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Notes and Comments

Arthropods on *Azadirachta indica* A. Juss. (Sapindales: Meliaceae) saplings

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Azadirachta indica A. Juss. (Sapindales: Meliaceae) is used to control erosion due to its drought resistance and well-developed root system capable of extracting nutrients from lower soil levels. Applying *neem extract* directly to the crops as a pesticide (high toxicity to insects, feeding deterrent, and/or repellent effects) can be an effective agricultural practice due to its main compound: azadirachtin (Amaral et al., 2019). Few insects' pests damage *A. indica* like as *Atta sexdens rubropilosa* Forel, 1908 (Hymenoptera: Formicidae) (Souza et al., 2009). The Importance Indice (*I.I.*) can determine the loss and solution sources for a system in certain knowledge areas (e.g., agronomy), when production is known (Demolin-Leite, 2021). Events (e.g., agricultural pest) can have different magnitudes (numerical measurements), frequencies, and distributions (aggregate, random, or regular) of event occurrence, and *I.I.* bases in this triplet (Demolin-Leite, 2021). In general, the higher the magnitude and frequency, with aggregated distribution, the greater the problem or solution (e.g., natural enemies versus pests) for the system (Demolin-Leite, 2021). However, the final production of the system is not always known or is difficult to determine (e.g., degraded area recovery). A derivation of the *I.I.* is the Percentage of Importance Index-Production Unknown (*% I.I.-PU*) that can detect the loss or solution sources when production in the system is unknown (Demolin-Leite, 2024a). The *% I.I.-PU* can detect the most important phytophagous arthropods and their natural enemies by observing their ability to damage the plants (phytophagous) or reduce the herbivore (natural enemies), their constancies and types of distribution on the samples (e.g., aggregate) (Demolin-Leite, 2024a). The study aimed to evaluate the numbers and damages of herbivorous insects and their natural enemies on *A. indica* saplings using *% I.I.-PU*.

This study was carried out in a degraded area (≈ 1 ha) of the "Instituto de Ciências Agrárias da Universidade Federal de Minas Gerais" in Montes Claros, Minas Gerais state, Brazil (16º 51' 38" S, 44º 55' 00" W, 943 m) from April 2015 to March 2017. According to the Köppen climate classification, the climate of this area is tropical dry, with annual precipitation and temperature between 1,000 and 1,300 mm and ≥ 24ºC. The soil is Neosol Litolic with an Alic horizon. The *A. indica* seedlings were prepared, in March 2014, in a nursery in plastic bags (16 x 24 cm) with reactive natural phosphate mixed with the substrate at a dosage of 160g and planted, at the same time, in September 2014. Each *A. indica* seedling was planted in a hole (40 x 40 x 40 cm) when they reached 30 cm in height – there was a 2-meter spacing between them. The soil was corrected, per role, with dolomitic limestone (50 g) with base saturation increased to 50%, natural phosphate (80 g), plaster (50 g), FTE (Fried Trace Elements) (10 g), and potassium chloride (2 g) based on the soil analysis. The sewer mud manuring was made while planting the *A. indica* seedlings (2 L). There were two extra applications (1 L each) eight and 16 months later, totalizing 4 L/hole. The mud of the dehydrated sewer was obtained from the Sewer Treatment Station, Company of Sanitation of Minas Gerais (COPASA S.A.), Juramento, Minas Gerais State, Brazil. The sewer mud had the following characteristics: 4.67% total humidity, 95.33% dry matter, 27.86% organic matter, 1.69% nitrogen, 16.20% carbon, and 9.59 C/N. The young 30 *A. indica* saplings (young trees in the vegetative period) were irrigated twice a week until the beginning of the rainy season (October). The phytophagous damage were evaluated: i) defoliation - leaf area loss on a 0–100% scale with 5% increments for removed leaf area (Silva et al., 2020) and ii) score damage by sap-sucking insects or mites: I = non-damage; II = appearance of yellow chlorotic spots (leaf with 1% to 25% of attack symptoms); III = some yellow chlorotic spots and/or starting of black sooty mold (leaf with 26% to 50% of attack symptoms); IV = several yellow chlorotic spots and/or severe blackening of leaves (leaf with 51% to 75% of attack symptoms); and V = yellowing or complete drying leaves (leaf with 76% to 100% of attack symptoms). The numbers of arthropods (phytophagous and natural enemies) were assessed visually were counted, between 7:00 A.M. and 11:00 A.M., by visual observation every two weeks on the adaxial and abaxial surfaces of the first 12 leaves expanded, per sapling. A few arthropod specimens (up to three individuals) per species were collected with an aspirator (two hours per week) at the beginning of the study (between transplantation and first evaluation, six months after), stored in flasks with 70% alcohol, and sent to specialists for identification. Each replication is the total of individuals collected on 12 leaves (three heights and four sides of the sapling) for 24 months. The chi-square test defined the distribution

