

The Effect of Chemical Defenses on the Density of Insect Herbivores Between High, Medium and Coastal Elevations of Al Jebel Al Akhdar

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Abstract: The study area is dominated by *Juniperus* sp and *Pistacia* sp, the two species, the study aimed to investigate the effect of chemical defences on the density of insect herbivores, and the results showed that the two species differed very much in their levels of phenols. *Pistacia* had the highest concentrations (overall 2308 ± 43 ppm, $n = 45$), with *Juniperus* quite a bit lower (1516 ± 43 , $n = 45$). Overall levels of phenols were highest in plants from the middle and lowest in those from the bottom elevation level. This effect of the level was significant, showing that the two species showed the same pattern with elevation. There were substantial differences among plots within levels. This has affected the numerical density of the insects, where it was observed that their density on the tested *Juniperus* sp shrubs was more than the intensity on the tested *Pistacia* sp shrubs.

Keywords: Phenolic defences; *Juniperus* and *Pistacia*; insect Herbivores; Al Jabal Al Akhdar, Libya

INTRODUCTION

Plants in natural populations are frequently attacked by a huge number of natural enemies, comprising both pathogens and herbivores at all periods of their life cycles. Pest stress can be devastating, as demonstrated by the epidemics of insects and diseases in farmed crops, and in the success of weed control by biological control (Levin, 1976). Outbreaks or episodes of intensive herbivory may lead straight to the local extinction of a plant species, or weaken plants and make them vulnerable to subsequent ecological pressures (Levin, 1976). The lowering of photosynthetic ability due to disease or partial defoliation reduces resistance to biotic stresses or infection by other pests, and decreases competitive capacity (Levin, 1976). From a user perspective, varied stands lead to mixed resources within which herbivores choose their favored individuals. It is well recognized that the amount of herbivory is strongly

determined by leaf chemical and physical characters counting how they differ within a given host species with rising plant diversity, competition or facilitation among species could activate changes in plant nutritional quality or the creation of secondary metabolites (Muiruri et al., 2019).

Mediterranean climate regions experience reducing rainfall with decreasing latitude towards the equator, and there is a gradual change in vegetation toward plants that are adapted to semi-arid and desert conditions (Dallman, 1998). The local vegetation of the Mediterranean lands is adapted to survive long hot summer droughts and prolonged wet periods in winter (Martinez, 2002). The amount of energy allocated by Mediterranean plants to growth and maintenance is comparatively high-low resource accessibility may either be a cue for a plastic response that leads to, or a selection pressure for the evolution of, a greater investment in protection

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against biotic agents, that is shown by greater investment in defensive chemicals such as phenols, alkaloids, etc. (Dallman, 1998). Lack of water might also be involved; perhaps by restricting growth rate, this might also lead to the accumulation of defense compounds for greater resistance against natural enemies. Therefore, the cost of producing chemical defenses might be predicted to be higher in Mediterranean species than in those living in more favorable biomes. Mediterranean habitats vary in several characteristics; if these include nutrient and water availability, we might predict differences in the chemical defenses of Mediterranean plant species. Thus, plants at different elevations should invest different amounts in defense because of the availability of water and nutrients (Martinez, 2002).

Mediterranean plants have experienced high levels of herbivory by wild and domesticated ungulates over thousands of years (Massei, Hartley, & Bacon, 2000). We might expect, therefore, the existence of evolved interactions with mammals, but actually, very little attention has been paid to plant-mammal interactions in Mediterranean ecosystems. Phenolic concentrations were consistently higher in winter than in spring, and in new rather than old leaves, just when browsing is more probable (Glyphis & Puttick, 1988; Massei et al., 2000). These compounds reduce the palatability of plants to mammalian herbivores, but there is no evidence that they are induced by browsing (Massei et al., 2000; Rohner & Ward, 1997), although regrowth foliage following browsing may have decreased levels of phenolics in other ecosystems (Bryant, Heitkonig, Kuropat, & Owen-Smith, 1991; Bryant, Reichardt, & Clausen, 1992). (Glyphis & Puttick, 1988) showed that levels of phenolic compounds in shrub leaves are higher in winter than spring, and in new than older leaves, in only half of the species they checked.

The current study was carried out in the Al Jabal Al Akhdar region of Libya that consists of a high density of evergreen species of shrub and trees. We analysed variation among individual plants, among plots, and among elevations in the phenol levels of the two dominant species, that there will be differences in phenol concentrations in *Juni-*

perus and *Pistacia* within elevation. After testing this prediction, I will go on to test the impact of that plant chemistry (in the form of tannin levels) have on the density and distribution of insect herbivores on *Pistacia atlantica* and *Juniperus phoenicea*. Insects herbivory rates are affected by plant traits (such as levels of chemical defenses).

