

Vegetation exclusion under *Casuarina equisetifolia* L.: Does allelopathy play a role?

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Abstract: A study conducted to determine the ecological status of vegetation under a *Casuarina equisetifolia* L. plantation revealed that the number of species types, density and biomass of the understorey were drastically reduced compared to an adjoining grassland area. In general, vegetation under *Casuarina* was characterised by the presence of a few dominants with a trend towards homogeneity. On the other hand, the vegetation in the grassland was heterogeneous, with conspicuous spatial pattern and, thus, more stable. Indices of richness (indicating numerical strength), evenness (representing spatial distribution), and diversity (combining both richness and evenness) also indicated a marked difference in the vegetation between the two sites. The Shannon index, indices of richness and evenness, and Hill's diversity measures were greater in grassland areas than in *Casuarina* plantation, thereby signifying a richer, more diverse and even vegetation in the grassland. In contrast, the Simpson index of dominance which shows an inverse relation with diversity, was greater under the *Casuarina* plantation. The reasons for the restrained vegetation under *Casuarina* were explored in terms of allelopathic interference of various tree parts (fresh as well as fallen) as one of the major factor. The leaf leachates collected under the canopy of *Casuarina* trees in the plantation were found to have deleterious effect on the growth of *Medicago sativa* and *Ageratum conyzoides* and were rich in phenolics. The extracts prepared from different tree parts such as needles, female cones and litter adversely affected the growth and dry weight accumulation in *M. sativa* and *A. conyzoides*. An appreciable amount of water-soluble phenolics, known phytotoxins, was estimated in the fog leachates and different plant parts under use. It is concluded that phenolics released from the green needles and litter of the tree adversely affect the understorey vegetation.

Abbreviation: IVI - Importance Value Index

Nomenclature: Duthie (1960)

Introduction

Casuarina equisetifolia L. (Casuarinaceae), hereafter referred to as *Casuarina*, commonly known as *horsetail tree*, is a fast growing, drought tolerant, nitrogen-fixing tree with wide adaptability to varied environmental conditions. As a native to Australia, it has been introduced in various parts of India and elsewhere primarily for fuel, land reclamation and as an ornamental tree. Monospecific plantations of *Casuarina* can be seen all over in India. Apparently, very poor vegetation is seen under the canopy of these plantations compared to the adjoining open area (Batish and Singh 1998). This is probably due to grouping of the sensitive species with strong interactive ability. The stand floor, however, is covered with litter composed exclusively of cladodes (flattened photosynthetic stem) or needle like branchlets, whorled reduced and scaly leaves or cone like female flowers and echinate fruits. The pres-

ence of litter on the ground surface may affect nutrient availability and its accumulation in soil (De Jong and Klinkhamer 1985, Facelli and Pickett 1991) and may also contribute allelochemicals to the soil leading to depletion in vegetation (Souto et al. 1994, Gonzalez et al. 1995, Pellissier and Souto 1999). The reduction in plant diversity under *Casuarina* can be due to resource competition (especially for nutrients) as well as allelopathy since both these mechanisms have been largely proposed to be the reasons for patterning of vegetation under natural ecosystems (Rice 1984, Grace and Tilman 1990, Seigler 1996). Allelopathy is known to occur in many natural and managed ecosystems and plays an important role in regulating plant diversity, non-random distribution of vegetation, zonation and organisation of plant communities (Muller 1966, Rice 1984, Seigler 1996, Pellissier and Souto 1999, Singh et al. 1999).

Keeping in view the renewed interest in conserving species diversity at the ecosystem level, it becomes essential to determine the ecological status of understorey vegetation and the reasons for its depletion before taking necessary conservation steps. Vegetation may be influenced not only by physical or competitive means but also by inhibitory organic metabolites leached from intact and living plant parts (del Moral and Muller 1969). Leaching is one of the important mechanisms of releasing allelochemicals from plants (Rice 1984, Einhellig 1985). Aqueous solutes such as fog, dew, mist and rain are the main agents for their release (Tukey 1966). Upon release, these allelochemicals play an important role in plant-plant interactions (Muller et al. 1968, Lodhi 1975, Horsley 1977). In north-western India, fog is a very common factor during the winters (months of December, January and February). The condensed fog drippings may leach organic metabolites from the plantations and these may have profound influence on the floor vegetation. Therefore, an investigation to assess the impact of fog drips was undertaken. The decaying needles and female cones present on the floor of *Casuarina* plantations may also release inhibitory substances, mainly phenolics, upon leaching and/or degradation. Keeping these things in mind, the study was therefore further extended to elucidate the role of leachable allelochemicals (from natural fog drips and fallen litter) as one of the causative factor for depleted vegetation under *Casuarina*.

