Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(5): 1813-1816
Received: 25-07-2018
Accepted: 26-08-2018

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Autotoxicity against seed germination, seedling emergence of *Quercus leucotrichophora* A. Camus Ex. Bahadur

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Abstract
The present study deals with parameters that could influence early recruitments including germination and seedling growth with emphasis on the establishment of *Quercus leucotrichophora* in Garhwal Himalaya, because plant secondary compounds (terpenoides and or phenolic compounds) may direct competition. Autotoxicity study in different plant part extracts concentration in laboratory and nursery. In this experiment, germination %, mean germination time, germination index were taken in laboratory while in nursery, germination %, mean germination time, germination index, different growth attributes and biomass estimation were done by giving different plant part extract treatments. Study reveals that the significant effect of different concentration of bark, green leaves and litter extract of *Q. leucotrichophora* on both germination and growth of its own seedling indicating an auto toxic effect in laboratory as well as in pot culture experiment. Both stimulatory and inhibitory effect of various extracts was marked and found more pronounced during early stage of germination. The study reveals that phytochemicals present in plant parts exerts positive as well as negative effect on seed germination parameters and growth parameters. The purpose of study was to examine Autotoxicity against seed germination and seedling emergence of *Q. leucotrichophora,* the patterns of seed germination and seedling establishment under varied condition.

Keywords: Autotoxicity, Rural development, Food crisis, Employment generation, seed germination

Introduction
Quercus leucotrichophora belongs to genus *Quercus* of family Fagaceae is a moderate-sized/large evergreen tree (Osmaston, 1927) [1] and is an important and commonest oak of the Western Himalayas. It is chiefly distributed in outer ranges of Western Himalayas from 1,000-2,400 m altitude extending eastward up to Nepal, but occasionally descending lower in moist situations (Negi and Naithani, 1995) [2]. This species has a great importance in sustaining the livelihoods of the people, regulating the ecosystem services (mainly recharge of springs) and maintaining biodiversity (Tewari *et al*., 2015) [3] and intimately linked with hill agriculture as they protect soil fertility, watershed, and local biodiversity (Singh *et al*., 2016) [4]. But its natural regeneration is facing serious threat (Troup, 1921, Thadani and Ashton 1995; Tewari *et al*., 2015) [5, 6, 7]. It is evident from the available literature that low regeneration has been attributed due to insect infestation in acorns by acron weevils, high anthropogenic pressure, for fuel wood, fodder and feeding and trampling of seedlings by wildlife.
Regeneration potential is the ability of a species to complete the life cycle (Singh *et al.*, 2015) [8]. The germination of seeds, depending upon their state such as inherent properties, reserved food material and nature of pericarp, represent their own microenvironments which have rarely been investigated. Chemically mediated interferences (allelopathy) can also influence plant recruitment by regulating inter and intraspecific interaction (Weir *et al.*, 2004) [9]. The compounds produced by plants, such as phenol compounds or terpenoides, could inhibit the establishment of seedlings. Recent studies suggest the implication of this phenomenon in the establishment of invasive species (Bais *et al.*, 2003; Vivacano *et al*., 2004) [10, 11] and in the dynamics in arid environments (Vila and Sardans 1999; Karageorgou *et al.*, 2002) [12, 13]. For example, secondary compounds (terpenoides and/or phenolic compounds) can affect root symbionts and site quality through interference with decomposition, mineralization and humification (Kuiters, 1990; Kainulainnen *et al.*, 2003) [14, 15] and they also can influence interspecific competition (Fernandez *et al.*, 2008) [16].
The allelochemicals cause both inter-specific and intra-specific interaction between organisms, which are mediated through chemical process. Allelochemicals may also regulate interspecific interaction. In this case autotoxicity and auto inhibition could reduce the intensity of interspecific competition and maximize the fitness of the dominant members of a population.

By releasing these allelochemicals the functioning of other organisms can affect either negatively or positively (Rice, 1984) [17]. Autotoxicity is considered as one of the factors that contribute to replant failure (Ren, *et al*., 2015) [18]. For all these reasons, autotoxicity has been argued as a cause of forest regeneration failure (Malik, 2003) [19]. In most of the studies related to allelopathy have been oriented towards heterotoxicity, the phenomena of autotoxicity are known to occur in a number of weeds and crops particularly in agrosystems (Chung and Miller, 1995; Singh *et al*., 1999; Yu *et al.*, 2003) [20, 21, 22]. For natural species, autotoxicity was observed in both perennial species (Li and Romane, 1997; Singh *et al*., 1999) [23, 24] and annual species (Canals *et al*., 2005[25]) [25].

