



## Research Article

## Secondary metabolites and nutrient balance in casuarinas: An insight into Protein Competition Model (PCM)

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**Abstract:** The total phenolics, total condensed tannins (TCT), nitrogen (N) and total protein (TP) in needles of *Casuarina equisetifolia* and *Casuarina junghuhniana* were studied to understand the carbon-nutrient balance (CNB) and the growth-differentiation balance (GDB) hypotheses. The carbon-nutrient balance (CNB) hypothesis postulates that phenolic levels in plants are determined by the balance between carbon and nutrient availability<sup>1</sup>. The growth-differentiation balance (GDB) hypothesis<sup>2</sup> considers factors that limit growth and differentiation. The production of phenolics dominates when factors other than photosynthate supply are suboptimal for growth (e.g., under nutrient limitation). Resource-based theories assume that the synthesis of defensive compounds is constrained by the external availability of resources and internal trade-offs in resource allocation between growth and defense. It is stated that growth processes dominate over the production of defensive compounds and that more carbon is left for defensive compounds only when plant growth is restricted by a lack of mineral nutrient (emphasized by the CNB hypothesis) or by any factor (according to the GDB hypothesis). Jones and Hartley<sup>3</sup> presented a protein competition model (PCM) for predicting total phenolics allocation and content in leaves of higher plants. Protein competition model (PCM) stated that “protein and phenolics synthesis compete for the common, limiting resource phenylalanine,” so nitrogen (N) rather than C is the limiting resource for synthesis of phenolics. In our study, the contents of Total Phenolics, and Total Condensed Tannin (TCT) in needles of *C. equisetifolia* were higher than the *C. junghuhniana*. However, Total protein and nitrogen (N) contents were higher in *C. junghuhniana* than *C. equisetifolia*. There was a significant negative correlation between Total phenolics, TCT and Total Protein, N contents. Therefore, it is found from the present investigation that *C. equisetifolia* follows CNB hypothesis. However, *C. junghuhniana* follows GDB hypothesis, since it contains low defense chemicals viz., phenolics & TCT and high nitrogen and protein contents. Hence, the adaptability of *C. equisetifolia* in coastal areas and *C. junghuhniana* in drier inland condition is realized.

**Keywords:** Secondary metabolites, nutrient balance, Casuarinas and Protein Competition model.

### 1. Introduction

Casuarinas are nitrogen-fixing trees. *C. equisetifolia* is one of the most extensively planted tree species covering an area of 8 lakh hac. in India. *Casuarina junghuhniana* was introduced into India and has attracted the attention of farmers in drier tracts of the country. The use of Casuarinas as pulpwood has gained importance in the last decade. Its recent use is in the generation of energy through gasifiers, to gain carbon credits under the Clean Development Mechanism. Casuarina species provide subsistence for the socially and economically backward coastal communities. Casuarinas are most preferred species for ecorestoration and environmental protection and hence

assume importance in the era of climate change, where all the physicochemical stresses are likely to get exacerbated. Ecological and physiological properties of casuarinas are well documented, very little has been done to explore secondary metabolism, production and growth performance with special reference to tannin contents and nutrient relocation. Tannins play very important role in herbivore defense, litter decomposition, nutrient recycling, nitrogen sequestration, microbial diversity and activity, humic acid formation, metal complexation, antioxidants and pedogenesis. Tannins comprise a significant portion of terrestrial biomass. Leaves, branchlets, and bark may contain up to 40 percent tannins by dry weight; and in leaves and needles tannin contents can exceed lignin

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levels. Traditionally, justification for the high metabolic cost associated with the production of tannins was attributed to improved herbivore defense<sup>4</sup>. According to PCM, the growth-defense trade-off depends not only on competition for a limited pool of available carbohydrates but also in competition for nitrogen as a component of common precursor compounds<sup>5</sup>. In this regard, information on tannins and nitrogen contents is necessary. The objective of the present study was to test the validity of the following hypothesis: The production of phenolics and Tannin contents in needles of *Casuarina equisetifolia* and *Casuarina junghuhniana* dominates under nutrient limitation.