Materials and methods

Study site

A nearly 20-year old plantation of *Casuarina* was selected around Chandigarh, India (30° 42' N, 76° 53' E, 333 m a.s.l.). The trees were spaced at a distance of 5 m within each row and at 4 m between rows. The average height of the trees was 18.8 ± 1.46 m and their diameter at breast height was 24.59 ± 3.53 cm.

Vegetation analysis

Vegetation analysis was done based on data obtained from quadrat sampling. Twenty temporary quadrats of 1 m² size were laid randomly under the *Casuarina* plantation and in a nearby open grassland area (for comparison) in three different seasons, i.e., summer, pre-monsoon and winter. The size and number of quadrats were pre-determined using the species-area curve method and minimum quadrat number method (Misra 1968, Ambasht 1990). All the plants occurring in the quadrats were sampled, identified and their density, biomass, relative frequency and dominance, and Importance Value Index (IVI) were calculated (Misra 1968, Ambasht 1990). IVI gives an overall

picture of the ecological importance of species with respect to communities and was calculated by adding per cent values of relative density, relative frequency and relative dominance (Curtis 1959). In addition, the abundance values of different species were computed to calculate indices of richness, dominance, diversity and evenness, and Hill's diversity numbers N1 and N2. These indices are given by the following formulae and were calculated using the statistical package of Ludwig and Reynolds (1989).

Richness Index (Margalef 1958):

$$R = S - 1 / \log N ;$$

Index of Dominance (Simpson 1949)

$$\lambda = \sum_{i=1}^S \left(\frac{n_i}{N} \right)^2 ;$$

Shannon Index of Diversity (Shannon- Weaver 1963)

$$H' = - \sum_i n_i / N \ln n_i / N ;$$

Hill's (1973) Diversity Numbers

$$N_1 = e^{H'} ;$$

$$N_2 = 1 / \lambda ;$$

Evenness Index (Hill 1973)

$$E = (N_2 - 1) / (N_1 - 1) ;$$

where S = total number of species, N = total number of individuals of all the species, n_i = number of individuals of the i th species.

The pattern of distribution of species under *Casuarina* plantation and in the grassland area was analysed by plotting IVI versus the species sequence to get lognormal pattern of distribution. Lognormal distribution is used to quantitatively describe the species-abundance relationships in communities having a large assembly of species because in such cases the relative abundances are a product of many independent factors (Ludwig and Reynolds 1989) and describe changes in the undisturbed and equilibrium communities (May 1981).

Chemical analysis of the soil

Litter-free soil was collected from the 0-10 cm profile of the study site. It was analysed for pH, electrical conductivity, organic carbon, organic matter, available nitrogen, potassium and phosphorus, and sodium according to Allen (1989) and Piper (1966). All the analyses were made three times. These are given in Table 1.

Table 1. Chemical properties of the soil under *Casuarina* plantation and an adjacent grassland area. EC- Electrical Conductivity ; OC-, Organic Carbon ; OM - Organic Matter. * and ** mean significant difference at $P < 0.05$ and $P < 0.01$, respectively.

Character	<i>Casuarina</i>	Grassland area
pH (1:2, w/v)	5.95 ± 0.4**	7.67 ± 0.06
EC (µS)	493.5 ± 10.5*	525.2 ± 15.2
OC (%)	1.98 ± 0.12**	0.63 ± 0.06
OM (%)	3.41 ± 0.28**	1.09 ± 0.16
Available Nitrogen (kg/ha)	160.88 ± 6.24**	68.99 ± 4.12
P ₂ O ₅ (kg/ha)	21.41 ± 2.28**	9.24 ± 1.35
K ₂ O (kg/ha)	627.65 ± 17.95**	224.45 ± 6.58
Na (kg/ha)	47.04 ± 3.54**	129.02 ± 5.12

Collection of fog-drip leachates

On foggy days in the month of January 2000, natural leachates aided by fog were collected from the *Casuarina* plantation. For this, a thick polythene sheet 4m x 4m was spread under the tree canopy and tied to the branches for 12 hours to collect the fog-drippings next morning. The fog-drips so collected are termed natural leaf leachates. Collections were made from five such places in the plantation to serve as replicates, whereas collections from an area without *Casuarina* tree served as control. The leachates so collected are termed as full strength (X) and diluted to X/5 and X/10 strength. These were used for a bioassay study with *Medicago sativa* and *Ageratum conyzoides*. These two species were selected on the basis of their sensitivity so as to serve as a representative of the understorey vegetation, i.e., *A. conyzoides* being totally absent under *Casuarina* whereas *M. sativa* has a small IVI.