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type (aggregated, random, or regular) of arthropods (phytophagous and natural enemies) using the R-package 'IIProductionUnknown' (Demolin-Leite and Azevedo, 2022). The data also were subjected to simple regression analysis, and their parameters were all significant ($P< 0.05$) using the R-package 'IIProductionUnknown' (Demolin-Leite and Azevedo, 2022) (Supplementary Material, Table S1). The data above were used in the Percentage of Importance Indice-Production Unknown (*% I.I.-PU*). The formula is: *% I.I.-PU*= $[(ks_1 \times c_1 \times ds_1)/\Sigma(ks_1 \times c_1 \times ds_1)+(ks_2 \times c_2 \times ds_2)+(ks_n \times c_n \times ds_n)]$x100 [see details in Demolin-Leite (2024a)].

The phytophagous insects *Tropidacris collaris* Stoll., 1813 (Orthoptera: Romaleidae) (38.89%), non-identified Tettigoniidae (Orthoptera) (16.73%), and *Trigona spinipes* Fabr., 1793 (Hymenoptera: Apidae) (14.43%) showed the highest *% I.I.-PU* on leaves of *A. indica* saplings (Table 1).

Table 1. Total number (*n*), damage (*Da.*), reduction of *L.S.* (*R.L.S.*), key-source (*ks*), constancy (*c*), distribution source (*ds*), number of importance indice (*n. I.I.*), sum of *n. I.I.-PU* (Σ *n. I.I.*), and percentage of *I.I.* by phytophagous arthropods and natural enemies on the saplings of *Azadirachta indica* (Meliaceae).

Phytophagous arthropods	***n***	***Da.***	***ks***	***c***	***ds***	***n. I.I.***	**Σ n. I.I.**	***% I.I.***
Orth.: Romaleidae, *Tropidacris collaris* Stoll., 1813	7	0.0334	0.0048	6	0.56	0.0160	0.0410	38.89
Orth.: Tettigoniidae N.I.*	5	0.0238	0.0048	5	0.29	0.0069	0.0410	16.73
Hym.: Apidae, *Trigona spinipes* Fabr., 1793	3	0.0143	0.0048	3	0.41	0.0059	0.0410	14.43
Col.: Chrysomelidae, *Diabrotica speciosa* Germar, 1824	2	0.0095	0.0048	2	0.48	0.0046	0.0410	11.11
Col.: Chrysomelidae, *Cryptocephalus* sp.1	1	0.0048	0.0048	1	0.54	0.0026	0.0410	6.28
Col.: Lagriidae, *Lagria villosa* (Fabr., 1783)	1	0.0048	0.0048	1	0.54	0.0026	0.0410	6.28
Phasmatidae N.I.*	1	0.0048	0.0048	1	0.54	0.0026	0.0410	6.28
Hem.: Aleyrodidae, *Bemisia tabaci* (Genn., 1889)	3	0.0000	0.0000	3	0.41	0.0000	0.0410	0.00
Hem.: Cicadellidae N.I.*	2	0.0000	0.0000	2	0.48	0.0000	0.0410	0.00
Dip: Otittidae, *Euxesta* sp.	13	0.0000	0.0000	3	1.00	0.0000	0.0410	0.00
Hem.: Membracidae N.I.*	1	0.0000	0.0000	1	0.54	0.0000	0.0410	0.00
Hem.: Pentatomidae N.I.*	1	0.0000	0.0000	1	0.54	0.0000	0.0410	0.00
Hem.: Cicadidae, *Quesada gigas* Oliver, 1854	6	0.0000	0.0000	6	0.23	0.0000	0.0410	0.00
Aca.: Tetranychidae, *Tetranychus ludeni* Zacher, 1913	8	0.0000	0.0000	2	1.00	0.0000	0.0410	0.00
Natural enemies	***n***	***R.L.S.***	***ks***	***c***	***ds***	***n. I.I.***	Σ n. I.I.	***% I.I.***
Ara.: Araneidae N.I.*	6	0.0000	0.0000	6	0.27	0.000	0.000	0.00
Hym.: Formicidae, *Brachymyrmex* sp.	20	0.0000	0.0000	12	1.00	0.000	0.000	0.00
Hym.: Braconidae N.I.*	3	0.0000	0.0000	3	0.43	0.000	0.000	0.00
Neur.: Chrysopidae, *Chrysoperla externa* (Hagen, 1861)	1	0.0000	0.0000	1	0.53	0.000	0.000	0.00
Col.: Coccinellidae, *Cycloneda sanguinea* L., 1763	3	0.0000	0.0000	3	0.43	0.000	0.000	0.00
Hym.: Formicidae, *Camponotus* sp.	264	0.0000	0.0000	30	1.00	0.000	0.000	0.00
Dip.: Syrphidae,*Episyrphus balteatus* (De Geer,1776)	3	0.0000	0.0000	3	0.43	0.000	0.000	0.00
Col.: Lampyridae N.I.*	19	0.0000	0.0000	14	0.42	0.000	0.000	0.00
Mant.: Mantidae, *Mantis religiosa* L., 1758	12	0.0000	0.0000	7	0.99	0.000	0.000	0.00
Hym.: Vespidae, *Polistes* sp.	72	0.0000	0.0000	16	1.00	0.000	0.000	0.00

