

## SUPPRESSIVE EFFECT OF *POPULUS NIGRA* L. LEAVES ON GERMINATION AND GROWTH COMPETENCE OF *TRITICUM AESTIVUM* L.

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### Abstract

A laboratory experiment was conducted to evaluate the allelopathic potential of *Populus nigra* leaves in different guided bioassay on the germination and growth of four different wheat varieties i.e. *Ghaznavi*, *Siran*, *Atta Habib*, and *Janbaz*. All the extract had differential toxicity against all the four wheat varieties. Hot water extract was observed to be inhibitory than methanolic and rain leachate extract obtained. Furthermore, litter and mulching experiments had also inhibitory effects on the studied parameters. Suppressing effects were linearly correlated with extract concentration. Growth-suppressive effects of the guided-assay were recorded in the order hot water extracts > methanolic extracts > rain leachate extracts. It is suggested that *P. nigra* leaves possess phytotoxic activities, and further investigations are therefore required against challenging weeds. Filed experiments may be carried out to further expand the perceived allelopathic activities of *P. nigra* on other crops.

**Key words:** *Populus nigra*, Allelopathic potential, Toxicity, Wheat varieties.

### Introduction

Allelopathy is a mechanism which describes the exudation of allelochemicals, which are secondary metabolites concerned with detrimental or stimulatory effects on neighboring plant species and other organisms (Callaway & Ridenour, 2004; Muhammad and Majeed, 2014; Khanh *et al.*, 2018). Knowledge about the allelopathic effects of plants is vitally important in agriculture for modification of crop growing practices. Moreover, the inhibitory effects of allelochemicals can be potentially utilized as bio-pesticides, an alternative to conventional pesticides because of their natural origin and less hazardous influence on the environment (Khan *et al.*, 2011).

Poplar (*Populus nigra*) is a commonly cultivated tree in Pakistan on agricultural lands for different purposes. They may often be grown as a shelter belt along cultivated crops in fields. Poplars like many other trees secondary metabolites may possess low molecular weight compounds which are supposed to show phytoinhibitory effects on other plants, fungi, and microbes (Chen *et al.*, 2009). The secondary metabolic compounds are responsible for the allelopathic interference of associated crops in agroforestry systems. *P. nigra* and other plants may possess diverse classes of biological molecules which upon release to their surroundings, can influence different physiological and biochemical aspects of neighboring plants (Zeng, 2008). To investigate the possible involution of allelopathy, laboratory bioassays are the first step to investigate. Therefore, allelopathic phenomenon amongst plants can be exploited by utilizing different laboratory screening bioassays (Fujii, 2003).

Wheat is among the principal grain crops cultivated throughout the world for human consumption, livestock forage, and biofuel. Annual production of wheat in Pakistan is estimated to be 21.8 MT over an area under harvest for this crop 8.4 Mha (Mahmood *et al.*, 2012).

Although greater efforts have been made during the last few decades to enhance the yield outputs of wheat and other crops, weed prevalence and many other biotic and abiotic stresses still pose challenges to such efforts. Since wheat crop and poplar trees are intercropped in several parts of Pakistan, investigation of the allelopathic effects of *Populus* sp. on wheat is necessary for farmers to either grow or not the crop in the vicinity of such trees. The aim of this study was therefore to know the phytotoxic effect of *Populus nigra* on four wheat varieties and to further explore the topic under field condition.

### Materials and Methods

For extract preparation, mulching and leachates experiment and seedling bioassay, methods reported by Hussain *et al.*, (2011) and Muhammad *et al.*, (2018) were followed.

**Effect of natural rain leachates:** Natural rain leachates were amassed by spreading 80 g dried leaves of *P. nigra* in an immense funnel and kept under the natural slow showery rain. For control, rainwater was accumulated directly in the flasks. The accumulation of rainwater (control) or rain leachates (for making test) was commenced when it had rained perpetually for about one hour to eschew aerial contaminants. The funnels containing *P. nigra* leaves powder along with amassing flasks were kept on a one-meter high bench to evade any contamination by splashing water from the soil. This slow shower rain virtually took 24 h during December for amassing ample "Natural rain leachates". Since these rain leachates were quite dilute, consequently a portion of this leachate was concentrated by 5 times utilizing rotavapor at 60°C. Two types of controls consisting of simple rainwater and distilled water control were made to assess the quality of simple rainwater.