Preparation of extracts

Aqueous extracts of freshly plucked needles, decaying litter, fallen female cones and understorey soil (below the litter layer) were prepared by soaking 10 g dried and powdered material of each in 500 ml of distilled water (conductivity < 0.05 µS) for 24h at 25°C. The extracts were filtered through a muslin cloth followed by Whatman #1 filter paper. Each of these extracts was diluted to 1% and all were kept at 4°C until used. The osmotic potential of extracts ranged from 5-20 milliosmols; and this much of osmotic potential does not affect plant growth (Chou and Hou 1981).

Growth experiments

Germination and growth studies were carried out in 15 cm diameter Petri dishes lined with a Whatman #1 filter paper having a thin absorbent cotton wad below. These were moistened with 10 ml of each of the extracts. 20 seeds of each of *Medicago sativa* and *Ageratum conyzoides* imbibed for 10 h in the respective extract solutions were then equidistantly placed in the Petri dishes. Four replications were maintained for each treatment. Treatment in a similar manner with distilled water served as control. All the Petri dishes were maintained in a seed germinator at 16/8 light/dark photoperiod, 25°C temperature and 75% humidity. After a week, the number of seeds germinated, seedling length (from tip of root to tip of shoot) and dry weight of seedlings were recorded. The whole experiment was repeated twice. In the present study, impact of extracts on germination and seedling growth was evaluated simultaneously with the objective of finding further effects on the growth of those seeds that could germinate in response to extracts.

Estimation of total phenolic content

Amounts of total phenolics were determined in the aqueous extracts prepared above using Folin-Ciocalteu reagent according to Swain and Hillis (1959). The estimations were repeated three times and data were presented on the basis of dry tissue weights.

Statistical analysis

All the laboratory experiments were completely randomised. The different treatments were subjected to one-way ANOVA and the comparisons between groups were made using two-sample *t*-test at the 5 and 1% levels of significance using SPSSPC (1986).

Results and discussion

Results indicate that there is a substantial decrease in species diversity under *Casuarina* plantations compared to the adjoining open grassland area. Compared to 53 species in the grassland area only 24 were recorded under the *Casuarina* plantation (Table 2). Of the 24 species, *Malvastrum tricuspidatum*, *Cynodon dactylon* and *Evolvulus alsinoides* were the most dominant and abundant with higher Importance Value Index, leading to patchiness and non-random distribution of species under the *Casuarina* plantation. In other words, the vegetation under *Casuarina* plantation was more towards homogeneity. In contrast, in the grassland area none of the species actually dominated, though *Cynodon dactylon* and *Parthenium hysterophorus* had the largest IVI value. The rest of the

Table 2. Number of species, biomass and amount of litter in the study site. ** means significant difference at $p < 0.01$.

Parameter	<i>Casuarina</i>	Grassland area
Number of species	24	53
Density (number of plants / m ²)	265.8 ± 41.5**	773.6 ± 191.1
Biomass of ground vegetation (g/m ²)	152.5 ± 33.0**	437.5 ± 18.1
Amount of freshly fallen litter (g/m ²)	255.0 ± 43.1	-
Amount of partially degraded litter (g/m ²)	670.6 ± 74.9	-

species were uniformly distributed with greater heterogeneity. The abundance pattern becomes clearer when species are ranked from most abundant to least abundant using lognormal distribution based on IVI values (Fig. 1). Moreover, the average density (number of plants per unit area) as well as the average biomass was less in the *Casuarina* plantation than in grassland area (Table 2).

