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ORIGINAL RESEARCH ARTICLE

Allelopathic effect of *Pinus roxburghii* on an understorey plant, *Bidens pilosa*

Neel Kant Sharma[1]*, Daizy R. Batish[1], Harminder Pal Singh[2] and R. K. Kohli[1,3]
[1]Department of Botany, Panjab University, Chandigarh 160014, India.
[2]Department of Environment Studies, Panjab University, Chandigarh 160014, India.
[3]Central University of Punjab, Bathinda 151001, India.

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Abstract: Allelopathic effect of *Pinus roxburghii* was studied on *Bidens pilosa* through laboratory and greenhouse experiments. The aqueous extracts of green needles, needle litter and bark were found to inhibit germination and initial growth of *B. pilosa* in laboratory bioassays. The inhibitory effect on germination root length, shoot length and biomass was also observed on amendment of powdered needle litter in soil in greenhouse pots. The inhibitory effect increased with increase in conc. of extracts or amount of litter amended. The amended soil was found to be rich in phenolics, the known potent allelochemics. The study indicates that such interactions may also be operational in forests of *P. roxburghii.*

Key words: Allelopathy; Aqueous extracts; Needles; Phenolics; *Pinus roxburghii*

Introduction

Allelopathy refers to the effect of one plant on the other through the release of chemical substances (Rice, 1984). These chemicals are released through volatilization, leaching, as root exudates, or as a result of decomposition of plant residues (Rice, 1984). The allelochemicals, upon release, undergo a variety of changes such as sorption, transformation, toxification and / or detoxification (Cheng, 1992; Blum *et al*., 1999). However, their effect depends greatly upon a variety of biotic and abiotic factors, soil type, presence of microorganisms and soil conditions (Blum *et al*., 1999). Allelopathy is an important ecological phenomenon and governs plant-plant interaction (Kim *et al*., 1997). It plays a significant role in vegetation dynamics and is partially responsible for success or failure of seedling establishment and survival in forest ecosystems (Pellissier and Souto, 1999). This process has been demonstrated to be capable of altering the structure, function, and diversity of plant communities (Muller, 1969; Whittaker, 1970). The complexity and interacting nature of allelopathic phenomena make it difficult to demonstrate its role in plant interactions and community organization (Zen, 2014). So, in most cases, the assessments of allelopathy have been done through bioassays of plant or soil extracts based on seed germination or seedling growth.

The present study was undertaken to assess the allelopathic effects of needle (foliage), needle litter and bark of *Pinus roxburghii* Sargent (commonly known as Chir Pine) on early growth of *Bidens pilosa* L., an understorey plant. *P. roxburghii* is native to the Himalayas and one of the important forestry trees.

*Corresponding Author:
Neel Kant Sharma,
Research Scholar,
Department of Botany,
Panjab University, Chandigarh, India.
E- mail: neelkantsharma23@gmail.com

Materials and Methods

Collection of Plant Material

The needles (foliage), needle litter (undecomposed) and bark of *P. roxburghii* were collected from a forest of Mandi district of Himachal Pradesh, shade dried and powdered in electrical grinder. The seeds of test plant, *B. pilosa* were collected from plants growing in wild in surrounding areas.

Laboratory bioassays

Aqueous extract were prepared from powder of needles, needle litter and bark. For this, 4 grams of needle powder was soaked in 100 ml of distilled water for 18 hours at room temperature. The contents were filtered through triple layered muslin cloth followed by Whatman No. 1 filter paper. This 4% (w/v) stock solution was further diluted to get lower concentration of 0.5, 1 and 2%. Pure distilled water served as control. 15 seeds of *B. pilosa* were placed in 15 cm diameter Petri dish, lined with filter paper and moistened with 10 ml of respective solution or distilled water (control). The effect of different concentrations was studied on germination and initial growth of *B. pilosa.* For each treatment, 5 replicates were maintained. The percent germination, root length, shoot length and biomass of seedlings were recorded after a week.

Greenhouse experiment

The needle litter powder of *P. roxburghii* was mixed in the ratio of 0, 5, 10, 20 and 40 grams of powder per kilogram of soil in pots. 15 seeds of *Bidens pilosa* were sown in each pot. After germination thinning of seedling was done to 5 plants per pot. The pots were watered regularly. The percent emergence, root length, shoot length and biomass of test plant

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were recorded after one month. Powder amended soil was analyzed for presence of phenolics as per Swain and Hillis (1959) after one month of amendment. Five replicates were maintained for each treatment. The replicates were kept in completely randomized manner.

Statistical analysis

The data were analyzed by one- way ANOVA at p≤ 0.05 applying *post-hoc* Tukey's test. The statistical analysis was performed using SPSS/PC software ver. 16.0 (SPSS Inc., Chicago, IL).

