

Efficacy of aqueous extracts of neem (*Azadirachta indica*) leaves in the control of date palm Boufaroua (*Oligonychus afrasiaticus*) in Algeria

Eficacia de los extractos acuosos de hojas de neem (*Azadirachta indica*) en el control de Boufaroua (*Oligonychus afrasiaticus*) en la palma datilera en Argelia

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ABSTRACT

In Algeria, the neem plant *Azadirachta indica* (Magnoliopsida: Sapindales: Meliaceae) has insecticidal and miticidal properties. The use of such a natural biopesticide, preserving the environment and human health, is necessary. We conducted this study to evaluate the efficacy of aqueous extracts of *A. indica* leaves in the control of *Oligonychus afrasiaticus* (Arachnida: Trombidiformes: Tetranychidae) (Boufaroua), one of the main pests of the date palm. The results obtained showed a highly significant effect ($P < 0.001$) in the control of this pest for the two doses applied (D1: 100% stock solution of aqueous extracts of neem leaves, D2: 25% dilution of the stock solution). The mortality rate against Boufaroua was 97.61% and 96.36%, respectively, for the two doses used, with no significant difference between them. These highly effective and widely available extracts can be used to control pest populations of date palm such as *O. afrasiaticus*. The results obtained for this biochemical control method are technically effective and economically affordable, given the widespread availability of neem over a large part of southern Algeria. It would be worthwhile extending neem cultivation to all regions where Boufaroua is abundant. This practice would help to protect human health and the environment.

Key words: biological control, biopesticide, mite, Sahara.

RESUMEN

En Argelia, el árbol de neem *Azadirachta indica* (Magnoliopsida: Sapindales: Meliaceae) tiene propiedades insecticidas y acaricidas. Su uso como biopesticida natural que preserva el medio ambiente y la salud humana es necesario. Llevamos a cabo este estudio para evaluar la eficacia de extractos acuosos de hojas de *A. indica* en el control de *Oligonychus afrasiaticus* (Arachnida: Trombidiformes: Tetranychidae) (Boufaroua), una de las principales plagas de la palmera datilera. Los resultados obtenidos mostraron un efecto insecticida altamente significativo ($P < 0.001$) en el control de esta plaga con las dos dosis aplicadas (D1: 100% es la solución madre de extractos acuosos de hojas de neem, D2: diluida al 25% de la solución madre). La tasa de mortalidad al aplicar este producto contra la Boufaroua fue respectivamente de 97.61% y 96.36% para las dos dosis utilizadas, sin que se observaran diferencias significativas entre las dos dosis aplicadas. Estos extractos son eficaces y ampliamente disponibles, por lo que pueden utilizarse en el control de plagas de la palmera datilera tales como *O. afrasiaticus*. Los resultados obtenidos para este método de control bioquímico son técnicamente eficaces y económicamente asequibles, dada la disponibilidad de la planta en gran parte del sur de Argelia. Sería conveniente extender el cultivo de neem a todas las regiones donde abunde la Boufaroua. Esta práctica contribuirá a proteger la salud humana y el medio ambiente.

Palabras clave: control biológico, biopesticida, ácaro, Sahara.

Introduction

One of the most serious pests of date palms is the mite *Oligonychus afrasiaticus* (McGregor), commonly called "Boufaroua". The adult mite, with four pairs of legs and a yellow to pink oval body, measures 0.2 to 0.4 mm long and is practically invisible to the naked eye (INPV, 2009). During its activity, the mite lays eggs on the dates where they are protected by dense white or greyish silky web

which is secreted by the adult at the time of oviposition (Bounaga & Djerbi, 1990). In Algeria, the activity increases rapidly in the spring, from May onwards, and becomes important when coinciding with the forming of date bunches (Bahlouli & Talmat, 2017). It feeds by puncturing the fruit epidermis and sucks the contents of the cells. The epidermis of injured fruits becomes rough, wrinkled, pigmented and reddish and dates develop poorly (Munier, 1973). The damage caused by the mite to Algerian palm

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groves was estimated at 30-70% of the date production in 1981 (Guessoum, 1986) and in some cases even wiped out the entire crop (INPV, 2009).