This research aims to study the effect of chemical defenses (phenols) on the types of insect herbivory among the top, middle and bottom elevations sampled in the Al Jabal Al Akhdar region of Libya.

MATERIALS AND METHODS

The study was carried out in the Al Jabal Al Akhdar in northeastern Libya (32°49'N; 21°51'E). The climate is Mediterranean: mean temperatures vary between 25 °C in February and 33 °C in July, and rainfall ranges between 520 and 600 mm per year (Faituri, 2002). The study area is dominated by *Juniperus phoenicea* and *Pistacia sp.* plants of each species were selected randomly and marked permanently in each of the nine plots at three different elevations (bottom 0-200 m, middle 200-400 m, and top 400-880 m above sea level). At each elevation, three plots were placed randomly that each was more than 1km away from the others. Ten fresh leaves per shrub/tree were collected from each marked plant: *Juniperus* ($n = 45$) and *Pistacia* ($n = 45$). Leaves were oven-dried at 60 °C for 48 hours, milled and analyzed at the Plant Protection Department at Omar Al Mukhtar University.

For collecting free-living insects, the methods used were sweep nets and beating. Collected specimens were killed by freezing, then pinned, labeled, and subsequently identified at the Natural History Museum in London. We selected elevations in a random order but were visited. The strategy was first to walk around the tree/shrub and observe what insects were present, and catching those which are reachable without disturbance. Then we worked through the foliage by hand, starting from the top, thoroughly and systematically turning over leaves

and branches initially until the range had been fully appreciated, all insects were collected from each tree/shrub, and after that, we counted the number of each type. It took more than one day to sample five plants. Every individual insect was given an identification code that tracked when, where, and on which plant it was collected. Some individual insects were maintained alive to ascertain whether the species was a herbivore of *Juniperus* or *Pistacia*. We placed a single insect in a Petri dish with some cut pieces of uneaten leaf material and a wad of wet tissue. The insects were checked periodically, and after 48 hours, we noted which ones have or have not fed. All were then frozen and pinned. The distinction between true herbivores and other insects is made clear in the results section. The assessment of the numbers of various insect pest species was done by carefully examining the selected host plants, leaf by leaf, and turning of leaves as well to collect any insects from the under-surface of the leaves. The insect herbivores collected from each plot were identified, counted and recorded. This study focuses on the insect herbivores that feed on the leaves of *Pistacia* and *Juniperus*, and others that attack the trunk, if found. There are insect herbivores that were not studied for the lack of time, such as those that feed on seeds, fruits, roots or that live under the bark.

Leaf samples were analyzed for total phenolics measured by detecting phenolate ions. Tannic acid was used as a standard, and the extraction of dried leaf material was carried out using 50% aqueous methanol. Phenolic content was expressed as % dry weight (calculated as mg tannic acid equivalents per mg dry leaf mass \times 100). The Phenolic assay was conducted according to the Folin-Ciocalteu method (Waterman & Mole, 1994). The protocol followed for this study was as follows: A standardized Phenolic solution was prepared with 10 ± 0.3 mg tannic acid in 100ml water. The precise amount used was recorded for use in later calculations. A 'blank' 4.2 ml cuvette was prepared with 3 ml DI water. A series of cuvettes

were prepared with 50-450 ml tannic acid solution in 50 ml increments and made up to 3 ml with DI water; and used to produce a standard line of tannic acid concentration by regression. For each sample to be analyzed, 15 ± 0.3 mg of ground leaf material was taken, and the precise amount used was recorded. Each sample was shaken with 10 ml of 50% methanol solution and phenolics extracted by standing in a sealed boiling tube in a water bath at 80°C for 30 minutes. Samples were immediately transferred to centrifuge tubes and spun at 3000 rpm for 15 mins, and 100 ml of each sample was placed in two cuvettes. These were made up to 3 ml with DI water and 0.25 ml saturated CaCO_3 solution and 0.25 ml Folin-Ciocalteu reagents added to each cuvette. The cuvettes were covered in parafilm and mixed, then left to stand. After 1 hr, the absorbance at 760 nm was measured for each cuvette against the blank prepared earlier. The mean value between the two replicates of each sample was taken. If the difference in absorbance between the two samples exceeded 0.05, the entire run was repeated, although in practice the two values were usually very close. The percentage of dry mass phenolics (%DM) in each sample were calculated by comparison with the standard line according to the formula:

$$\%DM = \frac{TA}{W} \times \left[\frac{A760 - c}{m} \right]$$

Where TA is the amount of tannic acid used in mg, W is the initial sample mass in mg, A760 is the mean absorbance for each sample, m and c are the gradients, and y-intercept of the standard line. Standard lines were produced at the start and the end of each day to confirm the consistency of measurements. Results are presented here as the parts per million of leaf dry weight that is made up of phenolics (or, more strictly, tannic acid equivalents).