## 2. Materials and methods

### 2.1. Collection of plant materials

One year old individual of *C. equisetifolia* and *C. junghuhniana* were chosen from a research plot of Institute of Forest Genetics and Tree Breeding (IFGTB) at Forest campus, Coimbatore, India. Thirty individual trees of each species were selected and labeled for sample collection. The height and growth conditions of the chosen trees were similar. Branchlets damaged by insects and disease or mechanical factors were avoided. Fresh needles of each labeled tree were collected and all samples were taken to the laboratory immediately after sampling and cleaned with distilled water.

### 2.2. Preparation of extracts

The collected samples were air dried and ground into a uniform powder. Dry powder of samples was

extracted with organic solvents viz., acetone, methanol and petroleum ether using soxhlet apparatus for 6 hours. The extracts were filtered over anhydrous sodium sulphate followed by concentrated using a rotary evaporator. The concentrated extracts were subjected to freeze drying in a lyophilizer till dry powder was obtained.

### 2.3. Chemical analyses

All chemicals were of analytical reagent purity grade. The condensed tannin was extracted and purified according to the procedure described by Asquith and Butler<sup>6</sup> as modified by Hagerman<sup>7</sup> and was freeze-dried and stored at  $-20^{\circ}\text{C}$  until required. The Procedures described by Lin *et al.*,<sup>8</sup> were used to determine total phenolics (P). P was measured with the Prussian blue method<sup>9</sup>. N content was determined with Nessler's reagent after Kjeldahl digestion of powdered samples with sulfuric acid and hydrogen peroxide<sup>10</sup>.

### 2.4. Statistical analyses

All measurements were replicated three times and analyzed using one-way analysis of variance (ANOVA) (SPSS 16.0 for Windows) with Total Phenolics, TCT, N and TP.

## 3. Results and discussion

Total Phenolics contents of needles of various solvent extracts of *C. equisetifolia* (7.5 to 9.6mg/g) and *C. junghuhniana* (3.5 to 7.1mg/g) is depicted in Fig. 1.

Table 1. Correlation coefficient of secondary metabolites and nutrient balance in Casuarinas.

	P:N	TCT:N	P:TP	TCT:TP
<b>Correlation</b>				
<i>Casuarina equisetifolia</i>	0.916288	0.820275	0.917753	0.999993
<i>Casuarina junghuhniana</i>	0.89138	0.953045	0.867277	0.998716
<b>Co-variance</b>				
<i>Casuarina equisetifolia</i>	3.4113	7.3822	21.34973	46.24582
<i>Casuarina junghuhniana</i>	4.145011	8.664411	23.44793	49.50047
<b>F test p=</b>				
<i>Casuarina equisetifolia</i>	0.110565	0.01983	0.608868	0.562178
<i>Casuarina junghuhniana</i>	0.544644	0.178354	0.146513	0.464071

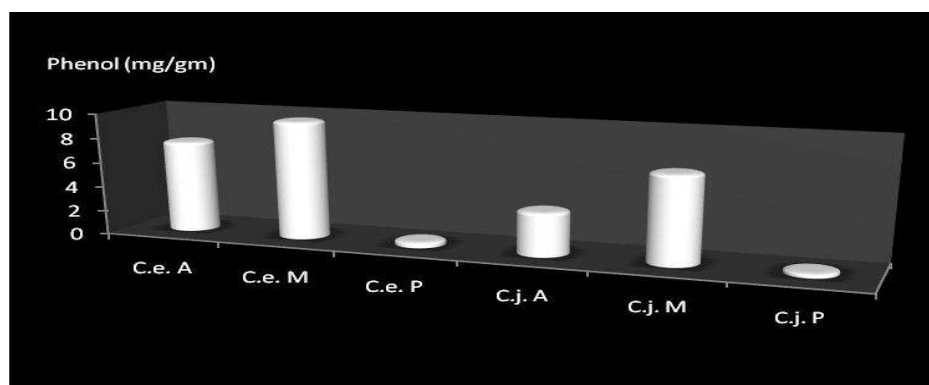
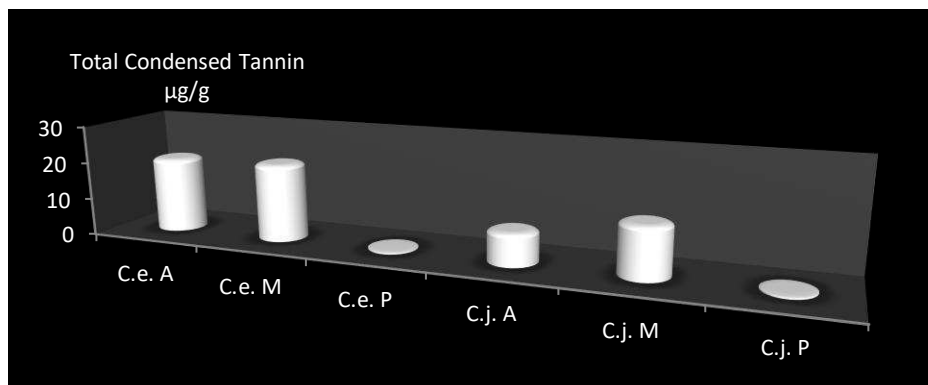