Chemical control remains the main mechanism to reduce the incidence of this pest in palm groves. Since the 1950s, control measures have been the application of sulphur dust mixed with lime or with wood ash at a rate of 1/3 - 2/3, on the bunches and the heart of the palm (N'Diaye & Tourneur, 1972). The effectiveness of sulphur has decreased significantly in recent years in some countries, probably due to the development of resistance in the pest. Other miticides are now used, with the risk of residues in fruits, resistance problems and elimination of beneficials (Gómez Vives & Ferry, 2005). Due to the increasing consumer concern regarding this residual pollution and the toxic effects of many synthetic insecticides, natural alternatives are becoming necessary (Bankole, 2004).

Trials in southern Tunisia using the predatory mite *Neoseiulus californicus* have shown that it is able to effectively control Boufaroua populations, especially when releases are made at the beginning of fruit colonization by the phytophage (Khoualdia *et al.*, 2001). Releases of *Stethorus punctillum* (Coleoptera: Coccinellidae) were carried out on date palms infested by *O. afrasiaticus* for the first time in Algeria (Idder & Pintureau, 2007). Their study showed that this ladybug plays an important role in the control of the mites, particularly when the trees are heavily infested.

Several botanical control alternatives have also been developed, including the use of plants with insecticidal, anti-appetent and repellent properties as well as mineral oils. Among these plants, neem (*Azadirachta indica*) (seeds, leaves, and bark) has been used to control populations of insects in several families as well as viral diseases (Déla *et al.*, 2014; Kossou *et al.*, 2007; Kulimushi Bwanampongo, 2014; Yarou *et al.*, 2017).

The objective of this study was to investigate the efficacy of the aqueous extracts of neem leaves in the control of the mite *Oligonychus afrasiaticus*.

Materials and methods

Experimental site

The experiment was carried out in a palm garden located in Sidi Amrane, a commune of Oued Righ, 120 km from El Oued, Algeria. The garden contained 50 palm trees, with 33 Deglet-Nour plants, 13 Degla, three Ghars, and one

Tantboucht. The presence of cover crops such as pepper and alfalfa and a few fig trees were noted (Fig. 1).

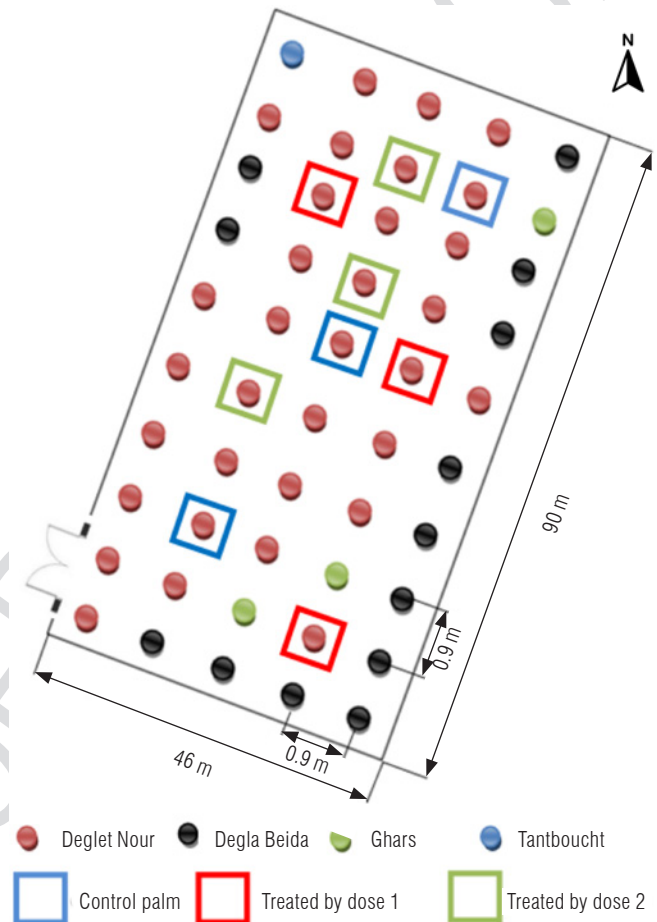


FIGURE 1. Plot diagram and experimental setup of the study site.

The distance between the palms and the rows was 9 m x 9 m. The average height of the date palms was 2 m. The plot was irrigated by submersion with water pumped from the Albian aquifer, a practice authorized by the government and used by all farmers in the Saharan regions given the lack of rainfall. The garden was surrounded by a windbreak composed of dry palms.