Material and Methods

Present study was conducted at College of Forestry, V.C.S.G. University of Horticulture and Forestry, Ranichauri, TehriGarhwal, having altitude about 2100 m msl, lying between 30^0 15' N latitude and 78^0 30' E longitudes under the mid hills of Uttarakhand, India in the year of (2014-15). Mean minimum and maximum monthly temperature during study period ranges from 2.0 ^{0}C to 25.9 ^{0}C, respectively with annual rainfall 1305 mm.

To study the auto toxicity of *Q. leucotrichophora* sundried green leaves, bark and leaf litter were collected from five trees (of natural forest nearby the Ranichauri campus). To prepare aqueous extract (2, 5 and 10%) 10g, 25g and 50g of sun dried green leaves, bark and leaf litter were spaked in 500 ml of distilled water for 24 hours at room temperature. The resultant solution was filtered with a three layers of Whatman no.1 filter paper and stored in a conical flask.

Seed germination studies were carried out in laboratory condition (temperature 25 ^{0}C) by placing 10 seeds above whatman filter paper (no. 1) and irrigated with respective bark, green leaves and litter extract.

In pot culture experiment plastic pots for germination studies and polypots for growth parameters studies (at three months interval) were filled with mixture of soil, sand and FYM in the ratio of 1:2:1. Each pot and polybag were irrigated with 50 ml each extract and control by distilled water. 20 seed of each replication were sown in the polybags at the depth of 2 cm. The weeding and watering of polybags were done manually when needed till the completion of the experiment. The seeds were considered germinated when the radicle had emerged out. Germination percentage, Mean Germination Time (MGT) as per Ellis and Roberts (1981)[26] (MGT =$\sum Dn / \sum n$, where n = number of seeds germinated on day D (D representing the no of days since the sowing of seeds) and Germination index (GI) was calculated as per Kendrick and Frankland (1969) [27] as Germination Index (GI) =Total germination per cent/Time (hours) taken for 50% at 28 days.

To assess the effect of leaf extract on periodic growth eight randomly selected seedlings in five replicates of ten treatments each were tagged and morphological traits *viz*. shoot length, collar diameter and number of leaves/seedlings were studied at three months interval till one year. Collar diameter was taken with the help of a digital calliper (Mitutoyo Absolute). Finallyt he tagged seedlings were up rooted, root length, shoot length and their respective biomass (oven for drying at temperature of 103˚C for 24 hours) were recorded.

Results

The data pertaining to autotoxicity of *Q. leucotrichophora* seeds revealed that highest germination percentage (85%) was found in seeds treated with 2%bark extract while significantly least (66%) in the seeds treated 10% green leaves extract as compared to control (79%). In general the plant part extract (bark, green leaves and leaf litter) had inhibitory effect on germination except the bark extract (2% and 5%) which had stimulatory effect on germination however, the effect was statistically at par with control treatment (table 1).The seeds treated with 5% green leaves extract had significantly highest mean germination time (MGT) as compared to control (18.24) while minimum in 5% litter extract (12.22 days). Maximum Germination index was recorded for seeds treated with bark extract 2% (0.17) which was statistically at par with control treatment (0.14) while 10% green leaf extract significantly reduced down the GI (0.09) (table 1).

Similarly, in pot culture experiment highest germination percentage (73.33%) was found in seeds treated with 2%bark extract while minimum (46.67%) in the seeds treated 10% green leaves extract as compared to control (63.33%). Stimulatory effect of all the extracts (bark, green leaves and litter) was observed on mean germination time (MGT) as compared to control treatment (19.18). Maximum Germination index was recorded for seeds treated with bark extract 2% (0.17) which was statistically at par with control treatment (0.14) while minimum for seeds treated with 5 and 10% (0.10) green leaf extract significantly (table1).