Results and Discussion

Different species of *Pinus* and other conifers have been reported to cause allelopathic effects on other species (Lodhi and Killingbeck, 1982; Kil and Yim, 1983; Kil *et al.*, 1991; Kil, 1992; Gallet, 1994; Singh *et al.,* 1999b; Kato-Noguchi *et al.,* 2009; Refifa *et al.* 2016). Melkania *et al.* (1982) have reported the allelopathic effects of leachates / extracts of *P. roxburgii* on some crop plants.

In our study, the germination and growth of test plant were affected adversely, in both laboratory bioassays as well as litter amended pot experiment. The percent germination, in general, was significantly affected at extract concentration of 1% and above. In general, the effect of green needles and litter extracts was almost similar whereas the bark extracts had slightly more inhibitory effect on germination (**Table 1**). The reduction in germination of target species in response to leaf and litter extracts of conifers has been reported by Jobidon (1986). The bark extracts of *Picea engelmannii* inhibited the seed germination of own and other conifers (Taylor and Shaw, 2011). Valera-Burgos *et al.* (2012) reported the negative effects of *Pinus pinea* litter on seed germination and seedling performance of three Mediterranean shrub species.

Wu *et al.* (2011) reported the allelopathic inhibition in germination and growth of herbaceous species in response to extracts of tree *Eucalyptus dundasii.* The inhibition of seed germination and seedling growth of target species in response to leaf litter extracts of *Ulmus pumila,* containing phenolics was reported by Pérez-Corona *et al.* (2013). In our study decrease in root length, shoot length and biomass of seedlings (**Table 2, 3 and 4**) was observed in concentration dependent manner in all three types of extracts. The overall response to needle and needle litter extracts were almost similar. However, the bark extracts were found to be slightly more toxic as compared to these two.

Table 1: Effect of green needle extract (GNE), needle litter extract (NLE) and bark extract (BE) of *Pinus roxburghii* on germination of *Bidens pilosa*

Concentration (%)	Germination (%)		
	Green Needle	Needle Litter	Bark
0	98.66±1.33a(0)	98.66±1.33a(0)	98.66±1.33a(0)
0.5	90.66±2.66ab(8.1)	91.99±2.49ab(6.8)	89.32±1.63b(9.5)
1.0	81.33±3.88b(17.6)	82.66±2.66b(16.2)	75.99±1.63c(23.0)
2.0	70.66±1.63c(28.4)	69.32±1.63c(29.3)	63.99±2.66d(35.1)
4.0	55.93±3.34d(43.3)	57.26±2.60d(42.0)	49.32±1.63e(50.0)

Data presented as mean ± standard error

Different alphabets within a column represent significant difference among treatments at p ≤ 0.05 applying *post hoc* Tukey's test

Values within parenthesis represent percent decrease over the control

Table 2: Effect of green needle extract (GNE), needle litter extract (NLE) and bark extract (BE) of *Pinus roxburghii* on root length of *Bidens pilosa*

Concentration (%)	Root Length (cm)		
	Green Needle	Needle Litter	Bark
0	3.05±0.11a(0)	3.05±0.11a(0)	3.05±0.11a(0)
0.5	2.83±0.04a(7.2)	2.78±0.06a(8.9)	2.65±0.06b(13.1)
1.0	2.40±0.07b(21.3)	2.39±0.06b(18.9)	2.26±0.06c(25.9)
2.0	1.73±0.07c(43.3)	1.86±0.05c(39.0)	1.77±0.06d(42.0)
4.0	1.13±0.05d(63.0)	1.21±0.04d(60.3)	1.04±0.04e(65.9)

Data presented as mean ± standard error

Different alphabets within a column represent significant difference among treatments at p ≤ 0.05 applying *post hoc* Tukey's test

Values within parenthesis represent percent decrease over the control

Table 3: Effect of green needle extract (GNE), needle litter extract (NLE) and bark extract (BE) of *Pinus roxburghii* on shoot length of *Bidens pilosa*

Concentration (%)	Shoot length (cm)		
	Green Needle	Needle Litter	Bark
0	4.02±0.06a(0)	4.02±0.06a(0)	4.02±0.06a(0)
0.5	3.81±0.07a(5.2)	3.79±0.06a(5.7)	3.51±0.09b(12.7)
1.0	3.02±0.07b(24.9)	2.98±0.05b(25.9)	2.79±0.09c(35.1)
2.0	2.38±0.09c(40.8)	2.27±0.07c(43.5)	2.16±0.06d(46.26)
4.0	1.74±0.06d(56.7)	1.65±0.06d(59)	1.34±0.07e(66.7)

Data presented as mean ± standard error

Different alphabets within a column represent significant difference among treatments at p ≤ 0.05 applying *post hoc* Tukey's test

*Figures within parenthesis represent percent decrease over the control

Table 4: Effect of green needle extract (GNE), needle litter extract (NLE) and bark extract (BE) of *Pinus roxburghii* on seedling biomass of *Bidens pilosa*