**Effect of hot water extracts:** 10, 20, 40 and 80g powder of dried leaves were soaked discretely in 250ml boiled water for 24 hours with infrequent shaking and then subsequently filtered to get aqueous extracts.

**Effect of methanolic extracts:** *P. nigra* leaves powder of 10, 20, 40 and 80g concentrations were soaked discretely in 250 ml methyl alcohol for 72 hours with infrequent shaking and filtered. The filtrate was then dried to concentrated extract on a water bath. The concentrated extract was again dissolved in 250 ml distilled water and was filtered.

**Litter effect:** Litter of rushed leaves of *P. nigra* in different concentration i.e. 2.5, 5.0, 7.5 and 10g and was kept in petri dishes containing single-layered filter paper. A 6 ml of water was poured into each petri dish while small fragments of paper were used as control treatment.

**Mulching effect:** For the mulching experiment, plastic pots of equal size were selected and filled with sterilized sand amended with crushed dried leaves of *P. nigra* (2.5, 5, 7.5 and 10g). Ten seeds of each variety were sown at a suitable depth in mulch-sand. Each treatment was replicated 5 times while sterilized sand served as control.

**Application of the extracts:** All these extracts were utilized against four wheat varieties i.e. *Ghaznavi*, *Siran*, *Atta Habib*, and *Janbaz* in standard filter paper bioassay using petri dishes of the same size. All the treatments were replicated 5 times, each with 10 grains of respective test varieties. Germination and radicle growth were recorded after 96 h incubation at 25°C. The data obtained were statistically analyzed by M.Stat-C software.

## Results

### Effect of rain leachates

**Percent germination:** ANOVA table interprets that varieties (V) and interaction between Treatments x Varieties means were significant. However, Treatments (T) mean were non-significant at  $p < 0.05$  (Table 1). Varietal means showed that germination was maximum (86.67%) was shown in variety *Atta Habib*, followed by *Siran* (78.67%), *Ghaznavi* (71.33%) and *Janbaz* (70.67%). Treatment means indicated that the highest percent germination (79.50%) was observed in distilled water control (DwC) condition, followed by rainwater control (RwC) and rain leachates (RI). T x V interaction denoted that highest germination percentage (94.00%) was found in DwC in variety *Atta Habib*, whereas, minimum germination percentage was observed in RI in variety *Janbaz* and *Ghaznavi* (64.00%) (Fig. 1). It was observed from the data that germination was inhibited by rain leachates.

**Radicle length (mm):** Analysis of Variance for radicle length shows that T and V means were significant, while their interactive effect i.e. T x V mean were non-

significant  $p < 0.05$  (Table 1). Significant inhibition in radicle length was recorded for all the four cultivars at RI treatments. Maximum radicle length (5.68mm) was observed in *Siran*, followed by *Atta Habib*, *Janbaz* and *Ghaznavi* (4.26, 4.07 & 3.32mm) statistically kindred to each other. Treatments mean exhibit that DwC and RwC treatments showed maximum radicle length (4.75mm & 4.57mm respectively), however, rain leachates treatment showed inhibition having a radicle length (3.68mm). According to T x V means maximum radicle length (5.71mm) was observed in variety *Siran* both in DwC and RwC. On the other hand, minimum radicle length was noted in variety *Ghaznavi* (2.06mm) in RI (Fig. 2).

