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# Toxicity of *Moringa oleifera* Lam. seed extracts at different stages of maturation on *Tetranychus urticae* Koch (Acari: Tetranychidae)

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**Abstract**
The study evaluated the acaricidal effect of aqueous extracts of green, mature and dried seeds of *Moringa oleifera* Lam. aiming to control *Tetranychus urticae* (Acari: Tetranychidae). Powder of green, ripe and dried seeds of *M. oleifera* were used in the preparation of aqueous extracts. Petri dishes containing *Canavalia ensiformis* (L.) D.C. leaf disc was used as arenas, with 10 replicates per treatment. First, the toxicity test was carried out, spraying the concentration of 20% (m / v) of each treatment on the mites in the arena. Later, the lethal concentration for *T. urticae* was estimated. The extracts were toxic to *T. urticae*, with higher mortality for green seed extracts, followed by mature seed extract. The green seed extract of *M. oleifera* showed lower LC50 among the tested treatments. This demonstrates that seed extracts, in the early stage of development, are promising for the control of *T. urticae*.

**Keywords:** Moringa, acaricidal plants, different maturation stages, spider mite

## Introduction
The spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a polyphagous species of great global economic importance, being reported in more than 1,100 plant species in 140 families of economic importance, such as beans, cotton, apple, strawberry, papaya, cucumber, tomato, fruit trees, ornamental plants, among others [1]. This mite has a habit of feeding on the leaf's abaxial surface, forming colonies that, when infesting the leaves, these initially turn yellow on the face opposite the colony. Subsequently, these areas are necrotic, with perforations in the leaves [2]. Under severe infestations, these can cause early defoliation, affecting productivity [3]. The occurrence of this mite is reported in regions of hot and humid climate, mainly during the hottest periods of the year [4].

Chemical control is one of the most used methods in the agricultural environment and, although these synthetic insecticides are relatively successful in agriculture, Van Leeuwen *et al.* [5] and Mercês *et al.* [6] claim that their intensive use can cause problems such as the resurgence of the target pest, the appearance of new pests and the selection of populations resistant to the active principle or the mechanism of action. This is due to the fact that these products used have a broad biological spectrum and persistence in the environment [5, 6].

However, numerous researches are being developed, with different techniques, to ease the use of pesticides. Among these techniques, the use of insecticidal plants stands out. Research related to the use of extracts and substances of plant origin as an alternative management has shown high efficiency in the control of mites and insects [4, 7]. It is known that plants are capable of producing secondary metabolites, which are substances responsible for its defense against insects, mites and pathogenic microorganisms. These substances can act in different ways on insects and mites, either by producing enzymes that degrade organism cells, forming rigid walls that are less susceptible to attacks or combining with their own structures that in any case, reduce their attack [6, 8]. Pavela *et al.* [9] report that the use of the extract of *Tithonia diversifolia* (Hemsley) A. Gray (Asteraceae), showed inhibitory activity in the oviposition of *T. urticae*, promoting its control in laboratory tests. Similarly, the aqueous extract of seeds of *Moringa oleifera* Lam. (Moringaceae) showed a larvicidal action on *Aedes aegypti* (L.) (Diptera: Culicidae), causing 100% mortality after 24 h of exposure [10].

In this context, studies related to different species of plants with insecticidal/acaricidal activity, aiming to understand the chemical composition of the metabolites present and the quantity of these in the different parts of the plants, the concentration to be used, storage time and temperature, types of packaging for storage of extracts and oils, residual effect of secondary

compounds, are necessary to prove their efficiency in the control of mites and insects. Thus, the objective was to evaluate the potential of *M. oleifera* seed extracts, at different stages of maturation, regarding their acaricidal activity on *T. urticae*.

## Materials and methods

The experiment was carried out at the Federal Institute of Education, Science and Technology of Espírito Santo - Campus Itapina (IFES-Campus Itapina), in climatic chambers of type B.O.D, at a temperature of 25 ± 1ºC, relative humidity 70 ± 10% and a photophase of 12h. Two types of laboratory tests were performed: toxicological activity (i) and estimation of lethal concentrations (LCs) (ii) of aqueous extracts of *M. oleifera* on *T. urticae*.

### *Tetranychus urticae* rearing

Mite rearing was established in plants of *Canavalia ensiformis* (L.) D.C. (Fabaceae) without any phytosanitary treatment, grown in pots. These pots were placed in cages made with anti-aphid screens in order to prevent the entry of other organisms, and they were placed in acclimatized room at a temperature of 25 ± 1ºC, relative humidity 70 ± 10% and a photophase of 12h.

