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Germination and Early Seedling Growth of Mustard and Wheat as Affected by Allelopathic Activity of Pinus Needle Extracts

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Abstract

An experiment has been conducted to assess the Allelopathic potential of Pinus needles on seed germination and early seedling growth of Wheat and Mustard. Phenomenon of Allelopathy could be defined as involvement of phytochemicals in the growth and development of surrounding plants, which could be direct or indirect. Moreover the effects could be positive or negative. Plants release phyto-chemicals in the surrounding soil and environment. Allelochemicals are primarily phenolic compounds which are involved in causing number of problems, such as reduction in crop production due to changes in chemical as well as microbial composition of soil, changes in the structure of ecosystem and failure in regeneration of natural forests. In the present study we demonstrate the toxic effects allelochemicals from Pinus needles on Wheat and Mustard seeds. The main aim of this study is to draw attention to the allelopathic potential of phenolic compounds present in pine needles in as the main cause of reduction in crop production.

Keywords: Allelopathy, crop production, mustard, phenolic compounds, wheat.

Introduction

Allelopathy is originate from the Greek words *allelon* and *pathos* which means to suffer of each other[1], and it could be understood as mutual suffering. Allelopathy could be explaind as useful and harmful effects of interaction between plants and microorganisms. Occurrence of weeds in natural and manmade ecosystems is a natural phenomenon. Weeds are reported to have negative impact on crop plants, they affect crop plants by sharing and utilizing the natural resources available in the soil environment. Weeds compete with agricultural plants for nutrients, water, light and space. Allelochemicals are mainly organic compounds released from leaves and other plant parts. Allelochemicals comprise of secondary metabolites and are predominantly found in perennial plants such as Pinus, these chemicals exert impact on other plants growing in their surrounding area and the phenomenon known as allelopathy[2]. Few researchers consider allelopathy, as harmful interactions while, few of them consider allelopathy to both damaging and beneficial interactions between the plants[3]. Reduction in germination and growth of agricultural crops has been acredited to phytotoxic chemicals released from the leaf litter and roots. Many species of weeds produce toxins that are inhibitory to other weeds and often to themselves[4].

Himalayas are one of the hot spot of biodiversity. Chir (*Pinus roxburghii*) is one of the five indigenous species of pine extensively distributed in the outer ranges and principal valleys of Himalaya between 450–2300 m elevation, and from North-West of Afghanistan to the North-Eastern part of India in Arunachal Pradesh[5] between 26°N–36°N latitude and 71°E–93°E longitude figure 1. Every year, the dried needles of Pine trees forms a dense carpet on the forest floor, which are gathered by the locals in large bundles to serve as bedding for their cattle, for the year round. Still a large quantity of these needles is left on the forest floor and with the rain water these needles are weathered and the lechates from them are mixed with the surrounding soil environment. Pinus needles are known to have terpenes in the oil which is reported to inhibit growth of fungi and bacteria[6]. In this context, a study was conducted to reveal the potential effects of allelochemicals from *Pinus* needles on seed germination and early seedling growth of wheat and mustard.

Material and Methods

The allelopathic potential of *Pinus roxburghii* needles was studied in laboratory conditions. The needles *Pinus roxburghii* were collected from various places of Nainital district. The location map of Nainital is given in figure-2. The aim of the present study was also to establish whether allelochemicals from Pinus needles have potential to inhibit seed germination and early seedling growth in studied plants and if there are any changes in the parameters like membrane permeability, malondialdehyde (MDA) content and electrolyte leakage.

Allelopathic extract was prepared from *Pinus roxburghii* needles collected from Nainital. Dried needles were fine powdered and extracted in 100 cm^3 of distilled water at room temperature for 24 h (2.5, 5 or 10 g). After 24 haours the extract was filtered through a filter paper.

