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Allelopathy Research Methods in Forestry

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CONTENTS

ABSTRACT

Forest ecosystems are very complex with numerous tree species and organisms and dominated by trees. Every part of forest ecosystem plays an important role in its development. In forestry systems, allelopathy affects many aspects of plant ecology [occurrence, growth and plant succession, structure of plant communities, dominance, diversity and plant productivity]. This review explains the allelopathy research methods used in forestry and forest ecosystems.

Key words: Allelopathy, bioassays, collection, extracts, forestry, leachates, methodology, preparation, samples

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1. INTRODUCTION

Despite the efforts to study the origin, mode of secretion, their transformations in soil, mechanisms of effects, chemical and physical properties of substances and biochemical interrelations of organisms are still in the initial stages. Hence, there are new possibilities of using allelopathy. However, if some external intervention occurs in ecosystems, allelopathic interrelations are clearly evident, proportional to the intensity of intervention. Allelopathy is least apparent but has greater role in forest management.

Ecology deals with mutual interactions among the organisms and environment. The mutual interactions of organisms via environment are called transmediopathy (*Trans*: via; *Medio*: medium or environment; *Pathy*: influence, operating via environment). These complex intraspecific and interspecific relationships can be divided in allelospoly and allelopathy. Allelospoly (*allelo*: mutual, *spolio*: to withdraw) includes mutual interactions of organisms via environment by withdrawing the matters and energy from environment. Allelopathy (*allelo*- mutually; *pathy*- influence, affect) includes mutual interactions of organisms through excretion of matter and energy to environment. Transmediopathy has two components (i). Endometabolites (Physiologically active matters excreted by tissues and organs inside the body and (ii). Exometabolites (Ecologically active matters excreted by tissues and organs out of the organism). The latter affects the producer and surrounding organisms. These matters were considered as unimportant waste products until recently, but their chemical compounds affect the development and behaviour of individuals and whole community. These natural interactions include positive, neutral or negative relationships. The extent to which tree species affect the growth of other tree species growing underneath by excretion of ecologically active matters are examined in this paper.

Table 1. Tree species used in this Review paper

Sl.No.	English name	Botanical name with author	Family	Economic uses
1.	Oak	*Quercus petrea* Liebl.	Fagaceae	Wood is used for construction purposes, shipbuilding and oak barrels for storing wine.
2.	Scot Pine	*Pinus silvestris* L.	Pinaceae	Wood is used for pulp and sawn timber products, used as a source of rosin and turpentine.
3.	Birch	*Betula spp (different birches can't identify which one)*	Betulaceae	Making plywood, leaves used to make a diuretic tea and extracts for dyes and cosmetics, birch sap (for making a traditional drink), birch oil (in leather making) etc.
4.	Spruce	*Picea abies* (L.) H. Karst.	Pinaceae	Used for wood and as the main Christmas tree in several cities around the world.
5.	Larch	*Larix decidua* Mill.	Pinaceae	Cultivated as an ornamental tree for planting in gardens and parks.
6.	Beech	*Fagus silvatica* L.	Fagaceae	Excellent firewood, chips of wood used for brewing beer, as ornamental tree.

Table 1. Contd.

Sl.No	English name	Botanical name with author	Family	Economic uses
7.	Loblolly pine	*Pinus taeda* L.	Pinaceae	Used for its timber
8.	Locust	*Robinia pseudoacacia* L.	Fabaceae	Wood used for furniture, flooring, paneling, fence posts, and small watercraft, firewood. In traditional medicine of India, different parts are used as laxative, antispasmodic, and diuretic.
9.	Alder	*Alnus acuminate* Kunth.	Betulaceae	Symbiotic nitrogen fixation, used for making electrical guitar body, dye from bark, tannin from bark used to tan leather.
10.	Field maple	*Acer campestre L.*	Sapindaceae	Wood used for furniture, flooring, wood turning and musical instruments and as ornamental tree.
11.	White ash	*Fraxinus Americana* L.	Oleaceae	Timber used for production of baseball bats and tool handles, solid body electric guitar, lobster traps etc.
12.	Hazel	*Corylus avellana* L.	Betulaceae	Poles used for wattle-and-daub building and agricultural fencing, hazelnuts are edible
13.	European White Elm	*Ulmus laevis* Pall.	Ulmaceae	Owing to its rapid growth, tolerance of soil compaction, air pollution and de-icing salts, the tree has long been used for amenity planting in towns and along roadsides
14.	Fir	*Abies alba* Mill.	Pinaceae	Essential oil used in perfumes, bath products, and aerosol inhalants, wood used for general construction and paper manufacture.
15.	Southern red oak	*Quercus falcate* Michx.	Fagaceae	Used for its timber
16.	Acacia	*Acacia auriculiformis* A.Cunn. ex Benth.	Fabaceae	Used as ornamental tree, wood for making paper, furniture and tools, contains gum, tannin etc
17.	Juniper	*Juniperus communis* L.	Cupressaceae	Used as an evergreen ornamental shrub, timber berries used for flavoring.
18.	Douglas fir	*Pseudotsuga menziesii* (Mirb.) Franco	Pinaceae	Wood is used for dimensional lumber, timbers, pilings, and plywood, railroad ties, mine timbers, house logs, posts and poles, fencing, flooring, pulp, and furniture, used extensively in landscaping, as a specimen tree or in mass screening.

2. BRIEF REVIEW OF FORESTRY RESEARCH

The literature reveals various effects of same tree species on each other depending on their age, distance, growth, site (soil, altitude, aspect, slope, moisture amount, heat and wind conditions, etc.). Their vitality, health condition, growth and their effects on surrounding organisms are connected to surrounding biochemical and biophysical effects of organisms. The impact of allelopathy has been on the structure of forest stands and dominant forest tree species. Allelopathy also plays an important role in the life of other components of forest ecosystem viz., (i). Introduction of forest tree species and (ii). Etiology and ecology of forest.

In USSR, Chernobrivenko (30) and Kolesnichenko (82) and others studied the interrelations/mutual effects of trees on growth of tree species, but overlooked the allelopathic interactions. The US researchers, isolated and studied the effects of individual isolated substances on respective tree species (36,43,45,115,125).

2.1. Radioactive Compounds

In research involving mutual influence of tree species on root secretions, use of radioactive substances was much favoured due to the speed and possibilities of its use under any conditions. Lavrinenko (95) used P^{32}.in his research on ash, which grew in monocultures and mixed stands irrigated with water containing radioactive phosphorus P^{32} and its content in leaves. The transition of mineral substances from one tree species to another by using H_3PO_4 radioactive phosphorus has been observed (139). It was also found that P^{32} applied through leaves of one plant can penetrate the neighbouring plant not only through roots growing into one another but also by mutual touching of root systems and via soil solution. Here, rhizosphere microorganisms play a great role. Transition from one tree species into another tree was in many cases stronger than transition between the trees of the same species. Radioactivity of oak leaves was 5-6 times higher, when phosphorus moved from pine secretions than when phosphorus moved from oak to oak (51). Exchange of mineral substances between the tree species occurs especially within the reach of root system, whose radius, is approximately equal to tree height. The knowledge that respective parts of tree species are being fed by certain roots is also interesting. It was reflected in the different radioactivity of twigs and leaves on the same tree species (90). Rakhteenko (139) reported findings of Akhromeiko and Zhuravlevova (2) that many tree species secrete much phosphorus and other mineral substances at night in soil (not used in plants metabolism), than they absorb. Nesterovich (118) reviewed the researches of Rakhtejenko (140,141) and other authors on this aspect. Majackyi (104) used the radioactive phosphorus by drainage tubes between the roots into 20 cm depth, while, Gonchar and Postrigan (51) applied it directly into the stem of 18-years old tree species in oak-pine stands.

Rakhteenko *et al*. (142) reported the absorbption of radioactive phosphorus and the effects of root leachates from other tree species on the intensity of photosynthesis in tree species. Kolesnichenko *et al.* (84) while studying the exchange of phosphorus between pine and birch, did not use radioactive phosphorus but determined colorimetrically the amount of organic and inorganic phosphorus after its extraction by 55-trichloracetic acid.

2.1.1. Volatile substances: Several scientists have researched on volatile substances of various tree species (34,121,166). In research of volatile substances, radioactive carbon C^{14} was used (39,40). Kolesnichenko (83) and Ivanov (65) used it as additional fertilization in form of $C^{14}O_2$. Rachkov (138) describes various methods of allelopathy research to use the radioactive atoms of carbon (C^{14}), phosphorus (P^{32}), sulphur (S^{35}), cobalt (Co^{60}) and strontium (Sr^{90}). Ovcharov (124) described the role of vitamins in allelopathy of plants.