### Preparation of Plant Extracts

To prepare the extracts, green, mature and dry seeds of *M. oleifera* were collected at IFES-Campus Itapina. After this procedure, the seeds, at the different stages of maturation, were weighed and then taken to an oven with forced air circulation, with a temperature of 60ºC, until they present constant weight. Subsequently, these were subjected to grinding with the aid of a knife mill to obtain a fine powder.

### Bioassay

To perform the bioassay leaves of *C. ensiformis* were used. The leaves were washed with distilled water and dried on paper towels, and then packed in plastic boxes of the gerbox type.

### i) Toxicity test

To obtain each solution, the vegetable powder (20 g) obtained from seeds of the different stages of maturation, were transferred, separately, to Erlenmeyers (100 mL), adding Tween® 80 adhesive spreader (0.05% v $v^{-1}$). Then, they were completed with distilled water to obtain 100 mL of the initial solution with a concentration of 20% (w $v^{-1}$). Subsequently, the mixture remained under stirring (magnetic stirrer), without heating, for 30 min at room temperature. After that time, the mixture was filtered with the aid of a filter paper funnel.

Each treatment consisted of ten repetitions, with 10 females of *T. urticae* per repetition, totaling 100 individuals per treatment. The repetitions consisted of Petri dishes (10.0 x 1.2 cm) containing a disk of pork bean leaf with 4 cm in diameter, with moistened cotton around it to keep the leaf turgescence and prevent the escape of the mites. Only the solvent was sprayed on the control [distilled water with Tween® 80 adhesive spreader (0.05% v $v^{-1}$)]. To make the applications, an airbrush SW-130K was used, connected to a compressor calibrated at a constant pressure of 25 psi, with a solution volume of 3 mL per repetition. The Petri dishes were kept in a climatic chamber type B.O.D. (25 ± 1ºC, 70 ± 10% RH and 12 h photo phase). The evaluations were carried out 24, 48 and 72 h after spraying. The mortality of each treatment was corrected based on the control, as proposed by Abbott [11]. The experiment was conducted in a completely randomized design, with the corrected mortality data submitted to analysis of variance and the means compared by the Tukey test ($p \leq 0.05$) [12].

### ii) Estimation of lethal concentrations (LC)

Aqueous extracts that caused mortalities equal to or greater than 80% were submitted to the bioassay to estimate the LC. The arena preparation methodology, number of repetitions, individuals per repetition and witness was similar to the previous test. For each treatment, concentrations were used spaced on a logarithmic scale (between the limits of 0.01 to 20%). Similar to the toxicity test, the arenas with the mites for estimating the LC were stored in a climate-controlled chamber, at a temperature of 25 ± 1°C, relative humidity of 70 ± 10% and a photophase of 12 h. The evaluations were carried out 24, 48 and 72 h after spraying the solutions of the different concentrations of the treatments. Lethal concentrations were estimated using Probit analysis [13], using the computational application R [12].

## Results and discussion

The different stages of maturation of *M. oleifera* seeds showed a difference on the mortality of *T. urticae* ($F = 44.611$, $p < 0.0001$) (Table 1). Based on the results obtained, it was verified that the green seeds of *M. oleifera* showed greater toxicity to the mite. The mature seeds of *M. oleifera* provided mortality close to 80%, while the dry seeds caused the lowest percentage of mortality (Table 1).

**Table 1:** Corrected mortality (mean ± standard error) of *Tetranychus urticae* treated with aqueous extracts obtained from *Moringa oleifera* seeds at different stages of maturation at a concentration of 20% (w $v^{-1}$)

| Maturation stages | Corrected mortality[a] |
|---|---|
| Mature seeds | 83.22 ± 3.53 b |
| Dried Seeds | 55.55 ± 4.31 c |
| Green Seeds | 98.18 ± 1.66 a |
| F | 44,611 |
| p | <0.0001 |

[a] Means followed by the same letter do not differ statistically by the Tukey test ($p \leq 0.05$)

According to the results obtained in the present study, it is observed that with the advancement of the phenological maturation stage of moringa seeds there is a significant reduction in the acaricidal action. This phenomenon can be associated with the fact of the degradation and, or reduction of the production of metabolic in the seeds of *M. oleifera*. In this regard, Gobbo-Neto and Lopes [14], in a review on the factors that influence the content of secondary metabolites in medicinal species, demonstrated that the age and development of the plant are among the main influencing factors, in addition to others, such as seasonality, circadian rhythm, ambient temperature, water availability, ultraviolet radiation, nutrients, altitude, air pollution and induction by mechanical stimuli or attack by pathogens. Studies related to the presence of secondary metabolites in plants report that the amount of these become smaller as the physiological maturation occurs [15].

