EFFECT OF NITROGEN AND BORON FERTILIZATION ON THE CHEMICAL COMPOSITION OF *PINUS PINEA L. NUT KERNELS*

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OBJETIVE

- **Evaluating the effect of boron and nitrogen fertilisation on the composition of total fat, fatty acids, protein and mineral nutrients in pine nut kernels**

MATERIAL and METHODS

Experimental plot

- Stand of **stone pine**
- Location: Municipality of **Coruche**, Ribatejo, Portugal
- Age: Planted in **1994/95** (2 blocks) **and 2004/05** (1 block)
- Soil: **Oligoeutric Arenosol** (WRB, 2015)




Experimental design

- Year of installation - **2018**
- Randomized complete blocks: **3**
- Useful trees in each plot: **4** (2 blocks) **and 5** (1 block)
- Experimental variables: **Nitrogen (N) and Boron (B)**
- Experimental treatments: **8**

T1 – N0B0 **T2 – N1B0** **T3 – N2B0** **T4 – N3B0**
T5 – N0B1 **T6 – N1B1** **T7 – N2B1** **T7 – N3B1**

- Nutrient levels:

N0=0; N1=30; N2=60; N3=90 kg ha^{-1} N (applied annually in early Spring as Urea 46% N)
B0=0; B1=2 kg ha^{-1} B (applied every 2 years in early Spring as Tecnifol Boro 11% B)

Sampling

- Ten healthy pine cones were sampled from each experimental treatment in 2018/19, 2019/20 and 2020/21
- The pine cone was dried and the pine nut in shell opened
- The chemical composition of pine nut kernels was determined

Analytical methods

- **Moisture -** by drying
- **Ashes -** by incineration
- **Total fat** - by Soxhlet extraction
- **Fatty acid composition -** by Gas chromatography (GC-FID)
- **Protein** - calculated from total N determined by Elemental Analyser (considering that the N content of proteins is 16%)
- **Mineral nutrients** - by Inductively Coupled Plasma (ICP-OES)

Statistical analysis

- **Analysis of variance (ANOVA II)** was used to assess the effect of B and N fertilisation on the variables studied
- **Tukey's HSD test** (p = 0,05) was used to establish differences among means

RESULTS

- The results obtained during the three years showed that no significant differences (p>0.05) were observed for moisture, ashes, protein and P, K, Ca, Mg, Zn and Cu concentrations due to the supply of N and B.
  On the other hand, the concentrations of Fe, Mn and B were affected by N fertilization, with the highest values observed when N was not applied.
- Also, N and B fertilization did not significantly influence (p>0.05) total fat and fatty acid composition, except for myristic acid (C14). The highest percentage of this fatty acid was obtained in T1 (without N and B application).
- During the three years, significant differences (p≤0.05) were observed between mean values of most of the variables evaluated (all minerals - except Mn -, moisture, ashes, protein, fat and 10 of the listed fatty acids).
- Fat was the predominant component (46.9 g/100 g DW), closely followed by protein (44.0 g/100 g) and the most abundant minerals were P (1101 mg/100 g DW) and K (866 mg/100 g DW) (Table 1).
- A total of sixteen fatty acids (FA) were quantified, including the 5-olefinic fatty acids, pinoleic, pinolenic and eicosatrienoic, characteristic for conifers. MUFA and PUFA totalized almost 90% of the FA (Table 2).

Table 1 - Mean values of moisture, ashes, fat, protein (g per 100 g DW) and minerals (mg per 100 g DW) obtained in eight experimental treatment, three replications and three years

Constituent	Mean	sm (±)	n
Moisture	6.7	0.14	68
Ashes	4.9	0.062	68
Fat	46.9	0.46	68
Protein	44.0	1.29	67
Phosphorus (P)	1101	14.9	67
Potassium (K)	866	46.1	67
Calcium (Ca)	33.0	3.95	67
Magnesium (Mg)	470	6.8	67
Iron (Fe)	9.8	0.20	67
Manganese (Mn)	12.1	1.40	67
Zinc (Zn)	11.0	0.27	67
Copper (Cu)	2.79	0.197	67
Boron (B)	2.09	0.064	67

DW - Dry weight; sm (±) - standard error

Table 2 – Mean values of fatty acid composition (g per 100 g of total fatty acids) obtained in eight experimental treatment, three replications and three years

Fatty Acid	Mean	sm (±)	n
Miristic C14[1]	0.073	0.0097	45
Palmitic C16	6.27	0.300	66
Hexadecenoic C16:1 (c7)[1]	0.097	0.0164	38
Palmitoleic C16:1 (c9)	0.11	0.011	63
Margaric C17	0.078	0.0078	59
Stearic C18	4.07	0.126	66
Oleic C18:1 (c9)	40.07	0.490	66
Pinoleic C18:2 (c5,9)	0.11	0.029	63
Linoleic C18:2 (c9,12)	43.86	0.669	66
Pinolenic C18:3 (c5,9,12)	0.30	0.028	66
Linolenic C18:3 (c9,12,15)	0.61	0.022	66
Araquidic C20	0.68	0.047	66
Gadoleic C20:1 (c11)	0.89	0.053	66
Eicosadienoic C20:2	0.52	0.030	65
Eicosatrienoic C20:3 (c5,11,14)	1.63	0.094	66
Beenic C22	0.17	0.025	55
SFA	11.30	0.321	66
MUFA	41.17	0.511	66
PUFA	47.04	0.724	66
Diagnostic Index	0.24	0.013	66

sm (±) - standard error; [1] only two years; SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids; Diagnostic Index = [(C18:2 c5,9 + C18:3 c5,9,12 + C20:3 c5,11,14) / (C18:1 c9 and c11 + C18:2 c9,12 + C20:2 c11,14)] x 10

CONCLUSIONS

- ❖ **The levels of N and B fertilization used did not affect pine nut kernels chemical composition, except for the percentage of myristic acid (C14) and the concentrations of Fe, Mn and B.**
- ❖ **The results suggest that the conditions (climatic and others) observed throughout the experimental period affect the composition of pine nut kernels.**
- ❖ **Nevertheless, further investigations carried out under diverse conditions are needed to validate these results.**

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