Fig. 1. Phenol content in Casuarinas.

(C.e – *Casuarina equisetifolia*; C.j – *Casuarina junghuhniana*; A – Acetone; M – Methanol; P – Petroleum ether)

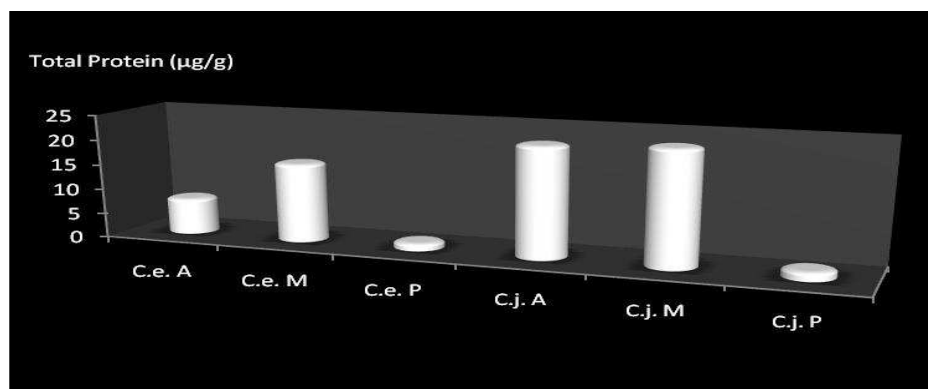
Total Phenolics content was highest in methanol extracts of needles of *C. equisetifolia* and *C. junghuhniana* (9.62 and 7.09mg/g). Total Phenolics were highest in *C. equisetifolia* than *C. junghuhniana*. The decrease in phenolics content may reflect an active turnover of phenolics and an increase in bound or non-extractable phenolics in *C. junghuhniana*. The same trend was observed in TCT content also. TCT of 20.97 and 20.35 $\mu\text{g/g}$  was found in methanol and acetone

extracts of *C. equisetifolia* whereas 13.70 and 8.65 $\mu\text{g/g}$  in methanol and acetone extracts of *C. junghuhniana* respectively (Fig. 2). Interestingly, very less phenolics were found in ether extract. However, inverse result was obtained with respect to protein and nitrogen contents. Total protein of 15.86, 7.29 and 1.42 $\mu\text{g/g}$  in methanol, acetone and ether extracts of *C. equisetifolia* respectively (Fig. 3). The same trend was observed for nitrogen content also (2.54, 1.16 and 0.23 $\mu\text{g/g}$ ) (Fig. 4).