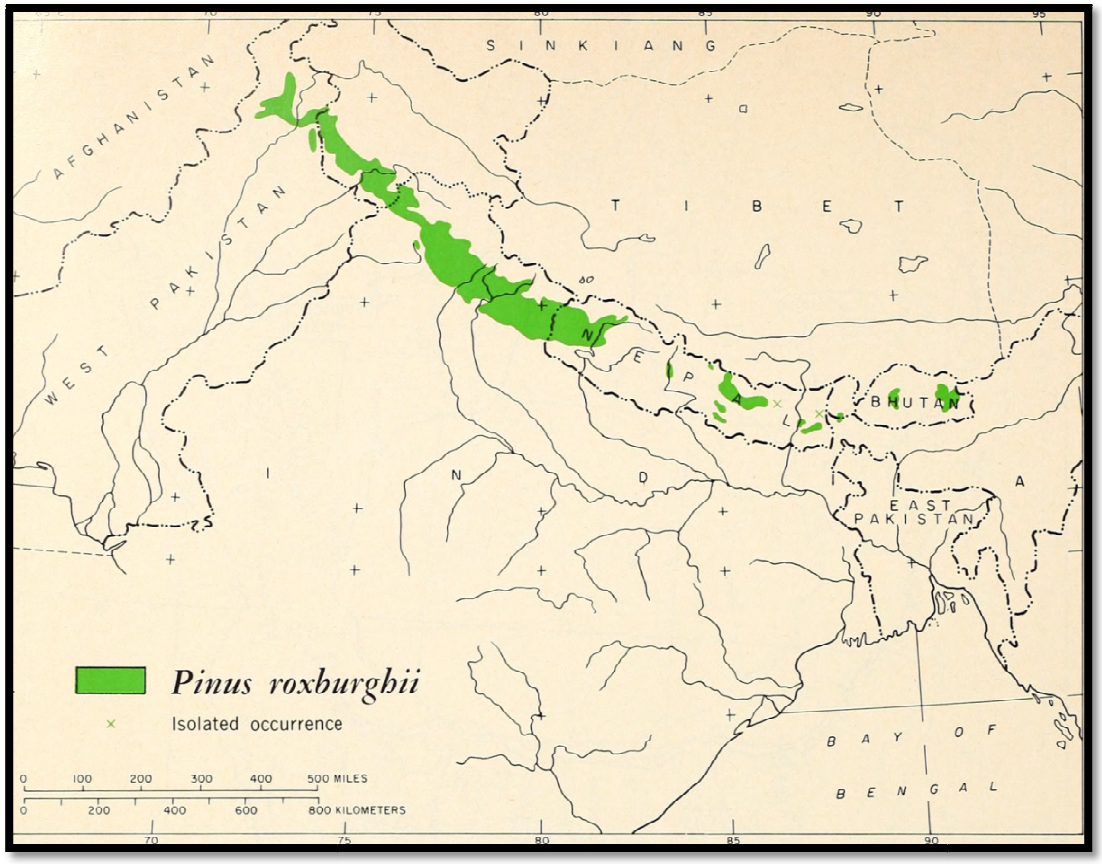


Figure-1
Distribution of *Pinus roxburghii* in Indian subcontinent[5]