The experiment was conducted on the Deglet-Nour variety due to its predominance and economic importance in the study area.

Estimation of the infestation rate of palms by *Oligonychus afrasiaticus*

In a phoenicultural garden, Boufaroua infestation of date palms varies from tree to tree. This degree of contamination can be visually assessed by the amount of webbing present in the date bunches of a date palm.

Initially, for consistency, we identified all Deglet-Nour trees with the same average infestation rate. We took three branches (one external, one internal, and one middle) per bunch for the nine trees selected for our experiment: six were treated with two doses (100%, 25%) and three served as controls. The selected branches, cut with pruning shears, were soaked in labelled bottles filled with 40% alcohol to kill the mites before being brought back to the laboratory for counting.

In the laboratory, the alcohol was filtered through filter paper and then, using a binocular magnifying glass, a total population count was made. This allowed for determining the number of mites per branch was known, then per bunch, which can be extrapolated to the entire palm tree.

Plant selection and preparation of aqueous extracts of neem leaves

The plant material used as an acaricide consisted of fresh neem leaves, collected from trees in Adrar (southwestern Algeria) in July. These leaves were finely ground using an electric mortar grinder.

The crushed material was soaked in water at a rate of 200 g per 1 L of water. The mixture was left to infuse for 24 h under ambient conditions and then filtered through a very fine mesh screen. The filtrate obtained constituted the aqueous extract of neem leaves. Two doses were obtained and applied to the pest *O. afrasiaticus* to determine the insecticidal effect of the extract. The first dose was the stock solution (100%) and the second was at 25%. The dilution was carried out according to the following formula:

$D1 \times V1 = D2 \times V2$, where

D1: dosage of the stock solution with 100% neem leaf extract,

D2: dosage of the diluted solution with 25% neem leaf extract,

V1: volume of the stock solution containing 100% neem leaf extract,

V2: volume of the solution diluted to 25% neem leaf extract.

Treatments

Firstly, it would be useful to study the effect of distilled water on Boufaroua. To this end, about 15 bunches of infested dates were sprayed with distilled water. After 48 h, no effect of the water on mite mortality was observed.

Secondly, in the laboratory, we counted the number of spider mites on date branches from the fruit garden under controlled conditions, before and after spraying with water. The effect of water on Boufaroua mortality was practically negligible.

Furthermore, the sparse rainfall in July and August, when mite infestation was at its peak, had no effect on the proliferation of pests.

The application of aqueous extracts of neem leaves was carried out on Deglet-Nour trees with three replicates for each dose. The first application was carried out on 23 July 2019. By spraying, we treated all the bunches of trees (except controls) selected in our sampling.

After one week, a second application was made with a quantity of 4 L per tree. The spraying was done with a 16 L battery-operated backpack sprayer. Three days later, we carried out the final sampling (control and treated trees) in order to evaluate the infestations and the effectiveness of the neem extract.

Socio-economic impact

Such a trial, if it produces significant results, would be worth sharing with the region's palm farmers. These farmers, who generally have very modest incomes, are often looking for alternatives to pesticides, which are relatively expensive, especially when applied to large areas with many trees.

Statistical analysis

The infestation rate of dates was analyzed using i) a one-factor analysis of variance (ANOVA) (treatment) and ii) a two-factor analysis (degree of infestation of the trees and treatment) in order to compare the infestation rates before and after the treatments. The comparison of means was performed using Fisher's PLSD test to distinguish the effectiveness of the different doses. All analyses were performed with XLSTAT (Version 2009, 1.02).

Results

Infestation of dates by mites *Oligonychus afrasiaticus*

Adult mite population counts carried out on control plants and plants treated with neem leaf extracts (Tab. 1) showed an increase in the number of adult mites on control plants over time, from 195 to 222 on average per branch. This increase appears to be due to climatic factors favorable to mite proliferation and multiplication, such as high heat and low air humidity.

TABLE 1. Infestation of date palms by mites *Oligonychus afrasiaticus* and efficacy of the neem extract applications.