2.2. Mutual allelopathic influences in mixed plantations

In natural communities interrelations of tree species had been formed over a long time. Rice reported much data on forest allelopathy from Communist countries (145,146) in his book 'Allelopathy'. Rakhteenko summarized the results of Belarus scientists and were published by Nesterovich (118). Mashinskyi (108) studied the changes and effects of root systems in 17- years old monocultures and mixed stands of pine, spruce, larch and locust and generated data to make tree dmixtures. Gubarevova (57) described the effects of number of interspersed tree species on the production of oak. The positive effects in admixture of 20-30%, changeds to negative (decreased height by 25% and stem girth by 20%) in admixture of 70-90%. Most researches studied the effects of various tree species growing close to each other. Kolesnichenko (81) described the information of admixtures formed with major tree species in the monograph (83). In belts plantations, various relations among the trees were observed (16,20).

In forests, pine and oak are mojor tree species, which grow in mixture with different trtee spacies. The interactions of various tree species with pine and oak are described below.

2.2.1. Pine

I. Pine + Birch: The biomass was higher (20-40%) in mixed stands than in monocultures (140). Tokar (177) reported higher production of mixed stands. Soviet researchers did more researches on the mutual influence of pine and birch.

Most of Soviet authors studied the pine and birch dealing with mixed stands and compared them with their monocultures. While some authors recorded strong negative interrelations, others described the positive effect of birch on common pine. Variability of interrelations depends on the site, climatic and soil conditions, tree species composition and age. Olejnikovova (122) stated that average biomass of pine growing in monoculture was higher (80.65%) than in 50% admixture with birch. Popov (132) recorded positive effects upto 5 % birch admixture on pine. Admixture from 5 to 25% of birch did not affect the pine, whereas, 25% of birch admixture negatively affected the stand. Also Rakhtejenko and Kabashnikovova (141) reported the increased production of pine-birch stand by 28.5% with 20% proportion of birch, and a reduction of production to 83.3% with 50% proportion.

Caboun (19) studied the 20-years old forest with various tree species growing close to each other and measured the height of trees, trunk thickness, trunk and total biomas, width of annual rings. He found marked differences in diameter, height, branching, formation of wood and total biomass of tree species. The greatest effects in mutual growth of pine and birch occurred in the locality near Liptovsky Mikulas. Based on

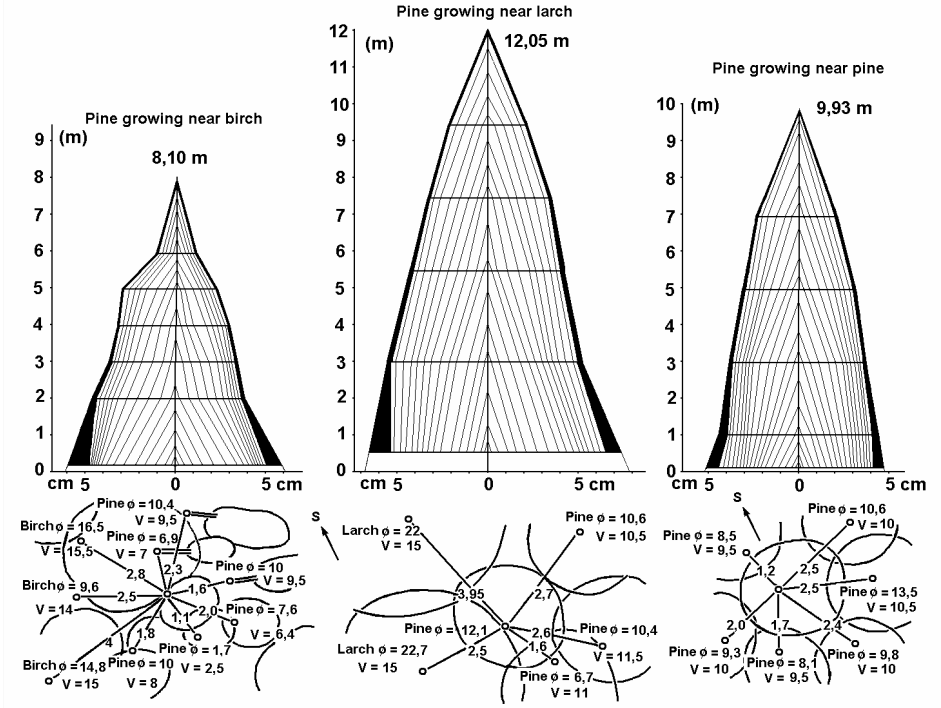

Figure 1. Stem analyses of pine growing near birch, larch and pine

the biometrical measurements, he found very significant inhibitory effects of birch on pine, ending in some cases with complete elimination of pine growing close to birch. Birch caused 100 % reduction in standing volume per hectare in pine growing at 4 m distance from birch than pine monoculture. When the crown of birch shelters the pine (without any contact between them), the pine started to dry (16,21,22, 24,25,27).

Negative effects of birch on pine was observed not only in diameter growth but also in asymmetric growth of pine, deformation of branches, declined growth of whole tree, reduced height growth and bending (deflection) of needles.

Goncar (50) analysed the relations between pairs of tree species, pine-birch and pine-oak, in his monograph. He concluded that horizontal structure of stand, placement of tree species on the plot and their number play an important role in the mutual relations of tree species.

II. Pine + Larch: The pine growing with larch had better growth under smaller proportion compared to monoculture. Levdik (97) noted positive effect of larch on pine: +16.1% on height growth and +9.3% on diameter growth. Therefore, 10-25% admixture of larch with pine is recommended. Kostenchuk (88) described the optimisation of forest structure. Fedotov (41) presented that standing volume for 48 years old larch-pine stand was higher by 48.14% than the larch-spruce stand of the same age. Standing volume per hectare for 56 years old larch-elm stand was higher (54%) than larch-birch stand. Several authors

confirmed these results (4,70,106,149). The larch stimulated the height of pine and standing volume per hectare by 60%. Positive effects of larch and negative effects of birch on pine were evident from the stem analyses (Fig. 1). Larch had positive effects on height and stem diameter of pine. The positive effects of larch in pine-spruce 69 years old stands were reported (71).

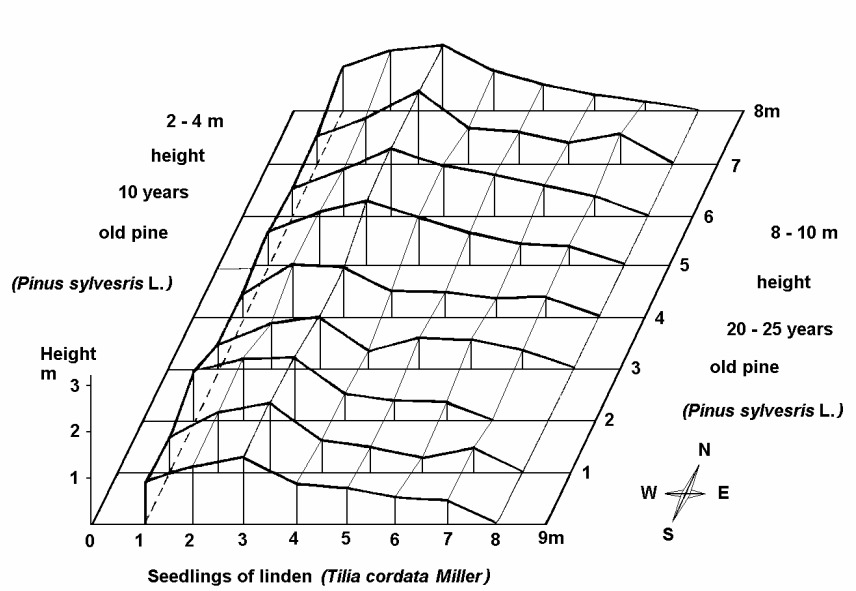

Figure 2. The influence of different old pine trees on young linden growing in Stupava sand

III. Pine + Other tree spp: caboun (19) reported that linden stimulated the growth of pine near Stupava, but pine decreased the height of linden. These effects depended on the age and size of pine and linden trees (Fig. 2). Other tree species can also have similar effects. Hornbeam, oak and locust deformed the twigs and total growth of closely growing pine trees; locust decreased the height. Besides negative or positive effects of some tree species were observed. Similarly stimulatory effect of spruce on pine growth were reported. A slight stimulation of pine growth in the vicinity of spruce was also observed (196) .The positive effects of larch in pine-spruce 69 years old stands were reported (71).