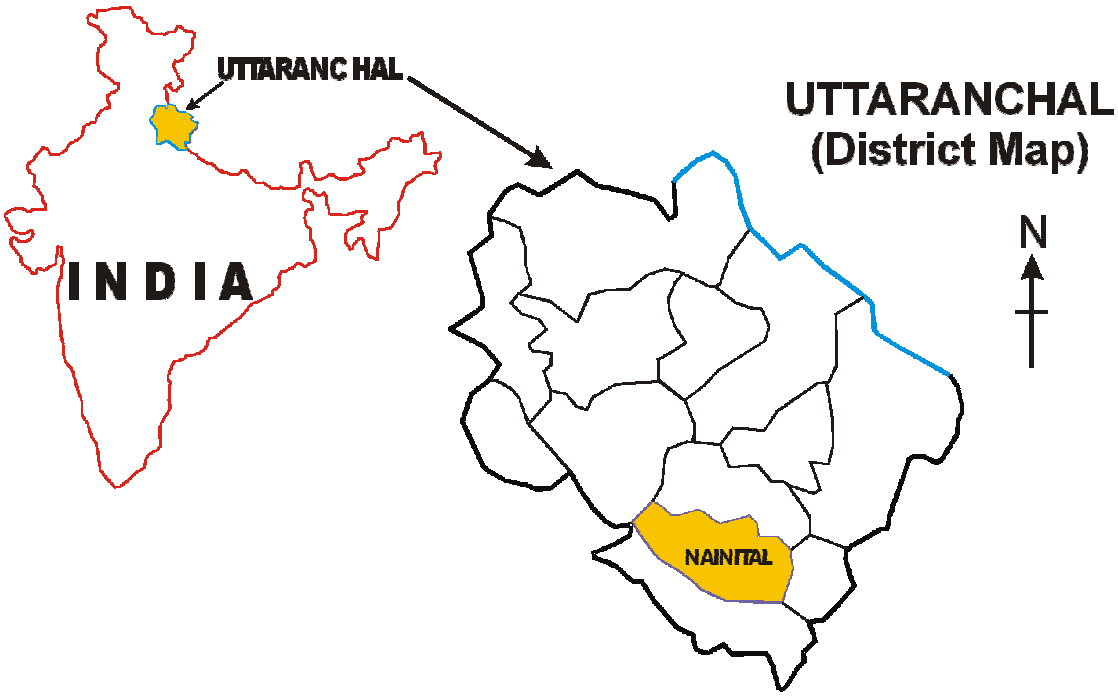


Figure-2
Location map of Nainital district from where the pine needle samples were collected

Wheat and Mustard seeds (*Brassica juncea* L.) were germinated in 10 cm Petri dishes on a filter paper moistened with DW (control) at 20 ^{o}C in darkness (50 seeds per dish). The effect of allelochemicals from *Pinus roxburghii* needles was studied in the presence of different concentrations of water extract 2.5 %, 5 % or 10 % (m/v).

Conductivity meter was used to measure the Electrolyte leakage of young seedlings and seeds. Seedlings (5- 10)/seeds (20) were placed in 15 ml DW at room temperature in darkness and conductivity in the medium was measured after 2 h. Results are expressed as % of total leakage from seeds or seedlings boiled for 20 min.

Lipid peroxidation in seed and seedlings was determined as malondialdehyde (MDA) content by thiobarbituric (TBA) reaction as described by[7].