Treatment	Trees studied	Sampled branches per tree	Average number of mites per branch		Percentage of mortality
			Before treatment	After treatment	
Control	3	3	195.56 ± 2.16	222.16 ± 4.83	27
Dose 1 (100%)	3	3	195.56 ± 2.16	4.66 ± 0.040	97.61 ± 1.07
Dose 2 (25%)	3	3	195.56 ± 2.16	7.11 ± 0.088	96.36 ± 1.01

Control – distilled water.

TABLE 2. Evaluation of neem extract dose in relation to mite mortality. Applied dose / Fisher's (LSD) test/ Analysis of the differences between the modalities are presented with a 95% confidence interval.

Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant
D0 vs D1	190.872	8.473	2.048	< 0.001	Yes
D0 vs D2	188.427	8.365	2.048	< 0.001	Yes
D2 vs D1	2.444	0.100	2.048	0.921	No

D0 – distilled water, D1 – neem leaf extract at 100%, D2 – neem leaf extract at 25%.

TABLE 3. Results of the Fisher's PLSD test.

Modality	Mean	Groups
D0	195.538	A
D1	7.111	B
D2	4.667	B

D0 – distilled water, D1 – neem leaf extract at 100%, D2 – neem leaf extract at 25%.

Efficacy of aqueous extracts of neem leaves

Table 1 shows the efficacy of neem leaf extracts in controlling *O. afrasiaticus*. The average number of mites per branch on treated plants decreased from 195 to 4.6 individuals for the first dose of 100% of the stock solution. In

addition, 7 individuals remained for the second dose at 25% neem extract, with a very high mortality rate of 96 to 97%.

The analysis of variance of mite mortality rate (Tab. 2) revealed a highly significant difference between the plants treated with the two doses and the control ($P < 0.001$). However, when the averages of the two doses (D1 and D2) were compared, no significant difference was found between them ($P = 0.921$) (Tab. 2). Both doses belonged to the same group B according to Fisher's PLSD test (Tab. 3).

Figures 2A and B show the dates infested with Boufaroua before and after treatments with aqueous extracts of neem leaves.

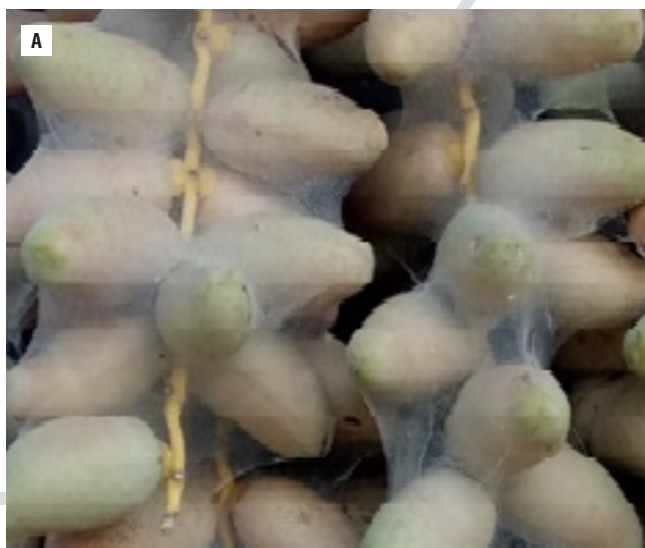


FIGURE 2. Infested dates **A)** before treatment, **B)** three days after treatment.

Discussion

The aqueous extracts of neem leaves significantly reduced the infestation density of date palm mites per plant compared to the control (distilled water), with a very high mortality rate.

The effectiveness of these extracts is thought to be due to the presence of azadirachtin, which is more concentrated in neem seeds (Gauvin *et al.*, 2003). Azadirachtin is the most abundant, powerful, and effective compound present in *A. indica* (Morgan, 2009), whose repellent, anti-appetent, fecundity-reducing and larval moult-inhibiting properties have been demonstrated (Lowery *et al.*, 1993; Mordue, 2004).

Azadirachtin is a terpenoid that acts as a growth regulator by antagonistically disrupting insect hormones (Banken & Stark, 1997), physiological processes and the hormonal cycle, inducing malformations in the moulting process and preventing normal development, optimal growth, and reproduction (Mordue *et al.*, 2005; Morgan, 2009). It also has anti-appetent effects on the natural movement of the intestine, causing paralysis and a decline in target organisms (Juan *et al.*, 2000; Senthil-Nathan *et al.*, 2004). It is rapidly absorbed by plant tissues, ensuring effective systemic action (Bernard *et al.*, 2008).