2.2.2. Oak

STIMULATORY EFFECTS

I. Oak + Linden: Oak with linden resulted in higher quality stands than with ash (127). After 32 year, standing volume of mixed oak-linden stand was 304 $m^{-3}.ha^{-1}$ than 255 $m^{-3}.ha^{-1}$ in oak monoculture. Mutual positive effect of oak and linden was reported in 17 years old stands (140). In monocultures, oak attained 3.5 m height, 3.8 cm diameter, while in mixed stands these values were 3.9 m and 4.5 cm, respectively. In monocultures, Linden

attained 3.1 m height, 3.4 cm diameter while in mixed stands it was 3.4 m and 3.8 cm respectively.

II. Oak + Spruce: Pavlovskyi (128) compared the total production of 68 years old mixed stand (710.9 $m^{-3}.ha^{-1}$) with the production of the same age oak stand (535.5 $m^{-3}.ha^{-1}$). Mixed stand had higher production (32.75%). Spruce had positive effects on oak (170). A slight stimulation of pine growth in the vicinity of spruce was also observed (196) .The positive effects of larch in pine-spruce 69 years old stands were reported (71).

III. Oak + Pine/Maple: Kolesnichenko and Krjukov (85) studied the effects of other tree species on oak based on the speed of accumulation of radioactive phosphorus. Remis (144) reported the marked stimulatory effects of oak on diameter increment of pine. Also, oak with Norway maple grows better than oak in monocultures.

INHIBITORY EFFECTS

I. Oak + Ash: Ash had negative mutual effects on oak. The mean standing volume in oak monoculture at 32 years age was 255 $m^3.ha^{-1}$, while in stand with ash it was only 23 $m^3 ha^{-1}$ (127). These differences continued throughout life cycle. Oak remained in the stand only due to tending, without which it would have disappeared and only ash would remain. Martinovic (105) studied effects of oak and ash (142), and found the optimal proportion (10-30%) of ash in oak stand.

II. Oak + Larch: Fedotov (41) found negative effects of larch on oak. Oak disappeared from the stand completely after 18-20 years. Chumakov (33) reported strong inhibitory effects of larch on spruce than effects of larch on pine and birch.

2.3. Tree Mixtures

Prat (133) and Runov and Egorova (153) reported about inhibitors and toxins. Maslov (107), Kotov and Shaposhnikova (89) and Demjanov (37) reported about the phytogenous field. Several authors studied the effects of underneath plant species on tree species (62,69,93,119,130,152,162,174), while some studied the effects of tree species on herbaceous undergrowth (76,99,100,101,109,192). The phytogenous field influences the 3-species of grasses around oak trees up to a distance of 5 m beyond the crown margin (156).

2.3.1. Tree + Understorey spp: Jakovlev and Nezabudkin (69) reported that 15- years old lupine had stimulatory effects on the 16 years old oak and increased growth by 75% in lupine + oak stands than in control without lupine. However, authors did not explain the factors responsible for this increase in oak productivity. Lupine in lupine+ spruce mixture increased the total growth, seed yield of spruce (151). Growth rate of spruce mixed stand with larch in upper and beech in lower layer was considerably higher than growth of larch in monoculture. In these mistures, higher growth rates of beech and larch continued till old age of 140 years (Assmann, 1968). He also found 38% higher growth of lupine in monoculture than in 80 years old stand of beech + lupine mixture. In 10-years mixture of lupine + Siberian larch, lupine increased the height and girth of Siberian larch by 10.5% and 31.8 % over the monoculture, respectively.

On the other hand, the fescue (*Festuca arundinacea* Schreber) decreased the growth and stem weight (50%) of transplanted loblolly pine plants than control (187). Golden rod (*Solidago altissima* L.) grass drastically reduces the growth of locust by 90%, but this grass did not effect the growth of alder (93). Even black locust was very sensitive to the effect of grasses.

2.4. Monoculture

Grümmer (56) reviewed the injurious effects of long-term accumulation of root secretions and substances from plants. Kolesnichenko (83) reported considerable reduction in yield of spruce and pine monocultures in Europe during their cultivation for 2-3 generations (over 200-300 years). In tree species such as field maple, ash and hazel, which succeeded after cutting of oak trees, the oak toxicity in soil was not evident. The monoculture stands have long term negative effects on the soil and slowly intoxicates the soil, with gradual changes in tree species composition. Thus, accumulated toxic substances would be absorbed by other plants and microorganisms associated with these plants and after some time composition of previous stand could be restored. Thus autointoxication (besides other anthropogenic effects) contributes to weakening of individual tree species, with dieback gradually (fir) or are attacked by pests and diseases (elm, spruce, oak, etc.). The results of Runov and Egorova (153) support this theory. They reported that (i) Soil in oak forests is more toxic for some microorganisms and plants, including oak field or meadow soil; (ii) Toxicity of soil under oak stands is stronger in older stands (the most toxic soil was found in 220 years old oak stand); (iii) Toxicity of soil in old oak stand reduced the growth of oak plants and together with other factors can prevent THE natural regeneration of oak; (iv) Under low soil moisture conditions, the toxic substances from root secretions and aboveground parts accumulate, however, in higher soil moisture these are decomposed and leached out; (v) Microorganisms play major role in decomposition of toxic substances. Baraneckyj and Ivchenko (6) and Baraneckyj (5) recorded similar accumulation of toxic substances from oak trees in the soil and their negative effects on the growth of oak trees.

2.5. Autotoxicity

An important regulator of stand structure is the autotoxicity of tree species secretions. Negative effect of plants of one species on soil cultivated in one place for a long time and problems connected with this issue are summarized in the monograph 'Allelopathic fatigue of soil' by Grodzinskii (53). The authors assumed that soil fatigue as a cyclic phenomena owing to multifarious factors:- (i). Unilateral development of soil micro flora, (ii). Allelopathy, (iii). Accumulation of toxins in the soil, (iv). Unilateral uptake of nutrients from the soil, (v). Development of pathogenic microorganisms, other pests and weeds, (vi). Change of pH and (vii). Disturbed soil structure.

2.5.1. Forestry: In forestry tree growth duration is much longer, hence, little data are available. Ivanov (65) evaluated the results of soil fatigue and possibility of autotoxication of plants by their own excrements. After the clear-felling of spruce stand, the soil had more biologically active compounds than in the soil of spruce stand. By removing the spruce stand, total microbial activity of soil was increased (92).

2.5.2. Agriculture : In agriculture soil fatigue is well known and connected with allelopathy (12,53,54,126,183). In agriculture, crops yields decrease the after one year continuous culture on the same field. The reduction in seed germination was 62 % in sugar beet and 67% in wheat (55). The seed germination of sugarbeet dropped from 74% to 33%, when sugar beet was cultivated on the same field for > 6 years. The species of soil organisms decreased by 5-15 times due to accumulation of toxic substances in the soil.

2.6. Root Exudates

Matveev (110) reported the effects of root secretions of tree species depending on soil moisture. Researches has been done on various ecologically active substances, their activity and survival in soil and also analyses of basic plant nutrients (29,32,44,158). The development of microorganisms in rhizosphere is regulated primarily by root exudates (150,182). However it is impossible to determine, whether they are active secretions or secreted by passive diffusion. Substantial nutritional substances and growth factors are also found in root secretions. Besides being a source of energy, they inhibit and stimulate the biochemical activities of microorganisms and enhances the spores germination of micromycetes. They influence the relations between the microorganisms colonizing the rhizopshere by exerting the selective effects on certain species. These relations can be used to increase the and improve the plant yield. Community of microorganisms in rhizosphere can be modified by secretions to favourably influence the growth, development and health of plants. On the other hand, microorganisms in rhizosphere produce various very active metabolites, which greatly influence the plant nutrition (macro and microelements). In addition, they produce growth substances (auxins, kinetins, gibberellins, ethylene, etc.), vitamins, amino acids, peptides, polysaccharides, and also various toxins. These substances may have stimulatory or inhibitory effects on the growth and development of plants. Tulemisova (181) reviewed the metabolites of rhizosphere micro flora. The changes in composition of root secretions of common pine with temperature and their effects on forest ecosystem are also important.

2.7. Tree Secretions

Soviet scientists also studied the tree species secretions. For example, Kaverzina, Prokuskin (1981) described the annual quantitative as well as qualitative changes in the composition of secretions of pine depending on the trees age.

