

Characterization of Aleppo pine, *Pinus halepensis* needles in the Sénalba forest, Djelfa : interaction with Insect pests

Sara HEZIL^{1*}, Hoceme DEGAICHIA¹, Gahdab CHAKALI²

Abstract

This study investigates the phytochemical characterization of Aleppo pine (*Pinus halepensis*) needles from the Sénalba forest in Djelfa, Algeria, focusing on their interaction with invasive forest pests. Qualitative phytochemical tests were performed on lyophilized needle powder to assess secondary metabolite composition, revealing notable differences between needles used as oviposition sites and those that were not. The analysis identified high levels of tannins (catechic and gallic) and flavonoids in needles associated with oviposition, indicating their potential role in attracting pest females. Terpenes and sterols were moderately present across all samples, while saponins were more abundant in reforested areas where oviposition occurred. Alkaloids, however, were consistently absent. These findings align with previous research demonstrating the richness of *P. halepensis* in phenolic compounds, influenced by various factors such as geographic location, environmental conditions, and extraction methods. The antioxidant and enzyme-inhibitory properties of flavonoids, along with the protective and regenerative effects of tannins, appear to contribute to their ecological significance. These compounds may act as olfactory cues, making reforested monocultures more detectable to pest females compared to mixed natural forests. This research highlights the critical role of secondary metabolites in host-plant selection by invasive pests and provides a foundation for further studies aimed at enhancing forest pest management strategies.

Keywords: Algeria, Forest, phytochemical characterization, *Pinus halepensis*,.

Caractérisation des aiguilles de pin d'Alep, *Pinus halepensis* dans la forêt de Sénalba, Djelfa : interaction avec les ravageurs insectes

Résumé

Cette étude examine la caractérisation phytochimique des aiguilles de pin d'Alep (*Pinus halepensis*) provenant de la forêt de Sénalba, à Djelfa, en Algérie, en mettant l'accent sur leur interaction avec les ravageurs forestiers invasifs. Des tests phytochimiques qualitatifs ont été réalisés sur de la poudre d'aiguilles lyophilisées pour évaluer la composition en métabolites secondaires, mettant en évidence des différences notables entre les aiguilles utilisées comme sites de ponte et celles qui ne l'étaient pas. L'analyse a révélé de fortes concentrations de tanins (catéchiques et galliques) et de flavonoïdes dans les aiguilles associées à la ponte, indiquant leur rôle potentiel dans l'attraction des femelles des ravageurs. Les terpènes et les stérols étaient présents de manière modérée dans tous les échantillons, tandis que les saponines étaient plus abondantes dans les zones reboisées où la ponte avait eu lieu. Les alcaloïdes étaient cependant systématiquement absents. Ces résultats s'accordent avec des recherches antérieures mettant en évidence la richesse en composés phénoliques de *P. halepensis*, influencée par divers facteurs tels que la localisation géographique, les conditions environnementales et les méthodes d'extraction. Les propriétés antioxydantes et inhibitrices des enzymes des flavonoïdes, ainsi que les effets protecteurs et régénérants des tanins, semblent contribuer à leur importance écologique. Ces composés pourraient servir de signaux olfactifs, rendant les monocultures reboisées plus détectables par les femelles des ravageurs par rapport aux forêts naturelles mixtes. Cette recherche met en évidence le rôle essentiel des métabolites secondaires dans la sélection des plantes hôtes par les ravageurs invasifs et fournit une base pour des études futures visant à améliorer les stratégies de gestion des ravageurs forestiers.

Mots clés: Algérie, Forêt, Caractérisation phytochimique, *Pinus halepensis*

* Main author

¹ Centre de Recherche en Agropastoralisme, Djelfa, 17000, Algérie

² École Nationale Supérieure d'Agronomie, El-Harrach, 16200, Alger-Algérie

INTRODUCTION

Aleppo pine (*Pinus halepensis*) is a key species in Mediterranean ecosystems, widely distributed across arid and semi-arid regions. Known for its resilience to drought, it thrives in rocky, sandy, and poor soils, making it essential for soil stabilization and reforestation efforts. Its role in mitigating desertification is particularly significant, as it provides critical ecological services, including habitat for wildlife and prevention of erosion in areas prone to degradation.

The selection of Aleppo pine for forestry and restoration projects is driven by several factors. Its adaptability to various environmental stresses, particularly water scarcity, positions it as a cornerstone of forest management in the Mediterranean region. Aleppo pine's rapid growth rate and ability to regenerate make it an ideal candidate for reforestation in areas where soil fertility is low, or in regions affected by desertification. In addition to its ecological benefits, Aleppo pine contributes to the local economy, as it is used for timber, resin, and ornamental purposes, while also offering potential for carbon sequestration in the context of climate change (Rousselet 2010; Tiberi et al. 1999).