Results and Discussion

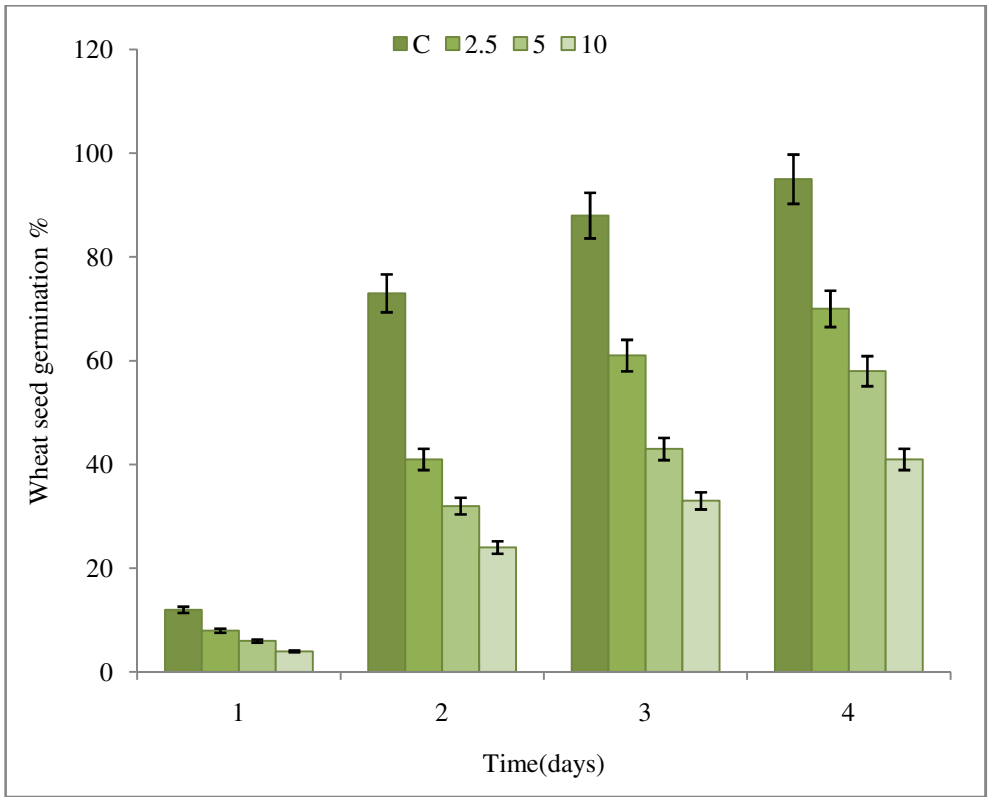
The results of present study shows that the seeds treated with the pinus needle extract show progressive enhancement in electrical conductivity with increased extract concentration and time of the experiment. Enhancement in electrolyte leakage shows damage in membrane integrity. Reduced germination of seeds could be a result of damage in the membrane integrity. Lipid peroxidation enhanced (determined as the increase in MDA concentration) in response to allelochemicals from *Pinus* needles (table 1). Lipid peroxidation occurred in both wheat and mustard seeds in response to *Pinus* needles allelochemicals. Highest level of malondialdehyde (MDA) was observed in seeds germinated in the presence of 10 % extract of *Pinus* needles. As MDA is indicator of membrane health, the increased levels of MDA in stressed seeds indicates occurance of lipid peroxidation and it could be associated with reduced seed germinability. Fresh weight of wheat and mustard seedlings is also affected by *Pinus* needles extract (table 1). Seedlings treated with extract showed reduced fresh weight as compared to control, at higher 5 and 10 % concentrations the effect was more pronounced. Control wheat and mustard seeds/ seedling showed reduced dry weight during experimental period (table 1). The seedlings of wheat and mustard, which were exposed to low and medium concentration of allelopathy extract, showed enhanced dry weight as compared to the control. Similar results were observed by[8] where phenolic compounds showed phytotoxic effect on wild mustard seedlings.

High germination ability (95 – 96 %) was observed in control wheat and mustard seeds after 4 d. Aqueous extracts of *Pinus* needles showed inhibitory effect on seeds germination in wheat and mustard figure 3 A and B. The level of inhibition increased with the extract concentration. There has been a similar trend found in both wheat and mustard in seed germination. At highest concentration (10%) the seed germination was 41 and 40 % in wheat and mustard respectively figure 3 A and B. Bogatek[9] found similar kind of results with sunflower extracts on mustard. This is suggested that Pinus needles have toxic allelochemicals which are responsible for the membrane deterioration, reduced seed germination and seedling growth.

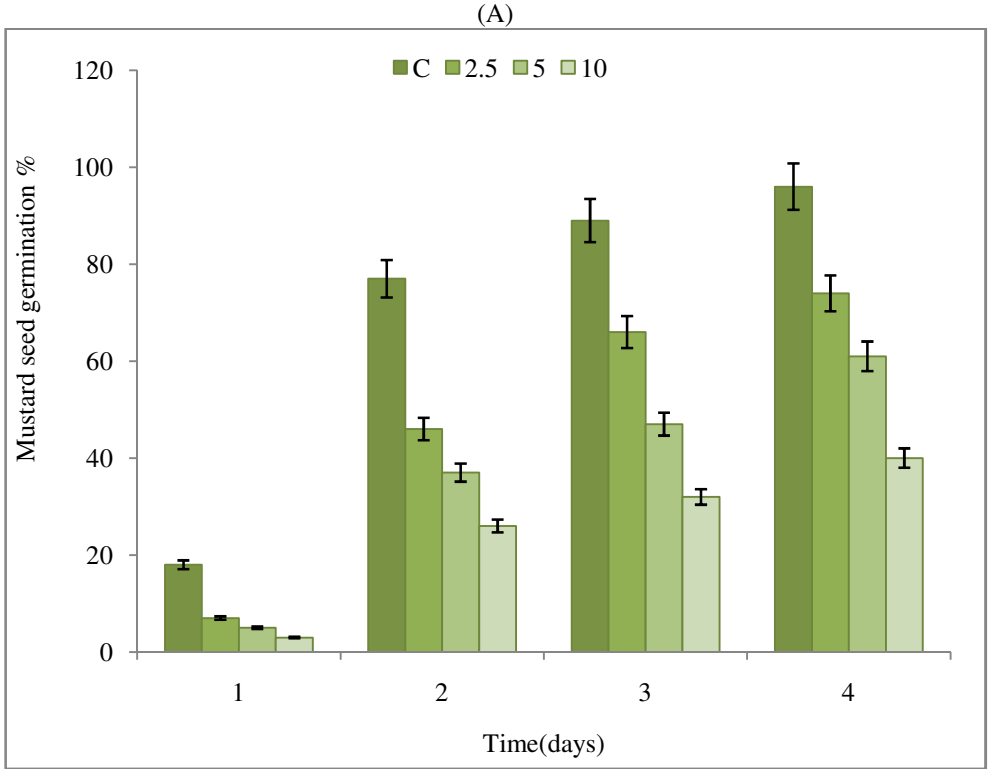
Reduction in radical and hypocotyl length was observed in wheat and mustard seedling in response to *Pinus* needle extracts Figure 4 A and B. Along with the reduced radicle and hypocotyls lengths the treated plants showed thicker roots. Similar results were found on mustard[9], bean and bottle gourd plants cultured in the presence of aqueous leachate of *Sicyos deppei* leaves[10].