**Plumule length (mm):** ANOVA for plumule length depicted that means value for V and T were significant at  $p < 0.05$ . Though, their interactive effect V x C were non-significant (Table 1). Among the wheat varieties under the study, shoot length varied among treatments such that shoot length of *Siran* was maximum (4.09mm). This was followed by that of *Atta Habib* (3.51mm), *Janbaz* (2.59mm) and *Ghaznavi* (2.24mm). However, T means showed that maximum plumule length (3.88mm) was recorded in DwC, whereas minimum plumule length was recorded in RI (2.71mm) statistically at par with that of RwC (2.73mm). V x I means clearly indicated that highest plumule length (4.72mm) was seen in variety *Atta Habib* (4.72mm) in DwC treatment, while minimum plumule length was observed in variety *Janbaz* (1.93mm) in treatment RI (Fig. 3).

### Effect of hot water extracts

**Percent germination:** Percent germination revealed significant differences among the mean value of different V, C and their interaction at  $p < 0.05$  (Table 2). Among different varieties maximum means germination (62.8%) was recorded in variety *Siran*, followed by *Atta Habib* (50.80%), *Janbaz* (44.00%) and *Ghaznavi* (43.60%). However, among concentration mean the highest percent germination (68%) was observed in control condition, which decreased gradually with increasing concentration i.e., 10g (57.5%), 20g (48%), 40g (47%) and 80g (31%). Interaction of V and C revealed that maximum percent germination (86%) was noticed in variety *Siran* at the control level (Fig. 4).

**Radicle length (mm):** ANOVA table regarding radicle length expressed significant differences among the mean values of C and their interactive effect V x C. However, V means were non-significant at  $p < 0.05$  (Table 2). The highest radicle length (25.65mm) was shown by control level, followed by 10g concentration (18.1mm), 20g (15.895mm), 40g (7.53mm) and 80g (1.27mm) respectively. Moreover, the means value for varieties showed highest radicle length (16.70mm) in variety *Siran*, followed by *Atta Habib* (13.8mm), *Ghaznavi* (13.23mm) and *Janbaz* (10.93mm) with no significant difference in the mean values. V and C showed maximum radicle length (33.8 mm) in variety *Siran* at control level while the minimum radicle length 0.9 mm was noted in *Siran* and *Atta Habib* at an 80g concentration (Fig. 5).

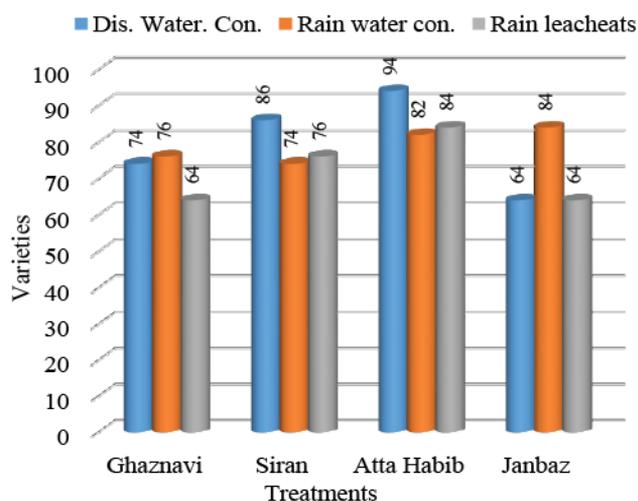


Fig. 1. Effect of rain leachates extract of *Populus nigra* leaves on germination (%) of test varieties. Each value is a mean of 5 replicates, each with 10 seedlings.

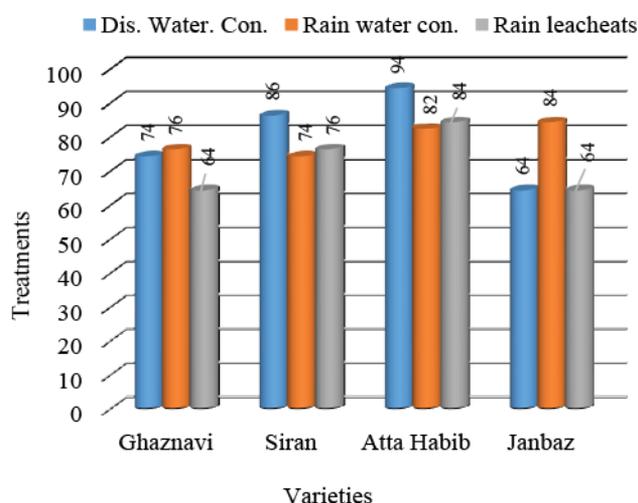


Fig. 2. Effect of rain leachates extract of *Populus nigra* leaves on radicle length of test varieties. Each value is a mean of 5 replicates, each with 10 seedlings.