2.8. Volatiles

Fedorovova *et al.* (1983) studied 31 compounds in volatile substances from Siberian larch, of which 25 were different terpenes constituting 84-90% of all volatile substances. The amount of these substances in the air during the day depended on the place of growth. The amount of volatile substances is directly proportional to the amount of secreted etheric oils, which are produced in low, medium and high mountain climatic zones. It appears in oxidization of air, there is about 13 mg of O_2 in 1 m^3 of low forest, 20-28 mg in medium forest and 35-45 mg of O_2 in 1 m^3 of air in high mountain climatic type of larch forest (42). Keiichi (77) determined the daily and seasonal variability in content of isoprene in secretions of oak.

2.9. Microorganisms

There are many microorganisms, which can fix atmospheric nitrogen or carbon in soil, while some microbes can make bound phosphorus available to plants. Silicate bacteria, owing to their unusually strong fermentation system, recover the silicon from sand and other minerals and use them in their own metabolism. At the same time, they release potassium and phosphorus for plants. The complicated interrelations in forest ecosystem are evident from the fact that there are about 50 000 microbial species. Actinomycetes comprise of about 600 species, which produces more than 3000 kinds of antibacterial substances. Till now these relations and composite compounds of microorganisms have not been properly examined. Secretions of fungi are even less examined and explained. There are amazing findings that secretions of truffle contain α and β androstenoles, ketone and androsterone, which affects the human sexual pheromone (aphrodisiac) and similar compounds are also found in parsnip and celery. In forest ecosystem these organisms secrete ecologically active substances and energy into the environment. The organisms modify these substances or influence each other.

2.10. Forest Protection

Several authors studied the possibility of using ecologically active substances of plant origin in forest protection. These substances act as attractants in insects, bactericidal effects against pathogenic microorganisms or fungi (59,165). The substances used in attracting or repelling insects have been sudied. Substances such as pheromones of insects aree used in forestry (184), to (a) Find population density and flight of certain pests and prognosis of their occurrence for future, (b) Attracting insects of one sex to same place, where individual can be killed directly or sterilized to reduce their population; (c) Controlling the pests by eliminating the communication between females and males. Males lose their orientation, if the territory is sprayed with pheromone. Fertilization will not occur and female will produce non-fertilized eggs, thereby reducing the population of pests. Besides, several substances are used as insect growth regulators are called juvenile hormones. These substances stop the sexual development of insect, which become incapable of reproduction. Zumr (197) reported that when pheromone Pheroprax was used against bark beetle *Ips typographus,* its predators were attracted. During the two years of study (1980-1981) 2279 predators (Coleoptera) were trapped in 65 traps. Repellence of some tree species can be used to control insect pests in tree stands. For example juniper's (*Juniperus communis* L.) high concentration of α- pinene, β- pinene, terpinene and limonene repels the bark beetles at 2% and 3% concentrations. While other compounds (camphene, borneol, nundecan, cedar oil, dipentene, heptane, methanol and butanol) were repellent in all concentrations. Wilde (189) and Novak (120) saw great scope of such substances in insect control. Many authors had advised the use of allelopathy in forest insect management (21,24,28,193).

Allelopathy in forest protection explains the interactions between tree stand and pests, that may increase the resistance in tree stands against pests. When tree species are attacked by pest, the trees mobilize their own defensive power by changing its own chemistry, to repel the insects and other pests.

Allelopathy of vertebrates in forest (mainly deer) is an unexplored area. In Polana

region and other parts of Slovakia, young conifers suffer great damage in winter, from intensive browsing by roe deer and red deer. Young artificial plantations are most damaged, but nearby growing young fir trees from natural regeneration, were not damaged. Allelopathic effects of tree species on red and roe deer can explain this phenomenon. Firs from natural regeneration secrete repellent substances, contrarily firs from artificial regeneration, attracts the deers for their delicacy. These observations are important for reforestation, regeneration and protection of forest. Connolly *et al*. (35) showed that substances of plant origin has great role in forest protection against red deer. Nesciarovic *et al.* (1981) reported that natural regeneration of spruce was more resistant and more vital than artificial spruce plantation.

The above facts reveals the immense role of allelopathy research in forest ecosystems, to understand the allelopathic interactions to manage forests for sustained high production and to conserve the basic ecological principles.

3. COMMON EXPERIMENTAL RESEARCH METHODS

The allelopathy Research methods used for woody species forest ecosystem are as under.

I. Allelopathic interactions between the woody species and individual components and adjacent forest ecosystems

(i). Tree–tree, (ii). Tree–shrub, (iii).Tree–ground flora (herbaceous cover), (iv). Tree–grass and agricultural plants (agroforestry), (v). Tree–microorganisms, phytopathogenic and mycorrhizal fungi, (vi).Tree– insects.

II. Allelopatic influence on different development stages of forest trees:

(i). Seed germination (germination, energy of germination), (ii).Seedling growth (height, diameter, weight of biomass, length and form of root, size of leaves), (iii) Forest regeneration, (iv). Young plants and (v). Adult trees-Biometrical quantitative and qualitatively indicators, tree vitality, health conditions, length of life act.

III. Allelopatic influence on different parts of trees

(i). Leaves, (ii). Branches, (iii).Stem and barks, (iv).Roots, (v).Flowers and fruits, (vi).Litter and (vii). Secretions of healthy and damaged parts of trees

IV. Properties (attributes) of allelopatic substances (matters)

(i). ***Physical properties***

(a). Volatile substances, (b). Liquid, or water soluble percolated substances and (c). Solid substances

(ii). ***Chemical properties and composition:*** (a). Phenolic compounds, (b). Mono and sesquiterpenes and (c). Alkaloids

(iii). ***Functions:*** (a). Defense matters - immunized (toxic, repellent), (b). Attacking matters – used for attack, (c). Regulation matters, (d). Communications matters, signal, or informative matters, (e). Trophic matters, (f). Sexual matters

(iv). ***Effects:*** (a). Attractants, (b). Repellents and (c). Toxic matters
(v). ***Origin of matters***
(vi). ***Allelochemicals concentrations in water, air, litter and soil***
(vii). ***Mechanism of action of allelochemicals in forest allelopathy***

VI. Allelopatic influence on different levels

(a). Ecosystems (natural till artificial- man made), (b). Population, (c). Species, (d). Individuals, (e). Individuals part, (f). Organs, (g). Tissue and (h). Cellular

VII. Allelopatic influence on natural and managed ecosystems.

(i) Primary and secondary succession.
(ii) Forest dynamics, forest regeneration, structure, ecological stability, forest protection, biodiversity.
(iii) Ecophysiological and physiological aspects of tree allelopathy (effect on photosynthesis, respiration, nutrition, vitality and other physiological functions of tree organs, tissues and cells.)

3.1 Collection, Preparation, Leachate/Extract preparation

Collection, Preparation, Leachate/Extract preparation has been described BY Jacob *et al* (67). The reliability of results depends on several factors viz., collection and preparation of plant samples and preparation of leachate/extract. These factors are further influenced by several sub-factors (temperature, composition, time of samples collection). The sample collected should be truly representative of the plant material.

3.1.1 Collection of Plant Material

Collect the plant part, whose inhibitory/stimulatory effects are to be studied. The method used for plant part collection differs with the plant part.

(i). Fresh leaf: Collect the leaves directly from the trees. While collecting from fully mature trees, select leaves from different parts of tree (lower, middle and top portions) to get a representative sample of the entire tree canopy. Besides, also consider the tree age. Leaves that are dead, in senescent stage and ready to shed should be avoided. The condition/ age of the leaf used, should be described; leaves of trees at different stages of degradation i.e. decayed, brown, green leaves have been used in bioassays (172). Bioassays with fresh leaves of varying age's viz., juvenile (15-20 days of age), mature (40-50 days) and senescent (showing signs of chlorosis) have also been reported (86). Carrying the sample in a paper bag prevents or slows down the microbial growth. Polyethylene bags at higher temperatures results in water condensation and favours microbial growth. The quick transport of collected plant material to lab and its storage in refrigerator (-4^0 C) are necessary, if used for bioassays.

(ii). Leaf litter: Collect the leaf litter by placing suitable litter traps erected on supports beneath the canopy of trees (Figure 1) This will prevent the litter from coming in contact with soil and its subsequent decay. Collect normally shed leaves only. Artificial drying of leaves by cutting the branches to hasten drying is not recommended.