Further, the vegetation structure and composition were also analysed through ecological indices, which are not only useful indicators of vegetation but also reduce the complexity of data on ecological studies (Whittaker 1953). A great variety of diversity indices are available,

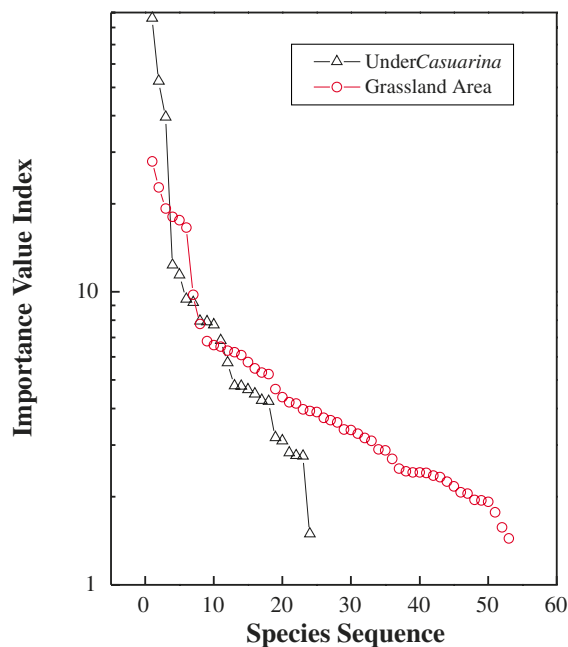


Figure 1. Species distribution curve between Importance Value Index and species sequence showing a lognormal pattern of distribution in *Casuarina* plantation and adjacent grassland area. Scale along Y-axis is logarithmic scale.

Table 3. Comparative values of different ecological indices under *Casuarina* plantation and grassland area. * and ** represent significant difference at $P < 0.05$ and $P < 0.01$, respectively.

Parameter	<i>Casuarina</i>	Grassland area
Margalef Richness Index (R_1)	4.12*	7.82
Simpson Index of Dominance (λ)	0.24**	0.05
Shannon Index of Diversity (H')	1.91**	3.46
Hill's Diversity Number 1 (N_1)	6.79**	31.80
Hill's Diversity Number 2 (N_2)	4.24**	21.59
Hill's Evenness Index (E)	0.60*	0.87

however, in order to avoid conceptual and technical problems and to get more precision, only a few are widely acceptable and applied (Ludwig and Reynolds 1989). The richness index indicating numerical strength of vegetation was found to be almost double in the open grassland than under *Casuarina* (Table 3). Likewise, the evenness index representing the spatial distribution of the vegetation was lower under the *Casuarina* plantation. In the present study, two widely used diversity indices (representing both richness and spatial distribution) viz. the Simpson Index of Dominance (λ) and Shannon Weiner Index of Diversity (H') were also calculated (Table 3). These indices of diversity are intrinsically more important and useful and are the least affected by patchiness and non-random spatial association of the species (Magnussen and Boyle 1995). The value of the Shannon Index was significantly higher in the grassland area than in the *Casuarina* plantations. On the other hand, the index of dominance, which has an inverse relationship with species diversity, was significantly greater under plantation than in the grassland, thereby indicating less diversity and homogeneity of the vegetation under *Casuarina*. Besides, two other diversity indicators termed as Hill's Diversity Numbers N_1 and N_2 representing abundant and most abundant species, respectively, were also significantly reduced under the *Casuarina* plantation relative to the grassland area. These diversity numbers are more acceptable and widely acclaimed due to their easier interpretability (Ludwig and Reynolds 1989). The study, therefore, clearly depicts a marked paucity of vegetation under *Casuarina* plantation when compared to the grassland area. There are a number of other reports indicating the presence of restrained vegetation under the plantations and attributed this reduction of vegetation to allelopathy (del Moral and Muller 1970, Kil and Yim 1983, Chou and Kuo 1986, Suresh and Rai 1987, Chou and Lee 1992, Kohli et al. 1996, Bhatt et al. 1997).

Table 4. Effect of natural leachates (aided by fog) collected under the tree canopy of *Casuarina* on seedling growth and weight of *M. sativa* and *A. conyzoides*. Values given are Mean \pm Standard Deviation; X, X/5, X/10 represent full, one-fifth and one-tenth strength, respectively. * represents significance from control at $P < 0.05$; ** represents significance from control at $P < 0.01$; ns represents no significance from control at $P > 0.01$

Treatment	Strength	Germination (%)		Average seedling length (mm)		Average seedling weight (mg)	
		<i>M. sativa</i>	<i>A. conyzoides</i>	<i>M. sativa</i>	<i>A. conyzoides</i>	<i>M. sativa</i>	<i>A. conyzoides</i>
Control		100	100	75.8 \pm 7.9	44.5 \pm 4.0	38.25 \pm 4.30	2.91 \pm 0.75
Natural	X	73.7 \pm 2.5*	73.5 \pm 0.6*	20.6 \pm 13.8**	11.2 \pm 0.9**	18.60 \pm 1.40**	1.12 \pm 0.21**
Leachates	X/5	92.5 \pm 2.9*	90.0 \pm 1.0*	51.2 \pm 16.1*	23.0 \pm 2.2**	26.70 \pm 1.80**	1.40 \pm 0.40**
	X/10	98.7 \pm 2.5	96.5 \pm 1.2	73.0 \pm 13.2ns	43.0 \pm 2.1ns	37.20 \pm 2.90ns	2.75 \pm 0.60ns

