. Adenekan MO, Okpeze VE, Ogundipe WF, Oguntade MI. Evaluation of *Moringa oleifera*
216 powders for the control of bruchid beetles during storage. Int J Agric Policy Res. 2013;
217 1(10):305-310.
- 218 26. Ileke KD, Ogungbite OC. Entomocidal Activity of Powders and Extracts of Four Medicinal
219 Plants Against *Sitophilus oryzae* (L), *Oryzaephilus mercator* (Faur) and *Ryzopertha dominica*
220 (Fabr.). Jordan J Biol Sci. 2014; 7(1):57-62.
- 221 27. Saleem S, Ul Hasan M, Sagheer M, Sahi ST. Insecticidal Activity of Essential Oils of
222 Four Medicinal Plants Against Different Stored Grain Insect Pests. Pak J Zool. 2014;
223 46(5):1407-1414.

- 224 28. Oliveira CFR, Luz LA, Paiva PMG, Coelho LCBB, Marangoni S, Macedo MLR.
225 Evaluation of seed coagulant *Moringa oleifera* lectin (cMoL) as a bioinsecticidal tool with
226 potential for the control of insects. *Process Biochem.* 2011; 46:498–504.
- 227 29. Martinez DST, Freire MGM, Mazzafera P, Araujo-Júnior RT, Bueno RD, Macedo MLR.
228 Insecticidal effect of labramin, a lectin-like protein isolated from seeds of the beach apricot
229 tree, *Labramia bojeri*, on the Mediterranean flour moth, *Ephestia kuehniella*. *J Insect Sci.*
230 2012; 62(12):1-11.
- 231 30. Oliveira CFR, Moura MC, Napoleão TH, Paiva PMG, Coelho LCBB, Macedo MLR. A
232 chitin-binding lectin from *Moringa oleifera* seeds (WSMoL) impairs the digestive physiology
233 of the Mediterranean flour larvae, *Anagasta kuehniella*. *Pestic Biochem Physiol.* 2017;
234 142:67-76.
- 235 31. Bhutada PR, Jadhav AJ, Pinjari DV, Nemade PR, Jain RD. Solvent assisted extraction of
236 oil from *Moringa oleifera* Lam Seeds. *Ind Crops Prod.* 2016; 82:74-80.
- 237 32. Santos BS, Farias PMA, Menezes FD, Ferreira RC, Júnior AS, Figueiredo RCBQ,
238 Carvalho Junior LB, Beltrão EIC. CdS-Cd(OH)₂ core shell quantum dots functionalized with
239 Concanavalin A lectin for recognition of mammary tumors. *Phys Status Solidi.* 2006;
240 11:4017-4022.
- 241 33. Barreto MB, Freitas JVB, Silveira ER, Bezerra AME, Nunes EP, Gramosa NV.
242 Constituintes químicos voláteis e não-voláteis de *Moringa oleifera* Lam., Moringaceae. *Rev*
243 *Bras Farm.* 2009; 19(4):893-897. Portuguese.
- 244 34. Santana CR, Pereira DF, Araújo NA, Cavalcanti EB, Silva GF. Caracterização físico-
245 química da moringa (*Moringa oleifera* Lam). *Rev Bras Prod Agro.* 2010; 12(1):55-60.
246 Portuguese.
- 247 35. Coelho JS, Santos NDL, Napoleão TH, Gomes FS, Ferreira RS, Zingali RB, Coelho
248 LCBB, Leite SP, Navarro DMAF, Paiva PMG. Effect of *Moringa oleifera* lectin on
249 development and mortality of *Aedes aegypti* larvae. *Chemosphere.* 2009; 77:934-938.
- 250 36. Ramsewak RS, Nair MG, Murugesan S, Mattson WJ, Zasada J. Insecticidal fatty acids
251 and triglycerides from *Dirca palustris*. *J Agric Food Chem.* 2001; 49(12):5852-5856.
- 252 37. Silva VCB, Ribeiro Neto JA, Alves SN, Lima LARS. Larvicidal activity of oils, fatty acids,
253 and methyl esters from ripe and unripe fruit of *Solanum lycocarpum* (Solanaceae) against
254 the vector *Culex quinquefasciatus* (Diptera: Culicidae). *Rev Soc Bras Med Trop.*
255 2015;48(5):610-613.