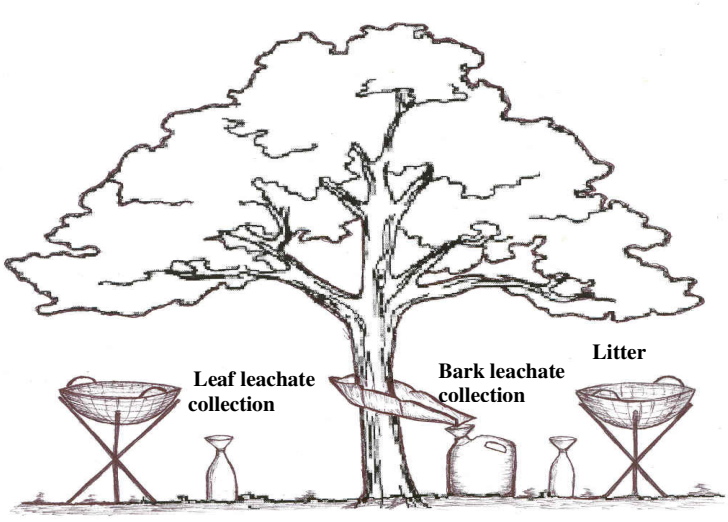

Figure 3. Method of collection of leaf litter, leaf leachate and bark leachate from trees

(iii). Bark: Collect the bark by scraping from the intact mature trees. The naturally flaked dry pieces of bark have also been studied (73).

(iv). Root: Collect the fresh roots from the field by cutting from the trees.

3.1.2. Preparation of Plant Samples

(i). Remove contaminants like soil etc. from the plant sample, which may influence the chemical composition of extract/leachate. Carefully remove the adhering soil/dust particles by dry wiping or with a soft brush. If not, wash gently with tap water only for few seconds, followed by quick rinsing bath in distilled water. Thereafter dry by shaking vigorously with hand and then wipe it gently dry with clean cloth or absorbent paper.

(ii). If fresh material is to be used, the sample should be extracted/leached immediately. Otherwise, dry it quickly to minimize enzymatic changes and respiratory losses. As the material could be of varying moisture content, it should be air dried (in shade) to uniform moisture content. Alternatively, when fresh material is used or comparison will be made between different parts with varying moisture content (e.g. leaf, bark, root), the moisture content in the material should be estimated and accounted during the leachate preparation. To avoid errors in results due to variation in water content, it is necessary to express results on dry weight basis (drying at 100^0C for 8 h) so that bioassay results can be explained quantitatively (116).

(iii). If the material is to be stored for later use, store it in polyethylene bags in refrigerator at 4^0C. This will prevent fermentation of the sample. It is important to consider that refrigeration alters the permeability of plasma membrane and allows the release of

allelochemicals from the intact leaves (163). While storing for dry grinding, transfer the shade dried material to paper or nylon bags. Alternatively to hasten drying, the material can be dried at 60^0C till constant weight in a stainless steel oven.

3.1.3. Preparation of leachate/extract

I. LEACHATE: It is prepared from the intact plant part (without its destruction). It will contain only easily soluble/releasable low molecular weight compounds.

(i). Solvent: To determine the presence of bioactivity, the method for leachate/extract preparation should be similar to natural conditions. In nature, allelochemicals are released in to the environment in water-soluble form. Therefore, the distilled water (ambient temp) should be used as solvent to ensures natural release of chemicals (164).

(ii). Dilution: Prepare aqueous leachate by soaking the fresh leaves/leaf litter/bark/roots in distilled water in 1:10 (w/v) ratio. This weight/volume ratio of 1:10, results in low osmolality, hence, inhibitory/stimulatory effects observed can be attributed to allelochemicals (147). Other ratios (higher or lower viz., 1:5, 1:15) can be tested to explore management/practical utility options (weed management with higher concentrations and alleviation of inhibitory effects through dilution).

(iii). Natural leachate collection: To simulate the natural conditions, leachates of leaf and bark can be collected directly from the field (66). In such cases, assessment of leachate concentration may not be possible. However, they can be used to simulate the field conditions.

- **(a). Leaf leachate:** Collect through fall (rain falling through the canopy), using a gauge designed for the purpose. It consists of polyethylene funnel connected to a collecting bottle and placed on the ground under the canopy of different trees (Figure 1). Gauges should be kept randomly under various trees of same sp. To prevent spatial variation beneath the tree canopy, change gauges location under each tree at regular intervals. Set up a similar gauge in the open, to collect rainwater, which will serve as control.
- **(b). Bark leachate:** Artificial spraying of bark with water may be done to collect the leachate. However, it is best to collect the natural bark leachate during the period of normal rains. Collect bark leachate using plastic collars designed locally and fitted to each tree at 75 cm above the ground. Fix the plastic collar to the trees using coal tar. The stem flow is channeled to jerry cans of 35 L capacity connected with polyethylene funnels (Figure 1). Wash several times the coal tar used for fixing the plastic collars with distilled water to ensure that it is free of any chemical. Use directly the samples collected specifying the time of collection, as leachate obtained after first rain may be more concentrated in chemicals than in subsequent collections. If collected leachates are to be used later, store them in refrigerator.

(iv). Soaking time: Several workers have soaked the plant materials for various periods. In general, soaking the leaves/bark/root for 24 h will leach out most of the allelochemicals.

In case of plant materials having waxy coating, prolong the soaking period. Specify the time of soaking of plant material, as it influences allelochemicals amount that leach out. Different periods of soaking has been tried in allelopathic studies (68). Add bactericides or fungicides to prevent microbial growth during the soaking period.

(v). Filtration: Filter the leachate through muslin cloth/cheese cloth/Whatman No.1 filter paper or via suction through Whatman No. 1 filter paper. Before filtration, it may be desirable to shake the plant material with solvent so that more chemical compounds are extracted in leachate solution.

(vi). Storage: Store the leachate at 7-8^{0}C to prevent decay, breakdown by bacteria (147) and fungal development.

I. EXTRACT: Extract is prepared after the destruction of plant cells by grinding and hence, contains cell constituents. To simulate natural conditions, extract should be prepared in cold water (ambient temperature). Studies have been done with fresh leaf extracts (11), air-dried leaf and litter extracts (91). The method of collection and preparation of the samples are similar to preparation of leachate. To prepare the extract, blend the plant material with distilled water in the desired ratio (w/v) and make desired dilutions in water.

(i). **Fresh leaf extract**: Weigh the sample of collected leaves, chop these into small pieces and homogenize in a mortar with a pestle (do not use mixer or grinder for chopping). Add to homogenized sample, distilled water in ratio of 1:10 (w/v) and mix thoroughly. Keep in dark at room temperature for 24 h. Filter the sample through Whatman No. 1 paper or centrifuge at 3,000 rpm for 30 minutes and use filtrate / supernatant immediately for bioassay. If needed, filtrate could be diluted further to prepare its different concentrations. In some studies, aqueous extract has been prepared by shaking the mixture for two hours followed by vacuum filtration (91).

(ii). **Leaf litter extract**: Dry the collected, naturally shed, leaf litter in oven at 60^{0}C until constant weight. Grind the plant material to powder in a grinding mill, pass through a 0.85 mm net sieve and then mix thoroughly to obtain homogenous sample. Store the powdered material at -20^{0}C until extraction. Weigh the sample of powdered material and add distilled water in ratio of 1:10 (w/v). Keep in dark at room temperature for 24 h. Centrifuge the sample at 3000 rpm for 30 min and use supernatant immediately for bioassay. If needed, dilute the filtrate further to prepare its different concentrations.

(iii). **Sterilization of extracts:** Some allelochemicals present in the extracts can be thermolabile components, so instead of heat sterilization, the extracts should be filtered using millipore filters (0.22 μm pore size filter) that are appropriate to the solvents (131).

3.2. Allelopathy in seed germination and seedlings vigour

Experiment: To assess the effects of aqueous leachates/extracts of tree leaf/bark/root and rhizosphere soil extracts on tree seeds/ seedlings

Mutual influence of tree species is apparent in seed germination. The germination and germination vigour is greatly influenced by above ground tree spp as well as soil extract. Allelopathy is very important for natural tree regeneration especially in natural forest ecosystems.

Konovalov and Luganskaya (87) reported negative effects (50% inhibition) of germinating seeds of pine on germinating seeds of spruce. Germination dropped by 50 per cent. Zaitsev (194) studied the seed germination of locust and maple. Golomedovova and Spiridonova (48) observed, that seeds of pine increased germination by 64.3% with germinating seeds of pea tree. Konovalov and Luganskaya (87) also studied the effect of vegetative organs on seed germination. Twigs of larch inhibited the germination of larch seeds. Only 55% of seeds germinated in comparison with control (without influencing of vegetative organs). Pine twigs stimulated the germination (128%) of larch seeds compared to control. Kalagurka (72) found mutual influences of germinating seeds of pine and hornbeam.