Ragasa *et al.* [16], when studying the chemical constituents present in moringa seeds, found that in dry seeds, there was the presence of only lipids, while in mature and green seeds there was a higher concentration of other compounds such as alkaloids. This fact is probably related to the physiological

maturation of the seeds that, while in formation, use the products of the other parts of the plants and, when they are more developed, become independent from the mother plant. In addition, there is a considerable increase in the amount of dry matter present, thus decreasing the number of metabolites present [17].

When estimating the lethal concentrations, there was an increase in the mortality rate of the mite with an increase in the concentration of the aqueous extract of green and ripe seed of moringa, adapting to the Probit model (Table 2). A greater slope of the curve was observed for green seeds, which consequently provided a lower LC50 for this material (6.94%), differing from the results observed for mature seeds, a fact reinforced by the absence of overlapping of the intervals of confidence (Table 2).

**Table 2:** Summary of the concentration-mortality curve and respective LC50 of the aqueous extracts of green, ripe and dried seeds of *Moringa oleifera* on *Tetranychus urticae*

| Seeds | n[a] | Slope ± SE[b] | $LC_{50}$ [c] [CI95 [d]] (% w $v^{-1}$) | DF[e] | $\chi^2$ [f] |
|---|---|---|---|---|---|
| Green | 260 | 3.99± 0.52 | 6.94 [6.07 -7.80] | 2 | 4.36 |
| Mature | 260 | 2.72 ± 0.37 | 12.00 [10.33 -13.67] | 2 | 2.15 |

[a] Number of insects used in the test; [b] Slope of the curve ± standard error; [c] Lethal concentration; [d] Confidence intervals at 95% probability [lower - upper]; [e] degrees of freedom; [f] Chi-square test

As noted in the results, extracts from moringa seeds, at different stages of maturation, have the potential to control *T. urticae*, especially young seeds (green). *Moringa oleifera* toxicity has also been reported for beetles like *Tribolium confusum* J. Du Val (Coleoptera: Tenebrionidae) [18] and *Callosobruchus maculatus* Fabr. (Coleoptera: Bruchidae) [19], verifying high mortality rates and reduced egg production when applied to adults in contact and fumigation tests using moringa root oil. Holtz *et al.* [20] found increased efficiency in the mortality of *T. urticae* using Moringa seed oil at different storage times.

Phytochemical analysis of moringa seed extract found large amounts of phenolic compounds, these being flavonoids and tannins [21] in addition to alkaloids, saponins, glycosides, among others [22]. Secondary plant metabolites are responsible for several actions that provide plant species with greater survival, such as antibiotic, antifungal and antiviral action, in addition to germination-inhibiting or toxic activities against other plants [23, 24]. However, there are also some proteins that play and activate these defense mechanisms, which can be located in different parts of plants or their fruits and when activated, they can express insecticidal action. Most of these plant defense mechanisms are concentrated in the seeds, given their great importance as a vehicle in the propagation and survival of species, and their tissues can accumulate such defensive substances naturally in their composition, or, after induction, conferring resistance to various organisms, such as mites, insects, fungi, bacteria, viruses, among others [25].

Studies using some of the compounds present in the extract of the seed of *M. oleifera* in isolation prove that they are effective in the control of agricultural pests. Among the various ways that flavonoids are supposed to act, in insects and, even in mite populations, they alter enzymatic and hormonal activity, block biochemical pathways and consequently reduce the assimilation of essential substances and the storage of nutrients [26]. According to Lenora and Senthilkumar [27], tannins reduce the rate of food intake, growth rate, feed efficiency and protein digestibility in several animals that have been studied experimentally. Alkaloids, in turn, can negatively influence the feeding of insects, the cardiovascular system with cardioinhibitory actions, interfere in the functioning and formation of the reproductive system, affect the development and exchange of integuments, among others [28, 29].

Saponins work by reducing food intake, delaying its passage through the intestine, degrading intestinal cells, forming indigestible bonds with sterols in food, paralyzing the food of pest organisms. In addition, there is the formation of complexes with cholesterol and consequent cellular toxicity, which causes the bad formation of insect ecdysis [30, 31].

## Conclusion

*Moringa oleifera* seeds, at different stages of maturation, have acaricidal activity and can be considered as potential tools in the management and control of *T. urticae*. Green seeds are more toxic to *T. urticae*, and it is possible to obtain them with a shorter interval of time compared to mature seeds. However, even with a slightly lower toxicity, mature seeds can also be used, which makes it possible to obtain moringa extract during almost the entire reproductive period of the plant, which is favorable for use aiming at sustainable agriculture.

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