Our results also show that the effect of the two doses applied of the neem extract (D1, D2) is nearly identical, as they belong to the same homogeneous group B according to Fisher's PLSD test (Tab. 3, Figs. 2A-B). The D2 dose is sufficient to control this pest.

This is confirmed by the results of Looli Boyombe *et al.* (2022), who concluded that the active principle of neem is found in all parts of the tree and that using any part of neem as a pesticide against *Spodoptera frugiperda* can result in a mortality rate of over 50%, even in an uncontrolled natural environment.

According to Biri and Sahli (2022), neem oil also causes several malformations and morphological anomalies in *Drosophila melanogaster* and inhibits adult emergence, which increases significantly as a function of concentration.

Indeed, Fortuné *et al.* (2018), in their study on the effect of *Azadirachta indica*-based aqueous extracts on aphid vectors of green pepper mottle virus, showed the efficacy of these extracts in controlling green pepper mottle virus and its vector (aphids). A similar study conducted on the efficacy of a mixture of medicinal plant extracts (*Gmelina*

arborea, *Eucalyptus citriodora*, *Azadirachta indica*, *Hyp-tis suaveolens*, *Vernonia amygdalina*, and *Cymbopogon citratus*) in protecting flowering cowpeas against *Megalurothrips sjostedti* (Trybom) revealed that the mixture of these medicinal plants was as effective as the synthetic insecticide treatment such as Decis (Adebayo *et al.*, 2007; Oparaeke, 2007).

The results obtained by Mondedji *et al.* (2014) confirm our findings and show that aqueous extracts of neem leaves are highly effective in managing insect pests (*Plut-tela xylostella*, *Hellula undalis*, and *Lipaphis erysimi*) and improving yields.

Additionally, according to Bélanger and Musabyimana (2005), neem extracts contain chemical substances active against insect pests in fields and stocks.

Moreover, unlike synthetic chemical insecticides, neem's use is safer for the environment because plant extracts are biodegradable and act as fertilizers (Faye, 2010). According to some authors, neem has no harmful effect on beneficial insects (Bélanger & Musabyimana, 2005).

Socio-economic impact

To further validate our results and enhance their credibility, we extended the product applications in the field by directly involving farmers in the process. The experiment was conducted in Ouargla, specifically in the Hassi Ben Abdallah region, where Boufaroua causes significant annual damage. A dozen medium-sized farms were selected to conduct demonstrations in collaboration with their owners. At the end of experiments, all the farmers were completely satisfied with the results. The use of pesticidal plant extracts, such as neem leaves, can increase yields at a lower cost-benefit ratio compared to existing synthetic pesticides on the market, thereby improving farmer incomes. The ease of preparing neem leaf extracts and their effectiveness in controlling *Oligonychus afrasiaticus* were important factors in persuading farmers in the study area to adopt this product. The production cost of this biopesticide is significantly lower than that of a chemical acaricide. It only requires planting a few neem trees in a palm grove to obtain the leaves, and it does not necessitate any special handling or storage equipment – simply storing it in powder form for several months suffices. Neem leaf extracts decompose rapidly in the environment, which limits the risk of environmental pollution and enhances the health quality of the produce grown (Faye, 2010). Moreover, the application of these biopesticides promotes a healthier, more balanced diet.

Conclusion

This study showed the effectiveness of *Azadirachta indica* leaf extracts in controlling *Oligonychus afrasiaticus* in the Oued Righ region of Algeria. The neem leaf treatment was highly effective on Boufaroua and represents an alternative to synthetic pesticides in integrated pest management of date palm.

Given these results, it would be desirable to extend this practice to all date palm growers in the Saharan regions where date palms are abundant.

Neem is well adapted to Saharan conditions. It would be beneficial to consider extending its use and making it accessible to all farmers. Additionally, adopting modern technological resources accessible to farmers would enhance pest control strategies.

Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

MAI designed the experiment. AA carried out the field-work. AA collected the data and redrafted the original draft. HII supervised the work. MAI contributed to the data analysis. MAI revised and wrote the final version of the manuscript. AA translated the final version. All authors reviewed the final version of the manuscript.

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