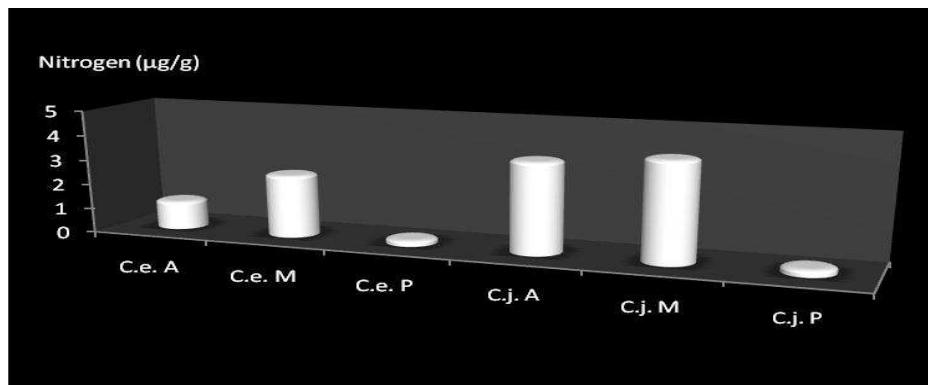
**Fig. 2. Tannin content in Casuarinas.**

(C.e – *Casuarina equisetifolia*; C.j – *Casuarina junghuhniana*; A – Acetone; M – Methanol; P – Petroleum ether)



**Fig. 3. Protein content in Casuarinas.**

(C.e – *Casuarina equisetifolia*; C.j – *Casuarina junghuhniana*; A – Acetone; M – Methanol; P – Petroleum ether)



**Fig. 4. Nitrogen content in Casuarinas**

(C.e – *Casuarina equisetifolia*; C.j – *Casuarina junghuhniana*; A – Acetone; M – Methanol; P – Petroleum ether)

However, higher amounts of protein and nitrogen were obtained in *C. junghuhniana* (25.02, 22.87 and 1.67 $\mu$ g/g of protein and 4.01, 3.66 and 0.27 $\mu$ g/g of nitrogen) when compared to *C. equisetifolia*. Higher contents of Total Phenolics and TCT in *C. equisetifolia* than the *C. junghuhniana* indicate that *C. equisetifolia* is experiencing more intense selective pressure than *C. junghuhniana*. Many soluble carbon compounds (polyphenols) are expected to be translocated from leaves. The GDB hypothesis<sup>2</sup> and the CNB hypothesis<sup>1</sup> assume that the synthesis of carbon-rich secondary chemicals is limited by the availability of photosynthesis (carbon). According to these hypotheses, growth processes in *C. junghuhniana* dominate over differentiation or production of carbon-rich secondary compounds as long as conditions are favorable for growth, but the GDB hypothesis is associated with the temporal variation in growth activity more directly than the CNB hypothesis<sup>11</sup>. If plant growth is active and therefore demands large amounts of carbon, allocation to carbon-rich secondary metabolites, e.g., phenolics, is predicted to decline; however, when growth is limited more than photosynthesis, allocation to defense will increase<sup>12</sup>. This supports the present study of low phenolics and tannin contents in *C. junghuhniana*. When growth is limited more than photosynthesis, allocation to defense chemicals will increase, this is realized in *C. equisetifolia* in the present study, hence higher contents of phenolics and tannins. Under the assumptions of the GDB hypothesis, allocation to phenolics should be low in *C. junghuhniana* which grows rapidly when compared to *C. equisetifolia*. The highest Total Phenolics content was found in *C. equisetifolia*. According to the PCM hypothesis, protein demand should be higher when the plant grows rapidly, and allocation to those phenolics that are derived from phenylalanine should simultaneously decrease<sup>3</sup>, as phenylalanine is the common precursor of either protein or condensed tannins synthesis<sup>13</sup>. However, the second major group of phenolics, the hydrolysable tannins, has Gallic acid as its precursor<sup>14</sup>. Therefore, depending on the relative strength of the synthetic route via dehydroshikimic acid to Gallic acid, hydrolysable tannins may or may not trade off directly with protein synthesis<sup>14</sup>. Hydrolysable tannins are thought to be metabolically cheaper than phenylpropanoids, and the synthesis may be a cost-saving defense strategy during a time when condensed tannins are not yet effective as defense<sup>14,15</sup>. However, the changes in contents do not necessarily reflect the quantitative allocation of tannins to the leaves, because of rapid turnover of labile compounds<sup>16</sup> and because the contents are affected by concomitant changes in proportions of other components of the leaves, e.g., structural leaf components<sup>17</sup>. The low nitrogen content was observed *C. equisetifolia* when growth is active.