*N.I.= non-identified. See details of this index in Demolin-Leite (2024a).

The sap-sucking insects and mites found did not damage *A. indica* saplings (score = I, non-damage). The chewing insect *T. collaris* damages *A. indica* saplings and confirms its polyphagy, which has been reported to attack several plants like *Acacia auriculiformis* A. Cunn. ex Beth (Fabales: Fabaceae) and *Casuarina glauca* Sieber (Casuarinales: Casuarinaceae) (Poderoso et al., 2013; Demolin-Leite, 2024a). The non-identified Tettigoniidae is related attacking *A. auriculiformis* and *Terminalia argentea* Mart. & Zucc. (Myrtales: Combretaceae) (Carvalho et al., 2020; Demolin-Leite, 2024a) and *T. spinipes* damages leaves and flowers such as on *Caryocar brasiliense* Camb. (Malpighiales: Caryocaraceae) trees and *Zantedeschia aethiopica* (L.) Spreng. (Commelinales: Araceae) plants (Carvalho et al., 2018; Demolin-Leite 2024b). However, these phytophagous insects on *A. indica* saplings occurred in low densities probably due to the compound azadirachtin, and, consequently, their natural enemies - *% I.I.U.P.* = 0.00% (Table 1). Azadirachtin is an insect antifeedant, growth disruptor, and insecticide (Amaral et al., 2019). However, among the natural enemies, *Camponotus* sp. (Hymenoptera: Formicidae) showed the highest number on *A. indica* saplings (Table 1), probably due to the two pairs of glands at the base of *A. indica* leaf which attract ants (Sornapriya and Varunprasath, 2019). The low densities of arthropods - herbivorous or not - on *A. indica* saplings probably, besides the several chemical compounds present in this plant, probably was due to *Camponotus* sp.. This aggressive ant react strongly to any disturbance of the tree which contains its nest like as observed in *A. indica* trees in Periyanaickenpalyam village, in South India (Sornapriya and Varunprasath, 2019).

The phytophagous insects *T. collaris*, Tettigoniidae, and *T. spinipes* were the most important ones on the leaves of *A. indica* saplings. However, these phytophagous insects occurred in low densities, probably due to the azadirachtin compound and *Camponotus* sp. ants.

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Supplementary Material

Supplementary material accompanies this paper.

Table S1. The phytophagous arthropods and natural enemies are distributed on Azadirachta indica (Meliaceae) saplings.

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