The physical and chemical properties of Aleppo pine also influence its ecological interactions. Its needle morphology, which is characterized by long, slender needles, plays a significant role in its resistance to various environmental factors. Moreover, the high resin content of Aleppo pine helps protect the

tree from pests and pathogens. However, under certain environmental conditions, the tree may still be susceptible to specific threats, such as the pine processionary, a pest that targets various pine species, including Aleppo pine. This pest's interaction with the tree is influenced by both the needle morphology and the volatile compounds released from the pine, such as limonene, which may attract or repel herbivores (Tiberi et al. 1999; Hodar et al. 2002).

Furthermore, the nitrogen content and other biochemical factors in Aleppo pine influence its growth and overall health, further enhancing its adaptability in challenging environments. These factors make it an essential species not only for forest restoration efforts but also for studies on plant-pest interactions and forest health (Paiva et al. 2011).

Aleppo pine is a vital species in Mediterranean forest ecosystems, offering numerous ecological, economic, and environmental benefits. Its ability to thrive in dry, nutrient-poor soils, combined with its rapid growth and resilience, makes it a key species in the fight against desertification and climate change. Understanding its biological and chemical characteristics is essential for optimizing its use in reforestation and forest management, as well as for protecting it from potential pests such as the pine processionary, which poses a threat to its vitality under specific condition.

MATERIAL AND METHODS

1. Choice of Host plant

The Aleppo pine (*Pinus halepensis*) studied in this research belongs to the Pinaceae family and is found in semi-arid regions. Only the tree's needles, which can reach up to 60 mm in length, were used in this study. Fresh needles were collected directly from the tree.

Two categories of needles were chosen: control needles and needles supporting egg masses of the pine processionary moth, to investigate the selection preferences of female moths for laying eggs. The methodology is based on the works of Samseny (2003), Diallo (2005), and Rachel (2005).

2. Alkaloid Characterization Reactions

Alkaloids are important natural compounds with structural diversity and significant pharmacological activity. They are nitrogenous organic substances with basic properties.

2.1. Solution to Analyze: 10 g of powdered Aleppo pine needles were mixed with 50 ml of 1/10 diluted H_2SO_4 in a 250 ml Erlenmeyer flask and macerated for 24 hours at room temperature. The mixture was filtered to obtain approximately 50 ml of filtrate.

2.2. Characterization: Alkaloid precipitation was tested with Mayer and Dragendorff reagents using a control substance (strychnine). The precipitate was categorized as abundant (+++), moderate (++), or faint (+).

3. Polyphenolic Substance Reactions

Polyphenolic compounds are complex and vary greatly. The presence of at least one benzene ring attached to a hydroxyl group is essential for this group's structure.

3.1. Solution to Analyze: 5 g of powdered Aleppo pine needles were infused in 100 ml of boiling distilled water for 15 minutes, and then filtered to obtain 100 ml of filtrate.

3.2. Characterization:

Tannins: Reaction with $FeCl_3$ showed a greenish or bluish-black color in the presence of tannins.

Catechic Tannins: Addition of concentrated HCl to the infusion caused a red precipitate to form, indicating the presence of catechic tannins.

Flavonoids: The presence of anthocyanins was indicated by color changes in the presence of sulfuric acid and ammonium hydroxide.

4. Cyanidin Reaction

The infusion was tested for flavonoids by adding hydrochloric alcohol, isoamyl alcohol, and magnesium chips. A color change to orange, violet, or red indicated the presence of free flavonoids.

5. Anthraquinone Derivatives

Anthraquinones belong to the quinone group, characterized by high oxidizing properties.

Solution to Analyze: 1 g of powdered needles was treated with chloroform and heated in a water bath. The hydrolysate was analyzed for the presence of anthraquinones.

Characterization: Anthraquinones were identified by the red coloration in chloroform or hydrolysate extracts.

6. Cardiotoxic Glycosides

1 g of powdered needles was mixed with ethanol and a neutral acetate solution of Pb and heated. The filtrate was tested for the presence of cardiotoxic glycosides through color reactions with specific reagents.

7. Detection of Narcotics (THC)

The presence of tetrahydro-cannabinols was tested by extracting with petroleum ether, followed by a reaction with potassium hydroxide, resulting in a violet color for a positive test.

8. Carotenoid Characterization

The presence of carotenoids was confirmed by the blue color that turned red upon the addition of antimony chloride solution.

9. Cardiotoxic Glycosides

A separate test for cardiotoxic glycosides involved extraction with chloroform, followed by the addition of

specific reagents. Positive reactions produced distinct color changes (orange, red-violet, or purple).