Changes in leaf N contents may directly impact the photosynthetic capacity of the species involved, as there is usually a direct relation between leaf N content and the maximum rate of photosynthesis<sup>18</sup>. In this study, Total Phenolics contents were inversely related to N contents. It is common to find a negative correlation between N and secondary compound contents, such as phenolics and tannins<sup>19,20</sup>. This pattern lends to support source-sink hypotheses, such as the CNB hypothesis<sup>1</sup> and the GDB hypothesis<sup>2</sup> that predict increased C allocation to secondary C compounds under low nutrient conditions.

The role that tannins play in soil processes is believed to occur largely through their ability to precipitate proteins, as well as their relative resistance to decomposition<sup>21,22</sup>. The amount of tannins entering the soil relative to the amount of proteins or N may be the key factor influencing soil nutrient cycling. Therefore, parameters such as Total Phenolics: N and CT: N ratios may be the best predictors of litter quality<sup>23</sup>. In green foliage, high Total Phenolics: N and TCT: N ratios may help reduce herbivory. In our study, Total Phenolics: N ratios in *C. junghuhniana* were higher and showed a positive correlation (Fig. 5) than those in *C. equisetifolia*. There was no significant correlation between Phenolics or TCT and TP or nitrogen in *C. equisetifolia* (Fig. 6). The highest levels of Total Phenolics: N in *C. junghuhniana*, indicating potentially increased grazer deterrence. Nutrient balance in *C. junghuhniana* with low tannin and high protein and nitrogen contents are the important nutrient conservation strategies for *C. junghuhniana*.

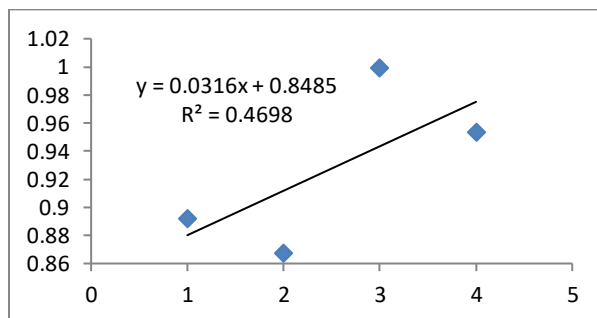


Fig. 5. Secondary metabolites and nutrient ratios in *C. junghuhniana*.

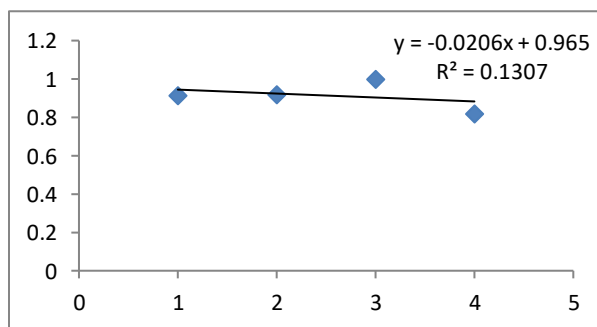


Fig. 6. Secondary metabolites and nutrient ratios in *C. equisetifolia*.

#### 4. Conclusion

It may be concluded from the present study that *C. equisetifolia* follows CNB hypothesis. However, *C. junghuhniana* follows GDB hypothesis, since it contains low defense chemicals viz., phenolics & TCT and high nitrogen and protein contents. High contents of Phenolics and TCT in *C. equisetifolia* may help herbivore defense, however, *C. junghuhniana* relocates nitrogen content from the needles to the growth of the plant and transfer C from Phenolics and Tannins for photosynthesis, since it grows under environmental stress conditions like drought/drier climates. Hence the adaptability of *C. equisetifolia* in coastal areas and *C. junghuhniana* in drier inland condition is appreciated. It is common to find a negative correlation between N and secondary compound contents, such as phenolics and tannins. This pattern lends to support source-sink hypotheses, such as the CNB hypothesis and the GDB hypothesis that predict increased C allocation to secondary C compounds under low nutrient conditions.

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