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Allelopathic Effect of *Populus Nigra* Bark on *Zea Mays* in Agroforestry Ecosystems

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I. Introduction

The phenomenons of allelopathy were explained where one plant exerts a negative effect on another through the production of germination and growth inhibiting substances. Agroforestry, which involves connecting woody plants with annual or perennial crops or livestock, increases the biophysical and/or socio-economic productivity of an agricultural enterprise (Bansal, 1988). However, farmers have expressed alarm about the harmful effects of trees on cultivated lands and standing crops. Although allelopathy the direct or indirect toxic effect of one plant upon another through the production of chemical inhibitors. Thus, Baker (1966) reported that the root and hypocotyl growth of cucumber seedlings were inhibited by *Eucalyptus globulus* which produces volatile materials. *Eucalyptus* however a potential industrial crop is not being recommended as an intercrop in agroforestry systems (Bansal, 1988), apparently due to the release of inhibitory compounds from the trees (Lisanework and Michelson, 1993). *Eucalyptus* reduces the growth of neighboring crops through the release of allelochemicals (May and Ash, 1990). The release of phenolic compounds adversely affects the germination and growth of plants through their interference in energy metabolism, cell division, mineral uptake and biosynthetic processes (Rice, 1984). Leachates from stemflow and litterfall are responsible for such an effect (Molina *et al.*, 1991). Lisanework and Michelson (1993) reported the the effects of leaf extracts of three Eucalyptus species on four Ethiopian crops. A number of trees do, however, negatively affect performance of crops through allelopathy. These include *Leucaena leucocephala, Populus deltoides, Eucalyptus* and *Acacia* species (Bansal *et al*., 1988; Ralhan *et al*., 1992; Bora *et al*., 1999; Singh *et al*., 1999a,b). Moradshahi *et al*., (2003) found that aqueous extracts of *Eucalyptus camaldulensis* has the potential to suppress growth of *Echinochloa crus-galli, Avena fatua*, and *Rumex acetosella.* Cao and Luo, (2005) reported that aqueous extract from bark and leaf, and volatiles from leaves of *Eucalytus citriodora* showed allelopathic effect on the growth of nine species, including the weeds i.e. *Bidens pilosa*, *Digitarie pertenuis*, *Eragrostics cilianesis*, *Setaria geniculata*, and crops such as corn, rice, cucumber, bean and *Stylosanthes guianensi*. Singh *et al*., (2005) stated that *Eucalyptus citriodora* oil completely inhibited the germination of noxious weed *P. hysterophorus*. Ercisli *et al*., (2005) studied that the Allelopathic effects of *Juglans regia* on yield, growth, chemical and plant nutrient element composition of the *Fragaria ananassa.* Shafique *et al.*, (2007) studied that the effect of aqueous extracts of 8 allelopathic tree species viz., *Accacia nilotica*, *Alstonia scholaris*, *Azadirachta indica*, *Eucalyptus citriodora*, *Ficus bengalensis*, *Mangifera indica*, *Melia azedarach* and *Syzygium cumini* was

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studied on germination of *Triticum aestivum*. Hence, an effort was made to analyze the bark for its allelopathic effect of test crops.