Other researchers studied the effects of herbaceous undergrowth on the germination of seeds of tree species. Strong inhibitory effects to prevent seed germination were reported (47, 49, 61, 74). Rebane (143) studied the effect of quantity and quality of litter on the germination of tree species seeds, similarly also Tkachenko and Kovalenko (175), Grodzinsky (52) and others studied the effect of active secretions from fallen foliage or litter (Zolotuchin, 195; Bojko, 10). Scherbatjuk (157) studied the effects of root secretions on the germination of larch seeds. The root secretions from maple reduced oak germination to 86.6% (57). Norby and Kozlowski (119) describe the results of the effect of extracts from September leaves of various plants on seed germination of pine (*Pinus resinosa* Aiton). Several other authors studied the effects of tree foliage washings on germination of seeds (110, 178,179).

The laboratory, pot culture and field bioassays aims to determine the inhibitory/stimulatory effects of plant-derived solutes from the leaf, bark and root leachate/extract of trees by observing the response that follows its application to test trees. Cited methods were described for allelopathy research in agroforestry (67). Bioassays are more sensitive to measure the response of germinating seeds or seedlings to allelochemicals and are the first step in allelopathic research. Separate experiments can be undertaken to examine the inhibitory/stimulatory effects of the leachate or extract of leaf (fresh and dry), leaf litter, bark and root or rhizosphere soil extracts.

The shoot and root macerates of tree species significantly influences the seed germination and seedling vigour of other tree species.

a) Materials and methods

Petri plates, plant part (leaf/bark/root/leaf litter), paper bag, polyethylene bags, seeds of test trees, filter paper, distilled water, Wareing blender, pH meter, Refrigerator, Stainless steel hot air oven, Glass bottle, Funnel, Jerry can, litter trap, Wooden poles, Coal tar, Electronic weighing balance, Measuring cylinder, Erlenmeyer flask, ethanol, detergent, sodium hypochlorite, mercuric chloride, centrifuge, agar, cloth, absorbent paper**,** mortar with a pestle, 2 mm sieve, millipore filters autoclave, aluminium foil, Millimetric ruler, Vapour pressure osmometer.

Procedure and observations

All experiments used seeds of woody species and followed the seed testing rules in forestry.

While comparing the lowest and highest germination energy - vigour in experiments to assess influence on spruce and pine seed of water macerates from vegetative organs of spruce, pine, fir, larch, beech, oak and locust almost 137.7% variation were found. Minimal spruce seed germination energy was in oak extract (54.2%) in comparison with 100 per cent in pure water. When comparing the germination energy of spruce seeds as influenced by the macerate from spruce twigs with macerates from other tree species, high inhibition, by oak extract (21.6%) and stimulation by beech extract (30.2%) was observed.

Germination and germination energy of pine seed was not influenced as much as germination especially in spruce. In such experiments extracts were replaced on the 9^{th} day. In several cases an abrupt reduction or increase of germination occurred. It is inferred that with daily replacement of extracts the effects would be more marked, as extracts contain easily volatile compounds and their effect changes easily.

Scherbatjuk (157) studied the effect of root secretions of various plants on the germination of larch seeds.

Certain tree species containing terpene, bitumen and tannic acids are harmful to plants when present in greater amounts (78, 79). Most of tree species and their bark are not obviously toxic and can be safely used in plant cultivation provided necessary amount of nutrients is supplied and soil is not too acidic (1). Baumann (7) suggested that tannic acids affect growth of plants. Tannic acids in bark after 6 month composting was nil (135). The effect of substances extracted from spruce bark by cold water on germination of seeds was verified during germination of the seed of spruce and lettuce (171). The reduction of germination was found to vary with fresh bark macerate. At a concentration of 1000 g of bark per 1 l of water the reduction was almost 40 per cent. The effect of macerate from seasoned and composted bark was in all cases negative, but, statistically insignificant.

A. **Petri plates:** Select Petri plates of uniform diameter for all treatments. To eliminate bacterial and fungal contamination, the best and most dependable method of sterilization is autoclaving at 120^0C and at 15 atmosphere pressure. If autoclave is not available, pressure cooker can be used. Sterilization may also be done in hot air oven but at a higher temperature (160^0C) and for more time (2 hours).

B. **Seeds sterilization:** The largest danger to the bioassay is from the pathogens-contaminating seeds. The bioassay conducted under conditions favourable to the development of these microorganisms, may have a negative effect on both seed germination and seedling development and thereby may change the bioassay results. Select uniform healthy seeds of test trees and surface sterilize with 0.1% (w/v) mercuric chloride for 5 min or 0.1 % sodium hypochlorite (Wilson, 1976). The sodium hypochlorite is preferred. Place the seeds in Erlenmeyer flask containing water solution of 95 % ethanol with 0.1 % detergent (v/v) and shake vigorously for 1 min. Allow seeds to settle and replace the ethanol solution with 0.1 % sodium hypochlorite. After 30 min, rinse seeds several times with sterile water to remove the sodium hypochlorite residues and then dry in the sheet of filter paper.

C. **Media and germination method:** The most frequently used medium for bioassays is filter paper. However, if the seeds of test plant require a longer time for germination, filter paper is unsuitable, as the addition of large quantity of extract may lead to flooding of seeds. The alternative is agar medium, which prevents the occurrence of anaerobic conditions and simultaneously minimizes seed dehydration. Add dry agar to water in beaker and mix (when preparing for example 1% agar medium, consider the final volume after addition of extract). Heat the beaker on hot plate and boil the mixture to dissolve the agar. Pour into a flask and autoclave. Allow the medium to cool to 60^0C, divide into parts as per experimental treatments. Add suitable amounts of sterile extract in different concentrations and mix. Add equal amount of water (instead of extract) to control medium. When the mediums are cool enough to touch, pour 15 ml into each Petri dish and tilt the dishes to obtain uniform spread. After the medium solidifies, wrap Petri dishes in the aluminium foil till needed for use (131).

The number of seeds used and the amount of leachate to be added will vary with the size of Petri plate. Maintain uniform and adequate moisture in all the treatments, during the study period by adding the leachate/extract on alternate days. The control will consist of Petri plates set up similarly but watered with distilled water. It is important to determine the amount of extract or water supplied to seeds, to avoid growth inhibition caused either by anaerobic conditions due to an excessive amount of the applied solution or medium dryness arising due to deficiency of solution. Correct the osmolality of the distilled water used in control, if needed, by adding safe solutes to replicate the extracts/leachate's osmolality (64,186).

i. (**Temperature and Light conditions**: Incubate the Petri plates at 20 or 25 ± 1^0C in BOD Incubator or in germination chambers for winter and summer test spp. respectively. Before beginning a bioassay, it is necessary to determine thermal and light conditions, under which it will be done. Temperature affects the rate/speed of germination and therefore maintain optimum temperature during bioassay. Sometimes alternate temperatures are used during day and night. Most seeds germinate well in the light or dark, but many plant species require light for germination. This is particularly true for small-seeded plants such as annuals. When such species are test plants, the incubators should be equipped with low intensity cool-white fluorescent tubes that are switched on during the day. However, even the germination of light requiring seeds can be inhibited, if they are exposed to continuous high intensity light. To avoid water losses by evaporation, maintain optimum humidity in incubator. If placed in the laboratory under open conditions, note down the ambient temperature and relative humidity (131).

ii. **Replications**: Replicate the treatments and control sufficiently, observing statistical principles. Avoid pseudo replication.

iii. **Germination:** Record the germination on the days specified for each test trees and other observations at end of the bioassay. Take measurements when inhibitory or stimulatory effects are maximum.

b) Observations to be recorded

Leachate/ Extract

(i). pH of leachate/extract
(ii). Osmolality of leachate/extract
(iii). Allelochemicals content in fresh leaf/bark/leaf litter/root/soil used
(iv) Allelochemicals content in leachate/extract
(v) Nutrient content of soil extracts

Test Plant

(vi). Germination count
(vii). Germination rate
(viii). Plumule length of seedlings
(vix). Radicle length of seedlings
(vx). Weight of plumule
(vxi). Weight of radicle

Calculations

Simultaneous calculation of several germination indices has been proposed to provide a better interpretation of allelochemical activity on seed germination (31):

Total germination (GT)

$$GT = \frac{NT \times 100}{N}$$

Where, NT = number of germinated seeds for each treatment at the end of assay and N = total number of seeds

Speed of germination (S)

$$S = (N_1 \times 1) + (N_2 - N_1) \times \frac{1}{2} + (N_3 - N_2) \times \frac{1}{3} + ... + (N_n - N_{n-1}) \times \frac{1}{n}$$

Where N_1, N_2, N_3.... N_n = number of seeds germinated at 6 (1), 12 (2), 18 (3)....and (n) hours (days) after the assay beginning.