Table 5. Effect of extracts from different plant parts or understorey soil of *Casuarina* on germination, seedling growth and weight of *M. sativa* and *A. conyzoides*. Values given are Mean \pm Standard Deviation; * represents significance from control at $P < 0.05$; ** represents significance from control at $P < 0.01$; ns represents no significance from control at $P > 0.01$.

Aqueous extracts	Conc. (%)	Germination (%)		Average seedling length (mm)		Average seedling weight (mg)	
		<i>M. sativa</i>	<i>A. conyzoides</i>	<i>M. sativa</i>	<i>A. conyzoides</i>	<i>M. sativa</i>	<i>A. conyzoides</i>
Control		100 \pm 0	93.8 \pm 0.9	63.0 \pm 8.9	43.7 \pm 3.1	36.20 \pm 8.74	2.83 \pm 0.76
Fresh needles	1	100 \pm 0	38.8 \pm 0.5**	37.2 \pm 10.9**	15.7 \pm 2.1**	25.80 \pm 2.80*	2.23 \pm 0.15 ^{ns}
	2	88.8 \pm 0.5*	32.5 \pm 0.6**	26.6 \pm 13.3**	15.0 \pm 4.0**	23.82 \pm 4.12**	1.53 \pm 0.15*
Fresh litter	1	100 \pm 0	28.8 \pm 0.5**	34.8 \pm 14.2**	16.0 \pm 2.6**	23.08 \pm 4.75*	2.13 \pm 0.25 ^{ns}
	2	67.5 \pm 1.0*	25.0 \pm 0.8**	32.0 \pm 7.8**	12.0 \pm 1.0**	20.22 \pm 3.77**	1.17 \pm 0.25*
Degraded litter	1	100 \pm 0	38.8 \pm 1.0**	56.0 \pm 16.4 ^{ns}	23.3 \pm 3.2**	29.78 \pm 1.80 ^{ns}	2.00 \pm 0.6 ^{ns}
	2	91.3 \pm 0.3	18.8 \pm 0.9**	31.8 \pm 8.3**	19.0 \pm 2.0**	24.18 \pm 3.57*	1.43 \pm 0.42*
Female Cones	1	100 \pm 0	67.8 \pm 0.5*	44.4 \pm 15.4*	24.0 \pm 7.9*	31.34 \pm 7.19 ^{ns}	1.97 \pm 0.45 ^{ns}
	2	70.0 \pm 0.8*	18.8 \pm 1.0**	30.0 \pm 6.1**	15.3 \pm 2.3**	21.44 \pm 5.23*	1.16 \pm 0.06*
Soil	1	100 \pm 0	97.5 \pm 0.6	57.0 \pm 14.7 ^{ns}	35.0 \pm 6.0 ^{ns}	31.74 \pm 9.56 ^{ns}	3.00 \pm 0.98 ^{ns}
	2	98.8 \pm 0.5	28.8 \pm 0.5**	40.8 \pm 7.2**	22.7 \pm 5.5**	23.84 \pm 4.05*	2.07 \pm 0.32 ^{ns}

The restrained vegetation under *Casuarina* plantation could be attributed to a number of factors like reduced light intensity, competition for resources, nutrients and allelopathy, which may operate or interact simultaneously or sequentially. Of these factors, allelopathy is probably the major mechanism responsible for the observed changes in vegetation because the soil under the *Casuarina* plantation was more nutrient rich than under the grassland (Table 1), and there have been reports that light and nutrients are not limiting factors under *Casuarina* plantations (Suresh and Rai 1987). Consequently, it was hypothesised that the paucity of vegetation under the *Casuarina* plantation is probably due to allelopathy by the release of secondary metabolites, which have a profound effect on the physiology, development, and growth of sensitive plants. The sources of release of

secondary metabolites (simple water soluble compounds, aldehydes, ketones, phenols, etc.) are living parts of the tree as well as the dead and decaying parts which fall onto the surface of the soil as litter. The most likely mode of metabolite release is leaching. In order to simulate natural conditions, fog leachates were collected from under the canopy of tree. The fog leachates exhibited appreciable phytotoxicity towards the germination and growth (seedling length and dry weight) of test plants *M. sativa* and *A. conyzoides* when used in full or one-fifth strength (Table 4). However, at one-tenth strength the inhibitory effect on test plants was marginal. Fog-drip was, therefore, capable of inhibiting growth of test plants as hypothesised and may have similar effects on the understorey vegetation and could reduce the diversity and density of the floor vegetation to some extent. Generally, fog drip is viewed