Concentration (%)	Biomass (mg)		
	Green Needle	Needle Litter	Bark
0	0.98±0.02a(0)	0.98±0.02a(0)	0.98±0.02a(0)
0.5	0.90±0.03ab(8.2)	0.92±0.02a(6.1)	0.86±0.02b(12.2)
1.0	0.81±0.01bc(17.3)	0.84±0.03ab(14.3)	0.75±0.01b(23.5)
2.0	0.72±0.01cd(26.5)	0.70±0.01bc(28.6)	0.61±0.01c(37.8)
4.0	0.62±0.01d(36.7)	0.59±0.04c(39.8)	0.47±0.04d(52.0)

Data presented as mean ± standard error
Different alphabets within a column represent significant difference among treatments at $p \leq 0.05$ applying *post hoc* Tukey's test
Values within parenthesis represent percent decrease over the control

Litter decomposition is claimed to be the most important source of allelochemical release (Reigosa *et al*., 1996). *P. roxburghii* forest floor is characterized by a carpet of fallen needles. The understorey of chir pine forests is characterized by sparse vegetation, which could possibly be due to allelopathic effect, at least partially. In our pot culture experiment, which was an attempt to simulate the natural conditions in *P. roxburghii* forests, the germination and growth of test plant were affected adversely in powder amended soil medium in all treatments as compared to control (**Table 5**). The germination and growth inhibition increased with increase in amount of litter powder amended. Kil and Yim (1983) reported that toxic substances released from *Pinus densiflora* inhibit seed germination and growth of other species in the forest. The phenolic compounds extracted from the aqueous extracts of *Pinus rigida* showed both inhibitory and stimulatory effect on *Cassia mimosoides* var. *nomame* depending upon the concentration (Kim *et al*., 1997). However, in our study all concentrations showed inhibitory effect. Kil (1992) demonstrated that growth of understorey plants was inhibited in soil mixed with pine needles. He attributed the growth inhibition of understorey plants to water soluble phenolics. He indicated that the allelochemicals may also affect indirectly by altering the symbiotic relationships with microorganisms, rhizosphere ecology and nutrient availability.

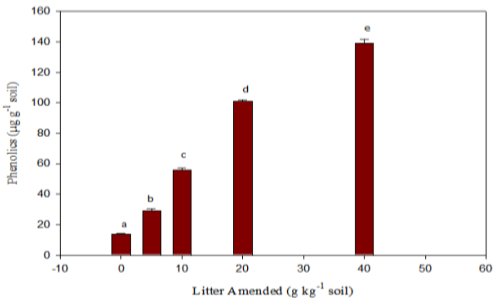
Soil analysis revealed the presence of water soluble phenolics in powder amended soil (**Figure**). The amount of total water soluble phenolics increased with increase in the dose of powder. The total phenolic content detection is relevant as phenolics act synergistically (Williams and Hoagland, 1982). These compounds affect plant growth directly or by altering the other properties of soil (Blum *et al*., 1999). Although the amount of phenolics measured in amended soil increased with increase in amount of amended powder, however it was not exactly proportional to amount of powder amended indicating the role of biotic factors (soil microorganisms) in toxification or detoxification. Singh *et al*.(1999a) attributed the presence of phenolics in litter and leaves of *Leucaena leucocephala* for observed inhibitory effect on *Zea mays*. The inhibitory effects observed in present study can also be attributed mainly to the water soluble phenolics, an important class of allelochemicals.

Table 5: Effect of *Pinus roxburghii* needle litter amended soil on seedling emergence and growth of *Bidens pilosa*

Litter amended (g kg^{-1}soil)	Seedling emergence (%)	Root length (cm)	Shoot length (cm)	Biomass (mg)
0	97.33±1.63a(0)	18.79±0.41a(0)	12.37±0.22a(0)	148.72±2.83a(0)
5	87.99±2.49a(-9.6)	19.09±0.39a(+1.6)	10.83±0.25b(-12.44)	138.88±2.19b(-6.6)
10	73.32±3.65b(-24.7)	13.16±0.26b(-30.0)	8.89±0.17c(-28.13)	89.62±1.51c(-39.7)
20	59.99±3.65c(-38.4)	7.91±0.24c(-57.9)	5.95±0.18d(-51.9)	28.40±1.85d(-80.9)
40	46.66±2.10d(-52.1)	4.27±0.11d(-77.3)	4.14±0.08e(-66.5)	9.56±0.22e(-93.6)

Data presented as mean ± standard error;
Different alphabets within a column represent significant difference among treatments at $p \leq 0.05$ applying *post hoc* Tukey's test;
Values within parenthesis represent percent increase (+) or decrease (-) over the respective control.

Figure: Total water soluble phenolic content in soil amended with *Pinus roxburghii* needle litter powder.



Vertical bars represent standard error
Different alphabets represent significant difference among treatments at $p \leq 0.05$ applying *post hoc* Tukey's test

Conclusions

The study concludes that *P. roxburghii* possesses allelopathic potential and affects the growth of under storey plant, *B. pilosa* in laboratory and greenhouse conditions. The present study indicates that similar allelopathic interactions might be occurring in plantations and natural forests of *P. roxburghii*. Allelopathy seems to be an important factor in determining the vegetation structure of these forests.

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