Table 1: the effect of autotoxicity of different plant parts extracts of *Q. leucotrichophora* on germination, MGT and GI in laboratory and nursery

‘		Laboratory			Nursery		
		Ger. %	**MGT**	**GI**	**Ger.%**	**MGT**	**GI**
T1	Control	79.00 ^{abc}	18.24^{b}	0.14^{ab}	63.33^{ab}	19.18 e	0.14ab
T2	Bark 2%	85.00^{a}	16.42^{bc}	0.17 ^{a}	73.33 ^{a}	20.14cde	0.17a
T3	Bark5%	83.00 ^{ab}	19.73^{ab}	0.13^{abc}	66.67^{ab}	20.51bcde	0.13^{abc}
T4	Bark10%	72.00 ^{bcd}	18.57^{b}	0.12^{bcd}	60.00^{abc}	21.63 abcde	0.12bcd
T5	Leaves 2%	71.00 ^{bcd}	20.15^{ab}	0.12^{bcd}	63.33^{ab}	19.62 de	0.12bcd
T6	Leaves5%	67.00 ^{cd}	24.22^{a}	0.10^{cd}	53.33^{bc}	24.20 a	0.10cd
T7	Leaves10%	53.00 ^{e}	20.94^{ab}	0.09^{d}	46.67^{c}	23.1^{ab}	0.10cd
T8	Litter 2%	78.00 ^{abcd}	18.87^{b}	0.14^{ab}	70.00 ^{a}	20.81^{bcde}	0.14ab
T9	Litter 5%	72.66 ^{abcd}	12.22^{c}	0.13^{abc}	66.67^{ab}	22.25abcd	0.13abc
T10	Litter 10%	66.00 ^{d}	17.41^{bc}	0.12^{bcd}	63.33^{ab}	22.78abc	0.12bcd
	CD@5%	12.70	5.27	0.039	13.91	2.64	0.04
	CV%	10.26	16.57	18.17	13.03	7.24	18.17

After 3 months of growth shoot length, collar diameter and number of leaves were varied among different concentration of leachates of leaf, bark and litter. The effect of all the extract (bark, green leaves and litter) was non-significant on

shoot length and number of leaves per plant of test crop, nevertheless, stimulatory effect was observed on collar diameter except the extract of green leaves 5% (1.30 mm) and leaf litter 10% (1.35 mm) which were at par to the control treatment (1.47 mm), maximum collar diameter was recorded for the seeds treated with bark extract of 10%.

Similarly after 6 month the effect of all the leachates was remained non-significant for shoot length and number of leaves of per plant and stimulatory on collar diameter except the pots treated with green leaf extract of 2% (1.59 mm) and 5% (1.63 mm) as compared to control (1.79 mm) while the maximum collar diameter was recorded for bark extract 10%(2.74 mm).

After 9 months of growth all growth attribute were found significantly different among treatments. In general stimulatory effect of all the extracts (bark, green leaves and leaf litter) was found on shoot length and collar diameter, except the litter extract (2% and 5%) which reduced down the collar diameter (1.78mm and 1.95 mm respectively) as compared to control (2.03 mm). Maximum shoot length was found in pots treated with 10% bark extract (15.35 mm) as compared to control treatment (11.38 mm) which was significantly least. Similarly maximum number of leaves per plant was recorded in the pots treated with 10% bark extract (7.09) as compared to control treatment (5.24) while minimum in litter extract 10% (4.52).

Finally after 12 months of sowing maximum shoot length was recorded in the plants treated with litter extract 2% (16.89 cm) as compared to control (13.51 cm) while minimum in green leaves extract 2% (13.28 cm) in bark extract treatment collar diameter was significantly higher than control similar result were found in leaf extract while in leaf litter treatment collar diameter was found less than control. Maximum collar diameter was found in the pots treated with bark extract 2% (3.79mm) and minimum in green leaves extract10% (1.98 mm). Number of leaf was found significantly more in all extract treatment than the control maximum number of leaves was found in bark extract 2% (8.20) and minimum in treatment control treatment (5.80).

Table 2: effect of auto toxicity of different plant parts extract on different growth attributes of 3 months intervals