10. Alkaloid Test

1 g of dried plant powder was macerated with 10 ml of 5% HCl. After filtering, Mayer's reagent was added, and a yellowish-white precipitate indicated the presence of alkaloids.

11. Sterols and Terpenes Test

5 g of dried powder was dissolved in petroleum ether, filtered, and evaporated. The residue was treated with acetic acid and concentrated sulfuric acid, forming a purple or brown ring at the interface, indicating the presence of sterols and terpenes.

This concise summary presents the various chemical tests used for phytochemical analysis of Aleppo pine needles, focusing on alkaloids, polyphenols, flavonoids, anthraquinones, and other bioactive compounds.

RESULTS AND DISCUSSION

The existence of physical and chemical links between insects and plants was recognized as early as the beginning of the 20th century (**Richardson, 1925 in Robert, 1986**). The identification of the host plant by the ovipositing females of phytophagous insects is perceived at a distance from the host plant through typical odors (**Robert, 1986**).

The hypothesis of a chemical barrier to colonization of the host tree suggests that insects locate their host through certain volatile compounds (**Dulaurant, 2012**). The same author notes that in mixed stands, the olfactory signal emitted is more complex than in pure stands. In this regard, **Jactel et al. (2015)** observed that the host plant selected by the pine processionary moth involves a wide range

of physical and chemical cues acting at different spatial and temporal scales.

Characterization reactions revealed several chemical groups in the powder of *Pinus halepensis* needles. We searched for the main classes of secondary metabolites in the leaves of *Pinus halepensis*, namely alkaloids, saponins, tannins, and flavonoids shown in Table 1.

Table 1. Characterization Reactions for Aleppo Pine Needle Powder

	Coloration	Interpretation
Case 1: Reforestation,- Control needles		
Catechic tannins (Stiasny reaction)	Greenish-brown precipitate	++
Gallotannins (Stiasny reaction)	Bluish-black	++
Flavonoids	Light yellow	++
Saponins	Persistent foam	++
Alkaloids	Negative	-
Carotenoids	Blue	+
Sugars and holosides	Red	++
Triterpenoid glycosides	Red-brown ring	+++
Terpenes and sterols	Brown circle	++
Case 2: Reforestation, needles with batches (pure stand)		
Catechic tannins (Stiasny reaction)	Greenish-brown precipitate	++
Gallotannins (Stiasny reaction)	Bluish-black	++

Flavonoids	Light yellow	+++
Saponins	Persistent foam	++
Alkaloids	Negative	-
Carotenoids	Blue	+
Sugars and holosides	Red	++
Case 3: Natural Forest –Control Needles (mixed stand)		
Catechic tannins (Stiasny reaction)	Light red precipitate	++
Gallotannins (Stiasny reaction)	Bluish-black	++
Flavonoids	Clear layer at the top of the tube	++
Saponins	Visible foam	+
Alkaloids	Negative	-
Carotenoids	Blue	+
Sugars and holosides	Red	++
Case 4: Natural Forest Needles with Batches (mixed stand)		
Catechic tannins (Stiasny reaction)	Brown precipitate	++
Gallotannins (Stiasny reaction)	Bluish-black	+++
Flavonoids	Color change	+++
Saponins	Visible foam	++
Alkaloids	Negative	-
Carotenoids	Blue	+
Sugars and holosides	Red	++

(+++): Positive reaction, (++): Moderately positive reaction, (+): Traces

Qualitative phytochemistry based on color reactions or precipitations by specific chemical reagents, conducted on reconstituted extracts from freeze-dried powder of each plant sample, provides preliminary data on the constituents of the extracts. The “+” sign indicates the presence of a chemical compound group in quantities above the detection threshold, while the “-” sign indicates a negative reaction. It shows the presence or absence of a secondary metabolite group.

Phytochemical tests revealed that; There is a high content of tannins and flavonoids in the needles supporting the egg batches of the pine processionary moth. There is a moderate amount of terpenes and sterols in all the needles. Saponins are present in moderate amounts in the needles supporting egg batches of reforestation. No precipitate was observed for the alkaloids test, indicating the absence of alkaloids.

In this regard, **Amari and Mechouche (2017)** note that the phenolic extract from *P. halepensis* resin is an important source of

phenolic compounds. Relatively high levels of total phenols (232 ± 12.72 mg EAG/gE), flavonoids (91.81 ± 2.66 mg EQ/gE), and proanthocyanidins (607.73 ± 19.28 mg EC/gE) were recorded, confirming the plant's richness in phenolic compounds. Several intrinsic and extrinsic factors—such as growth conditions, maturation process, sampling techniques, storage conditions (**Bergonzi et al. 2001**), climatic and environmental factors (geographic area, drought, soil), and extraction and quantification methods—can influence the results obtained.