II. Materials and Methods

Plant bark of *Populus nigra* L. 'Italica' was collected from Garden of Government Post Graduate College Mardan, District Mardan, Khyber Pakhtoonkhwa, Pakistan. Plants bark were then washed several time with water and dried in open air and under natural light. Leaf samples were ground and the powdered material were stored in plastic bottles at room temperature. 5g, 10g, 15g and 20g of *Populus nigra* powdered were mixed with 100ml distilled water and left for 24hr, 48hr and 72hr at the room temperature (average during day: 25°C) in dark conditions. Aqueous extract was obtained as filtrate (Figures 1, 2) of the mixture and final volume was adjusted to 100ml; this gave 5g, 10g, 15g and 20g aqueous extract. The extract was considered as stock solution (Figures 3, 4). 05 uniform and surface sterilized seeds (2% sodium hypochlorite for 15 min) of *Z. mays* were kept for germination in sterilized petri-dishes lined double with blotting paper and moistened with 10ml of 5g, 10g, 15g and 20g concentrations of aqueous extracts (Figure 5). Each treatment had 5 replicates (total number of test seeds: 10 x 5 = 50). One treatment was run as control with distilled water only. The petri-dishes were maintained under laboratory conditions (room temperature 25°C at mid day, and diffused light during day). The whole experiment was repeated once (Figure 6). After seven days, the seedling root length (cm), shoot length (cm) were measured (Figures 7, 8) while number of germination percentage, Fresh weight and Dry weight were measured. The data obtained was subjected to three way analysis of variance, Randomized Complete Block Design (RCBD) and the mean values were separated at $P < 0.05$ applying Least Significant Difference Test (LSD).

Figure 1 : Filtration of aqueous extract in lab

Figure 2 : Filtration of aqueous extract

Figure 3 : Stock solution of 5g, 10g, 15g and 20g extract

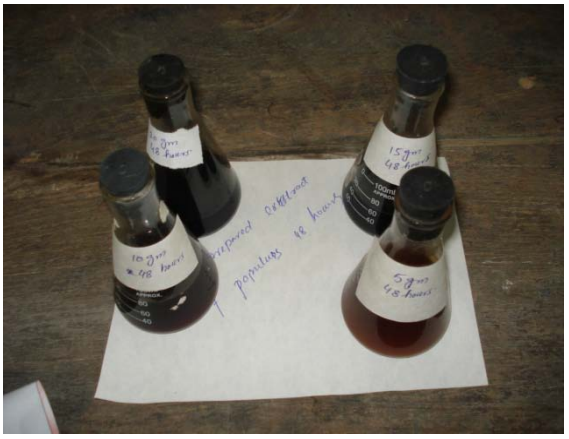

Figure 4 : Stock solution of 5g, 10g, 15g and 20g extract

Figure 5 : Seeds placed in petri dishes

Figure 6 : Seeds soaked in extract

Figure 7 : The germination of seed after seven days

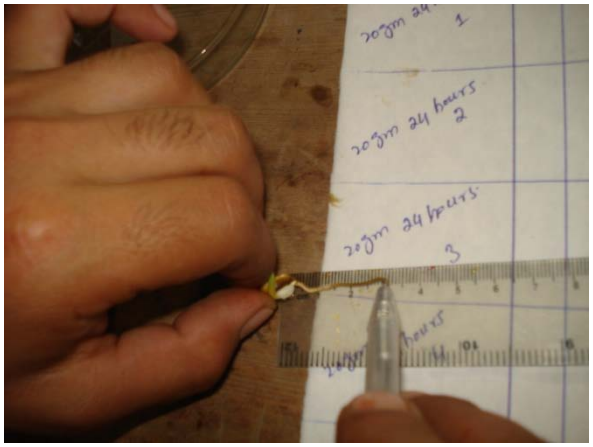

Figure 8 : Measurement of seedling root length (cm) and shoot length (cm)

III. Results

a) Germination

ANOVA (RCBD) (df 1, 56) showed no significant effects of concentration and duration on germination between group as well as within group. The coefficient of variation was found 35.32% for germination (%). (Tables I. a, b).