Table-1
Effect of various concentrations of aqueous *Pinus* needle extracts on wheat and mustard seedling growth (fresh and dry weight), electrolyte leakage and MDA concentration after 1 and 4 d of culture. Means ± SD of 3 replicates

		Treatments							
		Control		2.5		5		10	
		Day 1	Day 4	Day 1	Day 4	Day 1	Day 4	Day 1	Day 4
Mustard	FW (mg)	23	55	24	45	2	30	20	25
	DW (mg)	10	10	11	11	10	10	10	10
	Electrolyte Leakage. (%total)	7.1	8.8	17.2	18.9	18.1	24.2	19.9	49
	MDA (P mol g-1)	6.6	6.8	6.9	7.7	7	11.2	6.7	17.2
Wheat	FW (mg)	28	62	26	52	25	31	20	24
	DW (mg)	10	10	11	11	10	10	10	10
	Electrolyte Leakage (%total)	8.2	9.1	18.2	18.7	19.1	20.1	20.3	49.7
	MDA (P mol g-1)	7	7.2	7.1	8.8	7	10.9	6.3	19.2



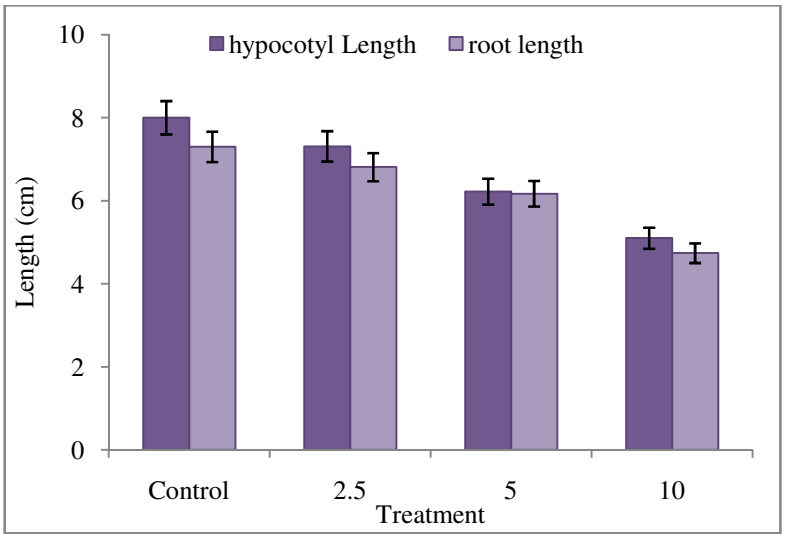
(A)