Table 6. Amounts of total phenolics in the natural leaf leachates and different parts of *Casuarina*. Different alphabets represent significant difference at $p < 0.01$ level of significance. * represents significant difference from control at $p < 0.01$ level of significance.

Aqueous leachates	Total phenolic content (mg.100g ⁻¹)
Fresh needles	39.0 ± 4.20 ^a
Fresh litter	13.9 ± 1.50 ^b
Degraded litter	9.45 ± 1.46 ^c
Female cones	9.00 ± 0.70 ^c
Soil	5.73 ± 1.99 ^d
	(µg.ml ⁻¹)
Natural (fog) leaf leachates from <i>Casuarina</i> plantation	165 ± 15.1*
Control (fog from open area)	25.7 ± 5.99

to considerably improve the moisture regime of the plantations, but it can also be an important mechanism of release of secondary metabolites from the foliar crown (del Moral and Muller 1969).

Interestingly, there was also a substantial amount of fresh and decomposing litter (comprising mainly of needles and female cones) under the *Casuarina* plantation (Table 2). This makes possible the release of metabolites /allelochemicals into the surrounding environment through leachate and the activity of microbes (Pellissier and Souto 1999). Molina et al. (1991) have shown that litter of several trees is toxic to native vegetation and depletes the understorey thus causing community structure disturbance. In the present study the extracts prepared from the fresh needles and litter were found to have inhibitory effect on the germination, seedling growth and dry weight accumulation of *Medicago sativa* and *Ageratum conyzoides* (Table 5). Extracts from fresh needles were most phytotoxic followed by fresh litter and decaying litter, respectively (Table 5). On the other hand, the soil extracts have the least affect. Such an observation is not surprising because the mineral soil layer below the litter has been reported to have less phytotoxic effect than litter itself (Wilt et al. 1988). Several studies have indicated that phytotoxic effects of plant extracts under laboratory conditions may also be due to negative osmotic effects besides allelopathic influence and hence there is overestimation of allelopathy (Stowe 1979, Wardle et al. 1989, Hardegree and Emmerich 1990, Asterita et al. 1996). However, in our studies the extracts have a very low concentration (1 and 2%) and less osmotic potential (20 milliosmols). At such a low osmotic potential, the

plant growth remains unaffected (Chou and Hou 1981). This clearly shows the presence of some inhibitory substances in the extracts leading to the phytotoxic effects on the test plants.

Though leachate removes a number of chemical compounds but phenolics are the most important chemicals which are ecologically significant (Appel 1993). They comprise tannins, phenolic acids and flavonoids, etc. In the present study an appreciable amount of phenolics was found in natural leachates (fog-drip) and different types of extracts (Table 6). Their amount was maximum in the needles followed by litter and minimum in the soil. The presence of phenolics in the litter and soil may also be responsible for the observed low pH in the soil under *Casuarina* (see Table 1). This may also have some effect in depleting vegetation cover under the plantation. Upon entering the soil, phenolics undergo various changes like retention, transformation and transportation either alone or in association with microorganisms leading to changed activity and toxicity (Blum 1998). These phenolics may have direct effects on vegetation or indirect effects by constituting a source of carbon in the immediate vicinity of seeds and consequent intensification of microbial activity (Pellissier 1993). Although in the present study, phenolics were not identified, but there are reports of presence of quercetin, iso-quercetin, and kaempferol and phenolic acids like gallic, protocatechuic acid and hydroquinone in the needles (Anonymous 1992). Further, Deng et al. (1996) have extracted and identified five allelochemicals viz. kaempferol-3- α -rhamnoside, quercetin-3- α -araboside, luteolin-3'-4'-dimethoxy-7- β -rhamnoside, kaempferol 3 β -dirhamnoside and quercetin 3 β -glucoside from the branchlets (needles) of *Casuarina*.

Based on this study, although we cannot at the present state of knowledge affirm that allelopathy is the main reason for the depletion of vegetation beneath *Casuarina*, there are some indications suggesting that depletion may be at least partially due to allelopathy

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