Table I (a) : Allelopathetic effects of *Populus nigra* bark on germination (%) counts of Maize

Concentration(g)	Duration			
	24h	48h	72h	Mean
Control	100	100	100	100
5	72	64	52	62.66
10	84	60	60	68
15	88	76	68	77.33
20	68	68	56	64
Mean	82.4	73.6	67.2	74.4

Table I (b) : Analysis of variance on germination (%) counts of Maize

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	4	2425.333	541.333	1.2368	0.2978
2	Factor A	4	3538.667	844.667	1.8743	0.111
4	Factor B	2	2738.667	969.333	2.1563	0.1375
6	AB	8	8321.333	1202.667	2.3617	0.0283
-7	Error	56	13674.667	442.762		
	Total	74	38898.667			

Factor A: Duration Factor B: Concentration

b) Plumule Length

ANOVA (RCBD) (df 1, 56) showed significant effects of concentration (F=28.1457) on Plumule length between group while the effect of duration (F=2.4125) showed significant effects within group and between group no significant was found. The coefficient of variation for plumule length was 46.45%. (Table II. a, b).

Table II (a) : Allelopathetic effects of *Populus nigra* bark on Plumule length of Maize

Concentration(g)	Duration			
	24h	48h	72h	Mean
Control	4.28	3.89	4.12	4.1
5	1.86	1.56	1.38	1.6
10	1.24	1.12	1.06	1.14
15	1.34	1.21	0.98	1.18
20	0.94	0.82	0.68*	0.81*
Mean	1.93	1.72	1.644	1.766

**: within groups, +: between groups*

Table II (b) : Analysis of variance on plumule length of Maize

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	4	2.609	2.6077	1.6359	0.1661
2	Factor A	4	52.134	11.036	28.1457	0.0000
4	Factor B	2	0.4235	1.1025	2.4125	0.0000
6	AB	8	3.1734	2.1592	1.4245	0.0622
-7	Error	56	22.491	1.0402		
	Total	74	82.578			

c) Radical Length

ANOVA (RCBD) (df 1, 56) showed significant effects of 5g concentration within group (F=17.2154) and between group (F= 12.8457) on radical length while the effect of 48h duration showed significant effects within group (F=4.8654). For radical length the coefficient of variation was 32.34%. (Tables III. a, b).

Table III (a) : Allelopathetic effects of *Populus nigra* bark on radical length of Maize

Concentration(g)	Duration			
	24h	48h	72h	Mean
Control	17.6	17.6	17.6	17.6
5	3.72	1.14	0.9*	1.92*
10	4.8	3.98	3.24	4.006
15	5.34	3.38	1.54	3.42
20	6.46	3.24	1.68	3.793
Mean	7.584	5.868	4.992+	6.148

Table III (b) : Analysis of variance on radical length of Maize

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	4	25.452	11.348	2.6547	0.0598
2	Factor A	4	2212.2541	634.735	17.2154	0.0000
4	Factor B	2	54.5245	26.977	12.8457	0.0000
6	AB	8	53.2587	12.014	4.8654	0.0000
-7	Error	56	192.2547	5.467		
	Total	74	2856.53			

d) Fresh Weight

ANOVA (RCBD) (df 1, 56) showed significant effects of high concentration within group (F=37.3254) and between group (F=18.5241) on fresh weight while the effect of 24h duration (F=4.6584) showed significant effects within group. The coefficient of variation for fresh weight was 13.21%. (Tables IV a, b).

Table V (a) : Allelopathetic effects of *Populus nigra* bark on fresh weight of Maize

Concentration(g)	Duration			
	24h	48h	72h	Mean
Control	2.954	2.954	2.974	2.961
5	1.785	1.564	1.356	1.568
10	1.654	1.657	1.574	1.628
15	1.745	1.584	1.487	1.605
20	1.324	1.234	1.245	1.268
Mean	1.892	1.799	1.727	1.806

Table IV (b) : Analysis of variance on fresh weight of Maize

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	4	1.685	0.209	2.4325	0.026
2	Factor A	4	8.745	2.462	37.3254	0.0000
4	Factor B	2	2.145	0.558	18.5241	0.0000
6	AB	8	3.548	0.358	4.6584	0.0000
-7	Error	56	3.425	0.163		
	Total	74	18.175			

e) Dry Weight

ANOVA (RCBD) (df 1, 56) showed significant effects of concentration (F=27.5684) within group on dry weight. The effect of duration (F=412.8457) showed significant effects within group while between the group (F=7.76352) significant effect was present. The coefficient of variation of dry weight was 13.31%. (Table V a, b).