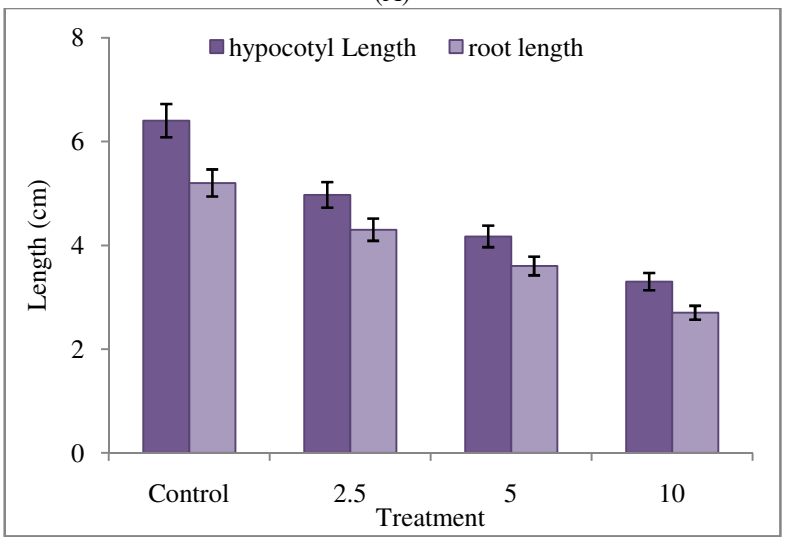
(B)

Figure-3

Germination of wheat (A) and mustard (B) seeds in the presence of diffrent concentrations [%] of water extracts from *Pinus* needles. Each reported value represents the means ± SD of 3 independent experiments



(A)



(B)

Figure-4

Growth (hypocotyls/radical length) of wheat (A) and mustard (B) seeds in the presence of various concentrations [%] of awater extracts from *Pinus* needles. Each reported value represents the means ± SD of 3 independent experiments

Production and mixing of allelochemicals to the soil environment is an important aspect in allelopathy. There are few reports on the mechanisms and processes involved in the production of allelochemicals. The primary effect of allelopathy could be a result from an association with plant litter in or on the soil[11]. Allelochemicals are reported to be present in almost all plant tissue, stems, roots, leaves and fruit[12,13]. These allelochemicals are released from the plant residues by different processes such as root exudation, leaching, decomposition and volatilization. The most regular source for these allelochemicals are leaves, whereas roots are supposed to consist of smaller amount and less effective toxic compounds. Plants growing on mountains at higher altitudes are reported to accumulate higher concentrations of phenolic compounds[14-15] which are known to inhibit plant growth[16].

Plant Growth is affected by various factors in the soil environment such as heavy metals like nickel and lead[17] and rhizobacteria from rhizosphere[18]. Soil environment is changed by burning of pine needles and thereby reducing the share of exchangeable bases like calcium potassium, and magnesium in the soil and this could affect the growth of plants[19]. There are reports that Chir Pine forests are decreasing very quickly and forest is under risk[20]. Researches on *P. densiflora* show that the toxic substances may inhibit germination of seed and growth of plants in the forest[21]. The source of these chemicals are needles of *Pinus*, pine roots, pine pollen grains and forest soil. allelopathic potential of five species of the Pinaceaes, *viz. P. densiflora, P. thunbergii, P. rigida, Larix leptolepis* and *Cedrus deodora* was studied[22]. Which exhibited inhibition of germination in all test species, but the highest inhibition in all

cases was in dry-mass. Figure 5 depicts the possible mechanism of induction of allelochemicals in pinus needles and its response as reduced crop production.