Flavonoids exhibit numerous activities, including antioxidant, anti-inflammatory, and enzyme-inhibitory effects (**Sharma et al., 2008**). Tannins promote tissue regeneration in cases of superficial wounds or burns and have a vasoconstrictive effect on small superficial vessels. In general, they are enzyme inhibitors. Some tannins, with structures similar to flavonoids, have vitamin-like properties (**Roux et al. 2007**).

Based on the properties of flavonoids and tannins, the females of the pine

processionary moth choose the pine needles that provide the best conditions for their offspring. Furthermore, some flavonoids may sometimes attract butterflies, with the presence and content of different glycosides drawing the female butterfly. The female pine processionary moth easily locates pure reforestation of *Pinus halepensis* compared to mixed natural pine forests through olfactory

signals, which make it more difficult to locate the host species in natural mixed biotopes. These ideas should be further developed through additional research investigations.

CONCLUSIONS

The phytochemical analysis of *Pinus halepensis* needles from both pure and mixed stands of reforestation and natural forests revealed key chemical compounds that influence the host selection behavior of forest defoliators. Notably, the presence of tannins, flavonoids, and saponins in the needles, particularly those supporting egg batches, suggests a strong chemical interaction between the insect and its host plant. These secondary metabolites, which play crucial roles in plant defense mechanisms, may also contribute to the moth's preference for certain types of pine stands.

The higher concentrations of flavonoids and tannins in needles supporting egg batches indicate that these compounds likely play a role in enhancing the suitability of the plant for reproduction, thus influencing the distribution and colonization of the moth. Additionally, the

study highlights the complexity of chemical signaling in mixed forest ecosystems, where the olfactory cues emitted by the host plants may differ from those in pure stands, potentially affecting the moth's ability to locate its preferred host.

These findings underscore the importance of understanding the phytochemical composition of host plants as a factor in insect-plant interactions, with implications for forest management practices aimed at controlling pest populations. Further research is needed to explore the functional roles of these phytochemicals in pest behavior and their potential use in integrated pest management strategies. The study also opens avenues for investigating the ecological dynamics of mixed vs. pure stands in forest ecosystems, contributing to a more nuanced understanding of forest biodiversity and insect dynamics.

ACKNOWLEDGMENTS

The authors gratefully acknowledge CFD (the Conservation of Forests of Djelfa) and DGRSDT (The General Direction for Research

and Technology Development) for their facilities and assistance. The authors declare no conflict of interest.

REFERENCES

- Amari M., Mechouche K., 2017- Contribution à l'étude des activités biologiques de la résine de *Pinus halepensis* Mill. Thèse. Master. 71p.
- Bergonzi M.C., Bilia A.R., Gallori S., Guerrini D., Vincieri F.F., 2001- Variability in the content of the constituents of *Hypericum perforatum* L. and some commercial extracts. Drug Dev. Ind. Pharm., 27: 491-497
- Diallo A., 2005- Etude de la phytochimie et des activités biologiques de *syzygium guineense* Willd. (myrtaceae). Thèse. Doc. Pharmacie. 100p.

- Hodar J. A., Zamora R., & Castro J., 2002- Host utilisation by moth and larval survival of pineprocessionary caterpillar *Thaumetopoea pityocampa* in relation to food quality in three Pinus species. Ecological Entomology, 27, 292–301.
- Paiva M.R, Mateus E, Santos MH, Branco M (2011). Pine processionary moth females use semiochemicals for host selection. IOBC/WPRS Bulletin 72 : 159-164.
- Rachel P.N.W., 2005- Propriétés pharmacochimiques de *Calotropis procera* Ait. (Asclepiadaceae) récolté au Mali : étude préclinique des effets anti-inflammatoires et antimicrobiens des extraits des écorces de racines. Thèse Docteur d'Etat en Pharmacie. 162p.
- Rousselet J., 2010- La chenille processionnaire du pin. L'arbre en ville : un hôte pour des invités non désirés. Inra Centre d'Orléans. 5p.
- Roux D., Catier O., 2007- Botanique, pharmacognosie, phytothérapie. Collection du cahier du préparateur en pharmacie, pp :141-146
- Samseny A.R.R., 2003- Contribution à l'étude phytochimique d'une plante traditionnellement utilisée comme poison d'épreuve au Gabon : le *Strychnos Icaja* Baillon (Mbundu) ; Loganiaceae. Thèse. Doc. Pharmacie. 89p.
- Tiberi R., Niccoli A., Curini M., Epifano F., Marcotullio M. C., & Rosati O., 1999-The role of the monoterpene composition in Pinus spp. needles, in host selection by the pine processionary caterpillar, *Thaumetopoea pityocampa*. Phytoparasitica, 27, 263–272