We analyzed the phenolic content in the two species using GLM implemented in SPSS16. The design of the analysis was *species + level + plot (level) + species*level*. The data were

not normal (Shapiro-Wilk = 0.955, df = 90, p<0.001).

RESULTS

The two species differed greatly in their levels of phenols: *Pistacia* had the highest concentrations (overall 2308 ± 43 ppm, n = 45), with *Juniperus* was quite a bit lower (1516 ± 43, n = 45). Overall levels of phenols were highest in plants from the middle, and lowest in those from the bottom elevational level (figure 1). This effect of level was significant, showing that the two species showed the same pattern with elevation (Table 1). There were substantial differences among plots within levels (Table 1). The density of insect herbivores on *Juniperus* was higher than that on *Pistacia* shrub, that possibly because of the low level of phenol in the *Juniperus* shrubs than in *Pistacia* shrubs figure (2).

Table 2:

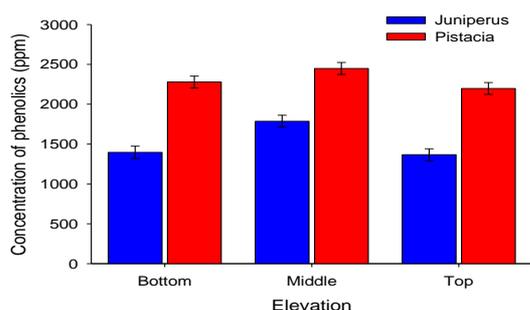


Figure 1: Phenolic content (parts per million) of leaves of *Juniperus phoenicea* and *Pistacia atlantica* shrubs/trees in the Al Jabal Al Akhdar region of Libya at three different elevational levels.

Table 1: Analysis of variation in the total phenolic content of *Juniperus* and *Pistacia* at three elevational levels in the Al Jabal Al Akhdar region of Libya.

Source	Tests of Model Effects		
	Type		
	Wald Chi-Square	df	Sig.
Species	165.616	1	.000
Level	21.924	2	.000
Plot(level)	33.589	6	.000
Level * species	2.371	2	.306

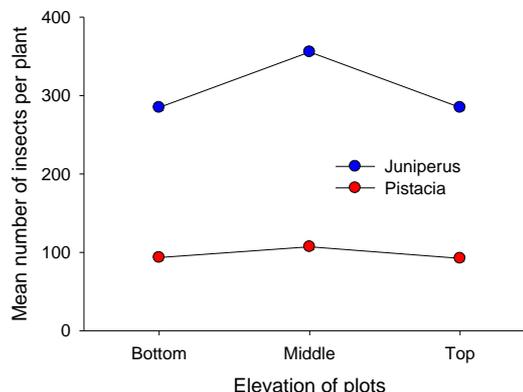


Figure 2: Average number (± s.e.) of individual insects discovered on marked trees of *Juniperus* and *Pistacia* in permanent plots at three different elevations in the Al Jabal Al Akhdar study site. The standard errors are too small to be seen.

DISCUSSION

Our data suggest that phenol concentrations were consistently higher in *Pistacia atlantica* than in *Juniperus phoenicea*, over and above spatial variation, among and within elevations. Overall levels of phenols were highest in plants from the middle and lowest in those from the bottom elevational level. This effect of level was significant, but there was no species-level interaction, showing that the two species showed the same pattern with elevation. There were substantial differences among plots within levels, but no effect on plant volume. Although there may indeed be spatial and/or temporal differences in the defensive chemistry of plants in response to the risk of herbivory (Coley, Bryant, & Chapin, 1985; Wold & Marquis, 1997) attempts to document such effects in Mediterranean areas have not been very successful (Dement & Mooney, 1974; Glyphis & Puttick, 1988). (Avi Perevolotsky, 1994) could not find any seasonal or age-related differences in the concentration of tannins in a variety of Mediterranean species, including *Pistacia*, *Phillyrea*, and *Quercus*. (Avi Perevolotsky, 1994) thought that constitutive were more likely in the Mediterranean than in-

ducible defenses. Although our study was not designed explicitly to test this, we observed constancy of defense chemical differences for these two Mediterranean plants over a wide range of environmental conditions.

