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 ± 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.

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. MATERIAL 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 ± 1ºC, relative humidity 70% ± 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 ± 1ºC, relative humidity 70% ± 10 and photophase of 12h.

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* (Ø = 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 ± 1ºC, RH of 70 ± 10% and photophase of 12h).

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) (Figure 1). There was an increase in the mortality of the mite with the increase of the storage time, reaching 74.16 ± 8.37% of mortality with 120 days of storage.

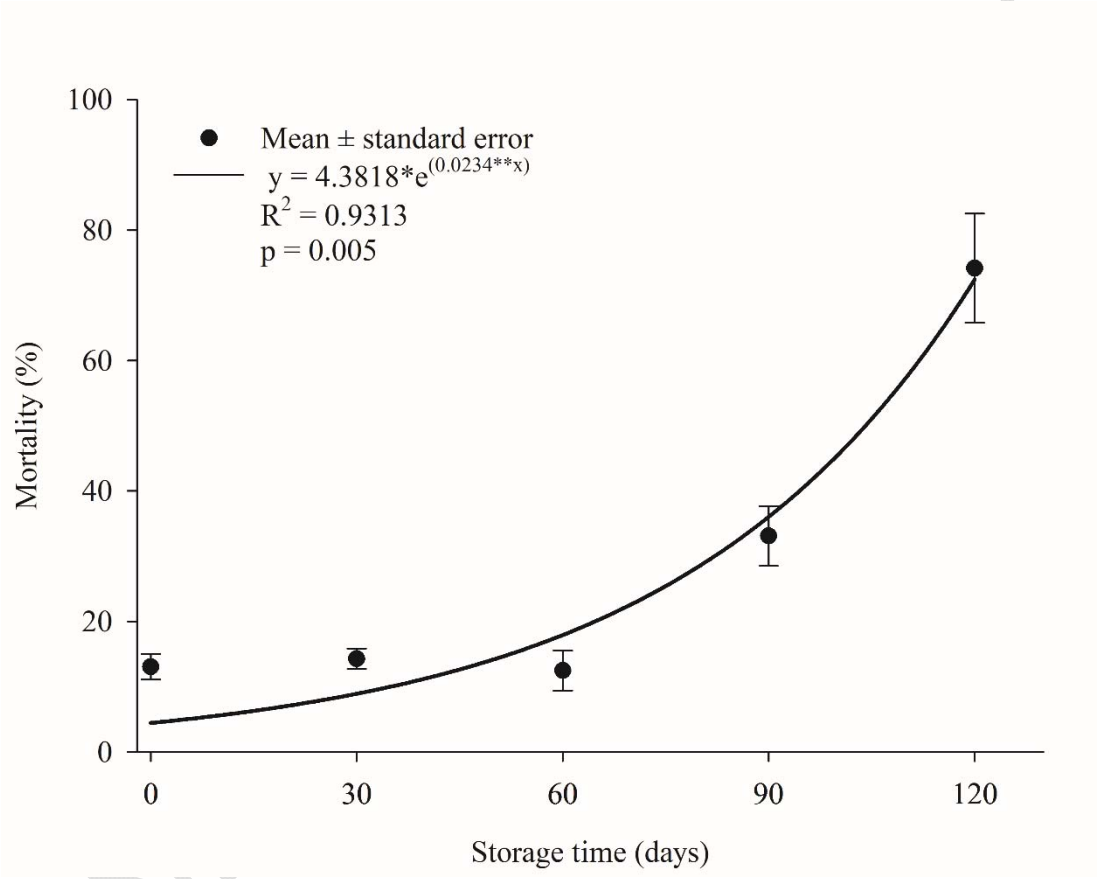

Figure 1. Mortality of *Tetranychus urticae* treated with *Moringa oleifera* oil with different storage times. ** and * significant regression coefficient at the 1 and 5% level, respectively.

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.

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 (≈ 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.

REFERENCES

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

02. Moro LB, Polanczyk RA, Pratissoli D, Carvalho JR, Franco CR. Potencial do uso de fungos entomopatogênicos no controle de *Tetranychus urticae* Kock (Acari: Tetranychidae) em mamoeiro: efeito de cultivares sobre a patogenicidade. Arq Inst Biol. 2011; 78:267-272. Portuguese.

03. Cazaux M, Navarro M, Bruinsma KA, Zhurov V, Negrave T, van Leeuwen T, Grbic V, Grbic M. Application of Two-spotted Spider Mite *Tetranychus urticae* for Plant-pest Interaction Studies. J Vis Exp. 2014; 89: e51738.

04. Paes JPP, Rondelli VM, Costa AV, Vianna UR, Queiroz VT. Caracterização química e efeito do óleo essencial de erva-de-santa-maria sobre o ácaro-rajado de morangueiro. Rev Bras Frutic. 2015; 37(2):346-354. Portuguese.

05. Pokle PP, Shukla A. Chemical control of two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) on tomato under polyhouse conditions. Pest Manag Hort Ecosyst. 2015; 21(2):145-153.

6. Sato ME, Silva M, Gonçalves LR. Differential toxicity of pesticides to *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) and *Tetranychus urticae* Koch (Acari: Tetranychidae) on strawberry. Neotrop Entomol. 2002; 31(3):449-456.