Treatments	3 month			6 month			9 month			12 months			Biomass (final)		
	Shoot Length (cm)	Collar diameter (mm)	Number of leaves	Shoot Length (cm)	Collar diameter (mm)	Number of leaves	Shoot Length (cm)	Collar diameter (mm)	Number of leaves	Shoot Length (cm)	Collar diameter (mm)	Number of leaves	Leaf biomass (gm)	Shoot biomass (gm)	Root biomass (gm)
Control	8.70	1.47^{de}	3.72	10.14	1.79^{de}	4.70	11.38^{e}	2.03def	5.24bc	13.51c	2.34def	5.80d	1.15^{c}	1.40^{bcd}	1.48^{d}
Bark 2%	9.70	1.79^{bcd}	4.00	11.37	2.20^{b}	5.18	12.70^{cde}	2.49bcd	6.03ab	15.65ab	2.84bcd	6.67bcd	1.46^{ab}	1.42^{bcd}	1.25^{d}
Bark5%	10.90	1.89^{abc}	4.20	12.54	2.00^{bcd}	5.22	14.65^{abc}	2.12cdef	6.02ab	16.02ab	2.41def	6.67bcd	1.44^{ab}	1.49^{bc}	2.11^{bc}
Bark10%	12.76	2.22^{a}	4.20	14.06	2.74^{a}	6.19	15.35^{a}	3.32a	7.09a	16.58a	3.79a	8.20a	1.65^{a}	1.79^{a}	2.68^{a}
Leaves 2%	9.37	1.70^{bcde}	3.50	10.84	1.96^{bcde}	4.49	12.25^{de}	2.38bcde	5.20bc	13.28c	3.00bc	6.28cd	1.37^{bc}	1.26^{cd}	2.26^{ab}
Leaves5%	11.10	1.58^{bcde}	3.70	12.16	2.21^{b}	4.54	13.27^{abcde}	2.74b	6.13ab	14.53abc	3.36ab	7.28abc	1.56^{a}	1.23^{d}	2.50^{ab}
Leaves10%	10.30	1.30^{e}	3.60	11.75	1.82^{cde}	5.12	12.81^{bcde}	2.09cdef	5.69bc	13.64bc	2.67cde	7.59ab	1.37^{a}	1.61^{ab}	1.62^{d}
Litter 2%	10.50	1.48^{cde}	3.72	12.33	1.59^{e}	4.95	14.62^{abcd}	1.78f	4.49c	16.89a	1.98f	6.75bcd	1.65^{a}	1.50^{bc}	2.57^{ab}
Litter 5%	11.20	1.35^{e}	3.90	12.91	1.63^{de}	5.11	14.33^{abcd}	1.95ef	5.99ab	15.25abc	2.17ef	6.60bcd	1.46^{ab}	1.50^{bc}	2.46^{ab}
Litter10%	11.20	1.98^{ab}	3.94	13.16	2.21^{b}	4.92	15.21^{ab}	2.54bc	4.52c	16.61a	2.75cd	6.68bcd	1.44^{ab}	1.35^{cd}	1.64^{cd}
CD@5%	NS	0.42	NS	NS	0.39	NS	2.40	0.49	1.34	2.41	0.53	1.25	0.23	0.25	0.48
CV%	20.54	19.39	26.28	15.67	14.83	19.54	13.75	16.60	18.51	12.43	15.19	14.36	12.5	13.31	18.29

Leaf biomass after 12 month was significantly varied in all extract treatment compare to control treatment maximum leaf biomass was found in bark 10% (1.65gm) and minimum in control treatment (1.15 gm) Similarly, maximum shoot biomass was found in treatment (T4=2.68). While minimum in treatment (T2=1.25).

Discussion

Present study pointed out the concentration dependent significant effect of bark, green leaves and litter extract of *Q. leucotrichophora* on both germination and growth of its own seedling indicating an autotoxic effect in laboratory as well as in pot culture experiment. Both stimulatory and inhibitory effect of various extracts was marked and found more pronounced during early stage of germination i.e., emergence of radicle and plumule. Bark extract (2%, and 5%) had stimulatory effect on its own germination in laboratory as well as in pot culture experiment while the bark extract (10%), green leaves and litter extract inhibited the germination. The results observed attributed to the allelochemicals present in bark, leaves and leaf litter of *Q. leucotrichophora*. Similarly, auto toxicity in aqueous extract of *Quercus ilex*on its own germination and seedling weight was reported by (Li and Romane, 1997[28]). The effect observed was both positive and negative effect on Mean germination Time (MGT) and Germination Index (GI), green leaves extract 5% in laboratory experiment, 5% and 10% green leaves and litter extract in pot culture significantly stimulated MGT while the litter extract 5% reduced MGT significantly in lab condition. Similarly, inhibitory effect of bark and litter extract (10%) and green leaves extract (2%, 5%&10%) and litter extract on Germination Index (GI) was found. Some previous studies on like *Pinus halepens is* needles extract having more dose effect than root effect on germination. Litter seems to be the more important parameter influencing germination rate (Fernandez *et al.,* 2008[29]). The effect of litter is both chemical and physical. The effect of all the leachates (bark, green leaves and litter) on shoot length and number of leaves was non-significant up to six months of sowing in pot culture while the effect of bark extracts(2%, 5% and 10%) was significantly stimulatory on collar diameter after 3 months. Similarly, the effect of bark extracts (2% and 5%) and green leaves extract (5%) on collar diameter was stimulatory. Inhibitory effect could not mark up to one year months on shoot length and number of leaves on the contrary it was stimulatory.

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