A vegetation of these plant communities usually consists of 20–25 shrub species in the Mediterranean region, but typically only six or seven species are dominant (*Quercus ilex*, *Erica multiflora*, *Arbutus unedo*, *Juniperus phoenicea*, *Viburnum tinus*, *Pistacia lentiscus*, and *Hedera helix*) (Rogosic, Pfister, Provenza, & Grbesa, 2006). However, utilization of the Mediterranean shrubs is often limited by secondary compounds such as tannins, terpenes, and saponins (A Perevolotsky et al., 1993).

Mediterranean woody evergreens are predicted to have strong primary (constitutive) defenses because, in contrast to deciduous woody plants, they keep more nutrients and carbon in their leaves. Herbivory is, therefore, more damaging to evergreens than to deciduous plants (Bryant et al., 1992; Herms & Mattson, 1992). Several studies have shown that the leaves of *Quercus* spp. are high in phenolic compounds (Wold & Marquis, 1997), and we found that *Pistacia* had even higher concentrations of phenolics than reported for *Quercus*, whilst *Juniperus* had much lower concentrations. Some studies seem to show that nitrogen content varies with season and leafage, but not among species (Dement & Mooney, 1974; Meletiou-Christou, Rhizopoulou, & Diamantoglou, 1994). Concentrated study of the defense chemistry of Mediterranean plants is necessary before generalizations can be made.

The commonest species on *Juniperus* of sampling was *Xylomeira* sp. (Bostrichidae), a species that attacks live and dead wood. This might be a significant herbivore that could affect the survival and life-history of juniper in Al Jabal Al Akhdar. In fact, nearly all the commonest species on juniper were beetles including many wood-boring species, such as *Agrilus* (*Xeragrilus*) sp. (Buprestidae) and

Purpuricenusdesfontainii (Cerambycidae). Clearly, juniper is a major resource for beetles in this area.

On *Pistacia*, on the other hand, the commonest species varied between years, with Orthoptera heading the list in the first sampling year: *Paracinipe* (*Acinipe*) *orientalis*, *Oedopodacae rulescens*, and *Scintharistano tabilis*. A set of wood-boring beetles were commonest in the second year of sampling, some of which were the same as those on juniper. This variability may indicate that the quality of *Pistacia* as a host varies among years, but we do not really have any real indication as to its basis.

Geography is clearly one of the major influences on the distribution of the insect herbivore fauna of Al Jabal Al Akhdar in the Mediterranean ecosystem. The number of recorded species broadly increases with elevation, while middle elevations had the greatest insect abundances. Insect damage to plants also increased with elevation, possibly due to a decrease in the number of *Pistacia*, which is observed in study area.

CONCLUSION

The study area is dominated by *Juniperus* sp and *Pistacia* sp, the two species selected for the study. Leaves were sampled for chemical analysis of their phenol (tannin) content. The results showed that Tannin levels were much higher in *Pistacia* than in *Juniperus*, and there were effects of elevation as well. Plants from middle elevation plots had the highest levels, while those from the lowest elevation at the coast had the lowest levels of tannins. The density of insects on *Juniperus* sp was higher than that on *Pistacia* sp.

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الدفاعات الفينولية في شجيرتي العرعر والبطوم ضد العاشبات الحشرية في منطقة الجبل الأخضر-ليبيا

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المستخلص: يسود منطقة الدراسة أنواع من نباتات *Juniperus sp* و *Pistacia sp*، وهما النوعان اللذان تم اختيارهما للدراسة وقد هدفت الدراسة إلى معرفة تأثير الدفاعات الكيميائية لهذه النباتات على الحشرات العاشبة، وأظهرت النتائج أن النوعين يختلفان كثيراً في مستوياتهما من الفينولات. وأظهر *Pistacia sp* أعلى التركيزات (إجمالي 2308 ± 43 جزء بالمليون، ن = 45)، مقارنة مع *Juniperus sp* (1516 ± 43 ، ن = 45). كانت المستويات الإجمالية للفينولات هي الأعلى في النباتات منها في الارتفاع الوسط والأقل في تلك الموجودة في مستوى الارتفاع السفلي، حيث كان التأثير كبيراً في هذا المستوى، وأظهرت الدراسة أن النوعين أظهرتا نفس النمط مع الارتفاع. كانت هناك اختلافات كبيرة بين القطاعات داخل المستويات. وقد أثرت هذه الاختلافات على الكثافة العددية للحشرات، حيث لوحظ أن كثافتها على شجيرات *Juniperus sp* كانت أكثر من كثافتها على شجيرات *Pistacia sp*.

الكلمات المفتاحية: الدفاعات الفينولية؛ العرعر والبطوم؛ الحشرات العاشبة؛ الجبل الأخضر، ليبيا.