Speed of accumulated germination (AS)

$$AS = \frac{N_1}{1} + \frac{N_2}{2} + \frac{N_3}{3} + ... + \frac{N_n}{n}$$

Where, N_1, N_2, N_3 , ..., N_n = cumulative number of seeds which germinated on time 1, 2, 3, ... or n, following set up of the experiment.

Coefficient of rate of germination (CRG)

$$CRG = \frac{(N_1 + N_2 + N_3 + ... + N_n)}{(N_1 \times T_1) + (N_2 \times T_2) + (N_3 \times T_3) + ... + (N_n \times T_n)} \times 100$$

Where, N_1 = number of germinated seeds on time T_1, N_2 = number of germinated seeds on time T_2 and N_n = number of germinated seeds on time T_n.

Response Index: The magnitude of inhibition versus stimulation is compared by Response Index (RI) as under:

If T > C then RI = 1 -(C/T)
If T = C then RI = 0
If T < C then RI = (T/C) – 1

Where, T is the treatment mean (number of seeds germinating or mean plumule/radicle length of germinated seeds) and C is the control mean. A negative RI (inhibition) corresponds to the proportional disparity in output (germination or radicle length) of plants in the treatment relative to output in the control. For example, a RI of -0.20 means 20% less germination or radicle growth in the treatment than in the control. A positive Rl indicates stimulation, while negative denotes inhibition (190).

c) Precautions (67):

i. For proper collection of plant samples the time of the day, season, phenological stage of plant growth and plant part are important, hence, these should be recorded. In case of leaf litter, mention the month, year and season of collection.

ii. In perennials, month, year and season (winter, summer, spring, rains) of sampling considerably influences the chemical composition of plants; hence, these must be mentioned (116). Allelochemical content is generally highest in extreme weather conditions i.e. summer and winter, while, it is always minimal after precipitation (rain, dew, fog, snow) particularly after rains due to their leaching losses.

iii. The rationale for using a specific plant part should be mentioned i.e. chemical composition, economic importance and role in agroecosystems.

iv. Remove contaminants like soil etc. from the plant sample, otherwise, these may influence the chemical composition of extract/ leachate.

v. During cleaning, the sample should be in water for minimum period to avoid leaching losses of water-soluble allelochemicals.

vi. Exclude damaged leaves while preparing leachate, to prevent the release of bound internal compounds that would not normally be leached with precipitation (147).

vii. Do not add excess leachate/extract or water to the Petri plates, as it cause water logging, which leads to anaerobic conditions for germinating seeds and rotting of seeds/seedlings.

viii. Do not use unsterilized seeds, as the fungal infection may adversely affect the germination and cause disparity in results.

ix. Ensure that all treatments (including control) receive uniform conditions of light, temperature etc.

x. Control osmolality effects either by using low osmolality extracts/leachates or by adding innocuous solutes to the distilled water used in control to replicate the extract's/leachate's osmolality (64,186).

3.3 Mutual allelopathic influence of young plants on the growth

Principle

Interrelations appear also with common growth of seedlings of various tree species. After the assessment of two- years long experiments with the seedlings of tree species, locust seedlings had the greatest negative effect on Scots pine, whereas only volatile substances had effect as tree species were growing separately in vegetative pots. Negative effect can be seen the best in graphs, where individual relations are equalled by regression straight lines and curves of polynomials of the 1st and 2nd degree ($y = a_0 + a_1x$ or $y = a_0 + a_1x + a_2x^2$) (???).

Inhibitory effect of volatile substances of aboveground part of locust tree on the seedlings of pine was so strong that at a distance of 15 cm from locust tree, seedlings of pine were not growing.

In natural forest communities interrelations have no significant effect, which was confirmed by the results of research by Lykovova and Matvejev (103). In natural linden oak forest oak, linden and birch have less effect on the growth of young trees of ash, maple and pine. Also Prudic (136) found the effect of linden on the growth of ash in floodplain forests to be insignificant.

a) Materials

Vegetation plots, seedlings of different tree species (Scots pine - *Pinus silvestris* L., Locust tree - *Robinia pseudoacacia* L.), soil, water for watering of plants, equipments (measuring tape, laboratory balance) for measuring height of seedlings, trunk thickness, trunk biomass, root biomass, total biomass, needles biomass.

b) Procedure Change tense as shown below while describing procedure and write step wise procedure as shown

Grow seedlings of different tree species beside each other in vegetation pots (16,17,18,21).

Plant trial plants of acacia around the pine (*Pinus silvestris* L.) plant (from all sides) and in different distances.

In one series of experiment, plant out the plants of pine (*Pinus silvestris* L.) and locust (*Robinia pseudoacacia* L.) in small boxes separately,

In the second experiment plant the test trees together.

c) Observations to be recorded on seedlings/grown up plants of different tree species)

i. Height (bimonthly intervals)
ii. Trunk thickness (bimonthly intervals)
iii. Trunk biomass (at harvest)

iv. Root biomass (at harvest)
v. Total biomass (at harvest)
vi. Weight of the needles

Response Index: The magnitude of inhibition versus stimulation is compared by Response Index (RI) as under:

If T > C then RI = 1 -(C/T)
If T = C then RI = 0
If T < C then RI = (T/C) – 1

Where, T is the treatment mean (number of seeds germinating or mean plumule/radicle length of germinated seeds) and C is the control mean. (190).

d) Results

Interrelations appear also with common growth of the seedlings of various tree species. After the assessment of two year long experiments with the seedlings of tree species it was found that locust seedlings had the greatest negative effect on Scots pine, whereas only volatile substances were effective (had effect) as tree species were growing separately in vegetative pots.

The effect was apparently stronger in case of seedlings and plants. Kolesnichenko (80) reported a negative effect of birch on 3-year-old plants of pine (-19%) whereas pine had positive effect on birch (+9%). Greater negative effect (-36%) was observed with secretions from the leaves of honeysuckle on pine small plants. With application of leaf as well as root secretions average growth of plants was worse by 34% in comparison with control and it ranged from –27% to – 43%. (recast this sentence)

In addition to the negative effects (locust tree on Serbian spruce, larch or Douglas fir) positive effect was also recorded (Douglas fir on height growth of Serbian spruce or Austrian pine on larch). While the growth of spruce and pine close to each other had not marked effect marked effect, spruce growing closely to locust tree, though it had more free space, was almost 10 times smaller than the spruce tree growing alone. The height of spruce grown separately was 10-15 cm while that of the one growing close to locust tree was only 1-3 cm. Separately grown spruce had total weight of 1.0 g, while spruce tree growing close to locust tree had only 0.1 g.

After the fourth year of establishment there appeared mutual effects of tree species on height of pine, linden, oak, locust and birch. The positive stimulatory effect of oak on pine was obvious. While in pine monoculture average height of pine was 88.36 cm, with 25% proportion of oak it was 94.05 cm, with 50% proportion 99.85 cm and with 75% proportion 111.39 cm. Increment or average height of pine trees grown with linden was higher when proportion was less than 25% but decreased sharply when it exceeded 50%.

In the second, drier experimental plot (what reflected also in the growth of plants and weed) the effect of various tree species on pine with their same proportion (were all the trees in same proportion-not clear) was observed. Pine grown as monoculture had average height of 62.2 cm. At 50% proportion of birch, average height of pine increased to 64 cm. Locust tree had a strong inhibitory effect, while with the same 50% proportion it reduced height of pine plants to 47.14 cm. On the contrary, when grown with oak, average

height of pine was 71.9 cm and with linden 85.27 cm. The difference between lowest and the highest value was more than 38 cm..

3.4 Allelopathy in forest regeneration and in nursery

Principle

The experiments of Mang were described by Assmann (3). Pine trees of 180-200 years age and 40 m high with diameter of 80 cm caused reduction of undergrowth forests almost by 61.2%, at the distance of 5 m by 34.6% and at the distance of 5-7 m only by 5.7%. This total reduction of undergrowth increment (for fir by 11%, spruce 27% and pine by 35%) can be explained as due to shadowing. But only 5-13% of total area was shadowed. Listov (1980) stated that the effect of pine trees on regeneration in Archangelsk region of USSR was so strong that there was no regeneration to the distance of 8 m from old trees. Similar phenomena are described in literature also for spruce, larch, maple and other tree species.