7. Wekesa VW, Vital S, Silva RA, Ortega EMM, Klingen I, Delalibera Júnior I. The effect of host plants on *Tetranychus evansi*, *Tetranychus urticae* (Acari: Tetranychidae) and on their fungal pathogen *Neozygites floridana* (Entomophthorales: Neozygitaceae). J Invertebr Pathol. 2011; 107(2):139-145.

8. Schlesener DCH, Duarte AF, Guerrero MFC, Cunha US, Nava DE. Efeitos do nim sobre *Tetranychus urticae* Koch (Acari: Tetranychidae) e os predadores *Phytoseiulus macropilis* (Banks) e *Neoseiulus californicus* (Mcgregor) (Acari: Phytoseiidae). Rev Bras Frut. 2013; 35(1):059-066. Portuguese.

9. Santos MD, Duarte ME, Valente ECN, Barbosa TJA, Silva ES. Eficiênia de isolados comerciais de *Beauveria bassiana* (Bals.) Vuillemin e *Metarhizium anisopliae* (Metsch.) Sorok (Hypocreales: Cordycipitaceae, Clavicipitaceae) sobre o ácaro rajado *Tetranychus urticae* Koch (Acari: Tetranychidae). Rev Bras Biocienc. 2016; 14:76-79. Portuguese.

10. Bestete LR, Pratissoli D, Queiroz VT, Celestino FN, Machado LC. Toxicidade de óleo de mamona a *Helicoverpa zea* e a *Trichogramma pretiosum*. Pesqui Agropecu Bras. 2011; 46:791-797. Portuguese.

11. Celestino FN, Pratissoli D, Machado LC, Costa AV, Santos Junior HJG, Zinger FD. Toxicidade do óleo de mamona à broca-do-café [*Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae)]. Coffee Sci. 2015; 10:329-336. Portuguese.

12. Regnault-Roger C, Vincent C, Arnason JT. Essential Oils in Insect Control: Low-Risk Products in a High-Stakes World. Annu Rev Entomol. 2012; 57:405-424.

13. Fotouo-M H, Du Toit ES, Robbertse PJ. Effect of storage conditions on *Moringa oleifera* Lam. seed oil: Biodiesel feedstock quality. Ind Crops Prod. 2016; 84:80-86.

14. Ferreira PMP, Carvalho AFU, Farias DF, Cariolano NG, Melo VMM, Queiroz MGR, 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 Ifes 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 rations 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, Alladoumbaye 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.

28. Oliveira CFR, Luz LA, Paiva PMG, Coelho LCBB, Marangoni S, Macedo MLR. Evaluation of seed coagulant *Moringa oleifera* lectin (cMoL) as a bioinsecticidal tool with potential for the control of insects. Process Biochem. 2011; 46:498–504.

29. Martinez DST, Freire MGM, Mazzafera P, Araujo-Júnior RT, Bueno RD, Macedo MLR. Insecticidal effect of labramin, a lectin–like protein isolated from seeds of the beach apricot tree, *Labramia bojeri*, on the Mediterranean flour moth, *Ephestia kuehniella*. J Insect Sci. 2012; 62(12):1-11.

30. Oliveira CFR, Moura MC, Napoleão TH, Paiva PMG, Coelho LCBB, Macedo MLR. A chitin-binding lectin from *Moringa oleifera* seeds (WSMoL) impairs the digestive physiology of the Mediterranean flour larvae, *Anagasta kuehniella*. Pestic Biochem Physiol. 2017; 142:67-76.

31. Bhutada PR, Jadhav AJ, Pinjari DV, Nemade PR, Jain RD. Solvent assisted extraction of oil from *Moringa oleifera* Lam Seeds. Ind Crops Prod. 2016; 82:74-80.

32. Santos BS, Farias PMA, Menezes FD, Ferreira RC, Júnior AS, Figueiredo RCBQ, Carvalho Junior LB, Beltrão EIC. CdS-Cd$(OH)_2$ core shell quantum dots functionalized with Concanavalin A lectin for recognition of mammary tumors. Phys Status Solidi. 2006; 11:4017-4022.

33. Barreto MB, Freitas JVB, Silveira ER, Bezerra AME, Nunes EP, Gramosa NV. Constituintes químicos voláteis e não-voláteis de *Moringa oleifera* Lam., Moringaceae. Rev Bras Farm. 2009; 19(4):893-897. Portuguese.

34. Santana CR, Pereira DF, Araújo NA, Cavalcanti EB, Silva GF. Caracterização físico-química da moringa (*Moringa oleifera* Lam). Rev Bras Prod Agro. 2010; 12(1):55-60. Portuguese.

35. Coelho JS, Santos NDL, Napoleão TH, Gomes FS, Ferreira RS, Zingali RB, Coelho LCBB, Leite SP, Navarro DMAF, Paiva PMG. Effect of *Moringa oleifera* lectin on development and mortality of *Aedes aegypti* larvae. Chemosphere. 2009; 77:934-938.

36. Ramsewak RS, Nair MG, Murugesan S, Mattson WJ, Zasada J. Insecticidal fatty acids and triglycerides from *Dirca palustris*. J Agric Food Chem. 2001; 49(12):5852-5856.

37. Silva VCB, Ribeiro Neto JA, Alves SN, Lima LARS. Larvicidal activity of oils, fatty acids, and methyl esters from ripe and unripe fruit of *Solanum lycocarpum* (Solanaceae) against the vector *Culex quinquefasciatus* (Diptera: Culicidae). Rev Soc Bras Med Trop. 2015;48(5):610-613.