Table V (a) : Allelopathetic effects of *Populus nigra* bark on dry weight of Maize

Concentration(g)	Duration			
	24h	48h	72h	Mean
Control	2.448	2.448	2.448	2.448
5	1.985	1.868	1.748	1.867
10	2.157	2.056	1.898	2.037
15	1.85	1.46	1.37	1.56
20	1.716	1.31	1.245*	1.42*
Mean	2.031	1.83	1.741+	1.866

Table V (b) : Analysis of variance on dry weight of Maize

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	4	0.656	1.164	4.1073	0.1622
2	Factor A	4	6.298	2.574	27.5684	0.0000

4	Factor B	2	0.327	1.163	12.8457	0.0000
6	AB	8	2.02	1.253	7.76352	0.0000
-7	Error	56	3.953	0.1253		
	Total	74	12.254			

IV. Discussion

In the present study allelopathic effects of *Populus nigra* bark was observed on germination, plumule length, radicle length, fresh weight and dry weight of *Z. mays*. Treatment with 5g, 10g and 15g extract has increased the germination with time. It is high in 24h treatment while 20g extract treatment has decreased the germination at 72h treatment. Overall 72h treatment decreased the mean germination in all concentration. At very low concentration increased in time has less effect on germination. The result show that at 24h the germination high with increase in concentration whiles at 48h the germination high with increase in concentration except 20g concentration and high duration the germination rate was low. It is evident from the result that higher aqueous extracts concentration of *P. nigra* bark exhibited more inhibitory effects on germination plumule length, radicle length, fresh weight and dry weight of test specie while higher duration present inhibitory effect on Plumule length and radical length as compare to control (Table I - V). The results of our study showed that the bark extracts of *P. nigra* present inhibitory effect in maize. Similar results have been reported by Ayaz *et al*., (1989); Khan *et al*., 2011a,c) and El-Rokiek and Eid, (2009) while studying the allelopathic effect of different plants. They observed that the foliar leachates have been more phytotoxic in nature. Comparative analysis between extracts and duration showed significant inhibitory effect of 48hr treatment on Plumule and radical length. In addition to it, the comparison of duration and concentration showed significant inhibitory effect of 15g concentration in 24hr treatment on fresh weight. The result shows that the inhibitory effects were increased proportionally with the extract concentration and duration. The present findings corroborate the earlier report by Bora *et al.,* (1999) who found that, the inhibitory effect of *Acacia auriculiformis* on germination of some agricultural crops was proportional to the concentration of the extract. Several reports address the allelopathic effect of various plants that significantly affected seed germination and seedling growth of several crops and weed species (Lisanework and Michelson, (1993); Ercisli *et al*., (2005); Shafique *et al.*, (2007), Akhtar *et al*., 2010) these studies showed that the extract of plant species decreased root growth of the majority of the crops. Similar findings were also reported by (Khan *et al*., 2011a,b; Jabeen and Ahmed, (2009) of different trees in common agricultural crops. Some recent studies indicating the phytotoxic/allelopathic effect of aqueous extracts of plants include *Chrozophora oblique* (Khan *et al*., 2011c) and *Rhazya stricta* (Khan *et al*., 2011a,b). All these studies indicated the release of phototoxic chemicals during the preparation of aqueous extracts. Based on this finding, a study was further extended to explore the impact of *P. nigra* bark as they possessed greater phytotoxicity on the emergence and growth of weed plants.

V. Conclusion

The present investigation revealed that its effectiveness on germination and growth suggests that bark of *P. nigra* may act as a source of allelochemicals after being released into soil or after decomposition. The presence of allelochemicals negatively affects the neighboring or successional plants. Further studies are suggested to clarify the possible physiological mechanism related to allelopathic effect on plants.

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