In forestry and agroforestry systems, allelopathic interference may result through exudates from root of trees. Studies of allelopathic activity of aggressor plant roots have not distinguished among effects due to phytotoxins originating from root exudates/dead root tissue/microbial rhizosphere products and that due to competition for nutrients. Studies with a “stair step apparatus," have been useful in deriving such information (8,102).

a) Materials

Plastic pots with single hole open in the base fitted with a rubber tube, Container for collection of runoff water, Sand, Nutrient solution, Seedlings of test tree

The effect of parent stand on the germination of spruce and pine seeds was simulated by applying water macerates from vegetative organs. There was no significant difference in germination, for example pine macerate reduced pine seed germination by 5.2%. Greater difference was observd for germination energy. Spruce macerate reduced germination energy of spruce seed by almost 30% compared to normal water. Besides increase in the energy of spruce seed germination as in the case of beech macerate (by 30.2%), pine macerate (by 20%) and fir macerate (by 15%), reduction of energy of spruce seed germination in comparison with spruce macerate was caused by oak macerate (by 21.7%) and locust (by 9.4%).

b) Procedure

Kolesnichenko (80) and co-workers, performed several. Experiments to study the role of allelopathy in growth and nutrition of plants, In 1961 he published interesting results of laboratory experiments with leaf secretions of birch, which increased oak photosynthesis by 16%, but root secretions of birch reduced it by 26%. Locust tree caused similar effects. In linden leaf secretions reduced oak photosynthesis by 8% while root secretions increased it by 9%. Positive effect of secretions from leaves as well as root was recorded for honey locust (by 10 and 8%).

Ecologically active substances of birch reduced total photosynthesis by 21% whereas oak increased photosynthesis of birch by 17%. In 1969, Kolesnichenko reported the amounts of accumulated nutrients in young oak trees influenced by other tree species.

He used radioactive phosphorus P^{32} as indicator. In 1976, he used more advantageous radioactive carbon in form of $C^{14}O_2$ for the same purpose. Spachovovova and Spachov (169) undertook studies based on the experiments of Kolesnichenko but they, besides the experiments in acclimatization chambers with drainage water, also recorded phosphorylase activity. Rink and Van Sambek (148) and Morris and Farmer (113) studied mutual influence on growth of seedlings.

Considerable differences in the growth of tree species (height and weight of plants, increment, weight of assimilatory organs, root, etc.) with common growth in vegetation pots were recorded (96). Gabriel (46) described strong inhibitory effect of chestnut on birch trees planting which died in its vicinity. With regard to the fact that all processes in plants are interrelated, breathing, transpiration, composition of water vapours, phosphorus compounds, nitrogen compounds or quality of ferments (83) were observed in the study of interrelations.

With the aim to find the effect of precipitation water falling through crowns of tree species on the growth of 2 years old plants, atmospheric precipitation was excluded and water macerates were applied i.e. by water in which we macerated for 18 hours foliaged twigs of pine, fir, spruce, larch, beech, oak and locust, the plants of the same tree species, which were growing in sand, soil and peat. Macerates were examined for pH, amount of evaporation residue**,** phosphorus, potassium and sodium.

In most cases change of concentration increases differences caused by the kind of macerate (not clear). The greatest differences between increments, due to difference in substrate, are for pine.

Sokolovova (167) described negative effect of parent stand on pine regeneration. Rysin (154) reported about root competition in regeneration. Saljajev (155) recorded reduced (40-55%) biomass in pine regeneration due influence of parent stand. Harborne (58) reported negative effect of sweet gum tree on southern red oak (*Quercus falcate* Michx.). Nesciarovic (114) recorded almost 30% lower biomass production of spruce under parent stand in comparison with open (free) plantation.

Experiment: To assess the allelopathic effects of leachate/extracts of different tree parts on tree growth

Principle

In forestry systems, rainwater passes through the foliage of tree component, leaching allelochemicals and transporting them to the soil and adjacent small, or young trees. Decomposition of fresh leaf lopping of trees applied as mulch or incorporated in the soil, could release toxic phytochemicals. Experiments to evaluate the growth of test trees in pots when irrigated with definite concentration of the aqueous leachate/extract of plant parts could be performed. This approach can be adopted for assessing:

i. Effect of different concentrations (w/v) of leachates/extracts of plant parts of a particular tree on various test trees.
ii. Effect of a specific leachate/extract concentration (usually 1:10 w/v) of leaf/bark of different trees on test trees.
iii. Effect of different time of soaking of leaves/bark of a particular tree or different trees on test trees.

a) Materials

Plastic pots (size depending on test trees) or trays of known dimension, Tree part (leaf/bark), Soil mixture, Distilled water, filter paper, Seeds of test crop, Leaf or bark of test tree, Buckets (for preparing leachate/extract), Blender, Millimetric ruler, pH meter, Vapour pressure Osmometer.

b) Procedure and observations)

Caboun established an experiment to find out the influence of water macerates from vegetation organs of grown-up trees on young plants of forest tree species (16,21,27). Five plants of one tree species were planted in three substrates - sand, peat, soil in circular vegetation vessels of 26 cm diameter. Two years old spruce (*Picea abies* Karst.), pine (*Pinus silvestris* L.), fir (*Abies alba* Mill.), larch (*Larix decidua* Mill.), beech (*Fagus silvatica* L.), oak (*Quercus petrea* Liebl.) and one year old acacia (*Robinia pseudoacacia* L.) plants were used for this experiment. Vegetation vessels were located under a fibreglass cover and watered by a mixture of water macerates from vegetation organs of the same species but grown-up ones (spruce, pine, fir, larch, beech, oak and acacia). For control water which was utilized for preparation of macerates.

The macerates were prepared by maceration of 1 mass amount of phytomass of fresh vegetation organs and little branches to 1 cm diameter which were cut to 4 cm bits in 20 water portions. Maceration time lasted 18 hours.

The vessel plants were watered by two concentrations of macerates: 1:20 and by a half concentration (50%) 1:40. At the same time an experiment was done with pine and oak plants which were watered by macerates of pine and oak which were prepared in ratio 1:10, 1:15, 1:20, 1:30, 1:40, 1:80.

Before the plants were planted their height and diameter of stems and length of roots had been measured. After the first and second year of experiment it means when this experiment ended, the heights of plants, stem diameters, sizes and weights of vegetation organs were measured. The substrates were subjected to analyses at laboratory. Also the macerates, which were used for watering, were delivered to laboratory to be analyzed.

We used 426 vegetative vessels with 2,130 plants of seven woody species. As 9 kinds of watering with two concentrations were used it was impossible to create repeating series and regarding the small sets the experiment was evaluated as an orientation one (16,17,21,26,27).

It is possible to see a lot of combinations between tree species and macerates. If is compare for example increment of spruce plants watered by macerates in concentration 1:20 with the increment of plants, which were watered by other macerates with the same concentration it can be seen that spruce watered by spruce has greater increment than fir or larch but lower than other tree species. From the said is possible assume also interrelations of tree species in ecosystems under certain conditions.

In addition to the effect of macerate on the growth of plants Caboun (14, 15) studied also the effect of watering on soil and the effect of individual tree species on soil. Was found the effect of various concentration for the plants of pine and beech, which we watered by the macerates with concentration 1:10, 1:15, 1:20, 1:30, 1:40 and 1:80 what is relative concentration of solutions 200%, 150%, 100%, (1:20), 75%, 50% (1:40) and 25%.

Obtained results can be used in further research, nursery production and establishment of new forest stands by artificial as well as natural regeneration as well as in tending of young forest stands.

Observations to be recorded

Test Plant

i. Plant height
ii. Biometric observation on growth and yield, depending on the seedlings
iii. Shoot and root biomass

Leachate/Extract

iv. pH of leachates/extracts and water used
v. Osmolality of leachates/extracts and water used
vi. Allelochemicals content in the leachate/extract

Soil

vii. Physicochemical properties (initial) of the soil used [(pH, cation exchange capacity (CEC), texture, water holding capacity, bulk density, porosity)]
viii. Nutrient status (organic matter content, available N, P and K) of the soil used.
ix. Soil moisture content (before start and at fortnightly intervals)
x. Soil microbes (bacteria, fungi and actinomycetes) population (initial, middle and at end of the experiment)

Microclimate

xi. Weather conditions during experimental period

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