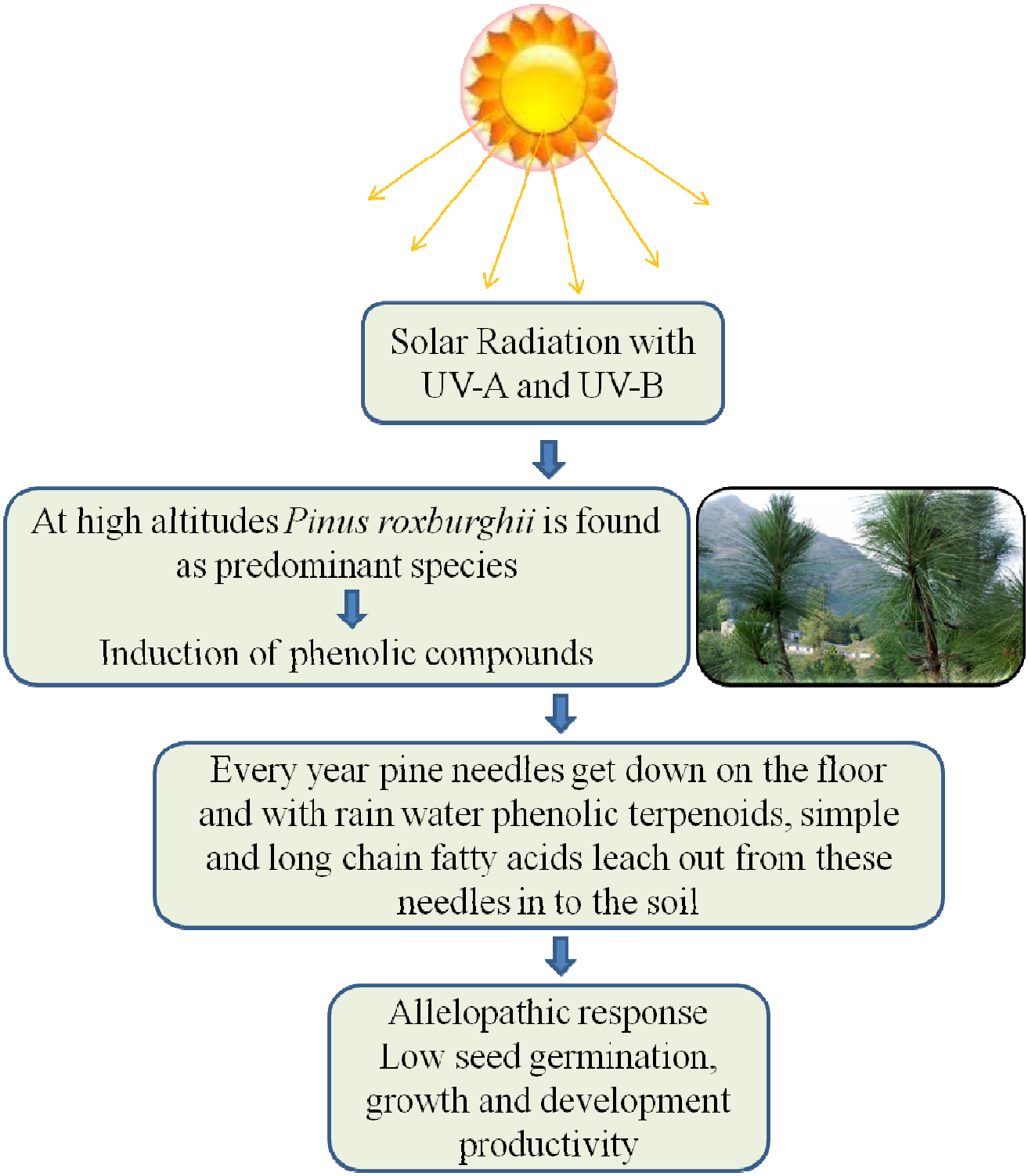


Figure-5
Induction of allelochemicals

Conclusion

Wheat and mustard plants are affected by allelopathic activity of extract from *Pinus* needles. Present results indicate that *Pinus* needles extract is having high concentration of allelochemicals which are responsible for reduced seed germination this is also accompanied by deteriorated membrane of seeds. *Pinus* needles extract showed allelopathic activity again st wheat and mustard. Predominance of *Pinus roxburghii* in the mountain region in India is a known phenomenon. Every season the fallen needles form a bed on the forest floor. In rainy season allelochemicals from pinus needles get dissolved with water and mixed in to the soil. These allelochemicals are added to mountain soils every year and these could be a possible cause for reduced crop production in mountain regions of India and World. Predominance of *Pinus roxburghii* could be a possible cause for reduced crop production in mountain regions of India and World. Further work is needed to appraise the potential inhibitory effects of allelochemicals from pinus needles.

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