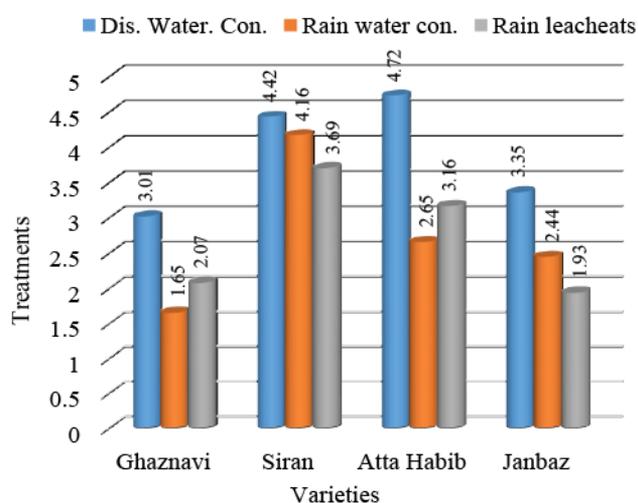


Fig. 3. Effect of rain leachates extract of *Populus nigra* leaves on plumule length of test wheat varieties. Each value is a mean of 5 replicates, each with 10 seedlings.

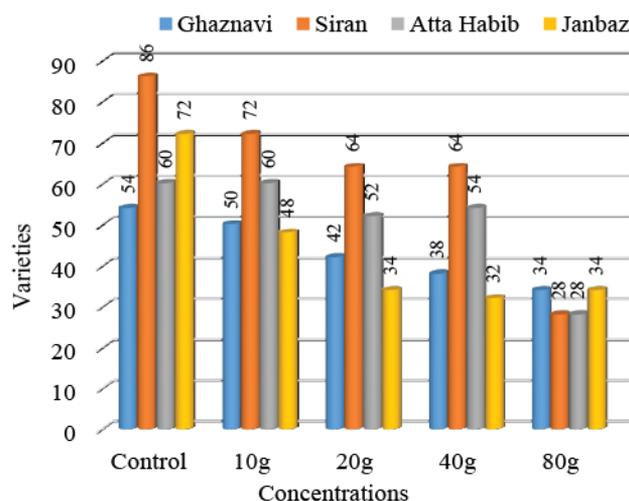


Fig. 4. Effect of hot water extract of *Populus nigra* leaves on germination (%) of test species. Each value is a mean of 5 replicates, each with 10 seedlings.

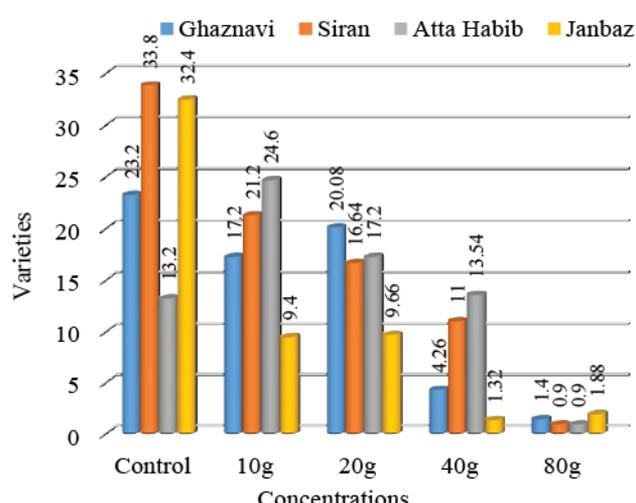


Fig. 5. Effect of hot water extract of *Populus nigra* leaves on radicle length of test wheat varieties. Each value is a mean of 5 replicates, each with 10 seedlings.

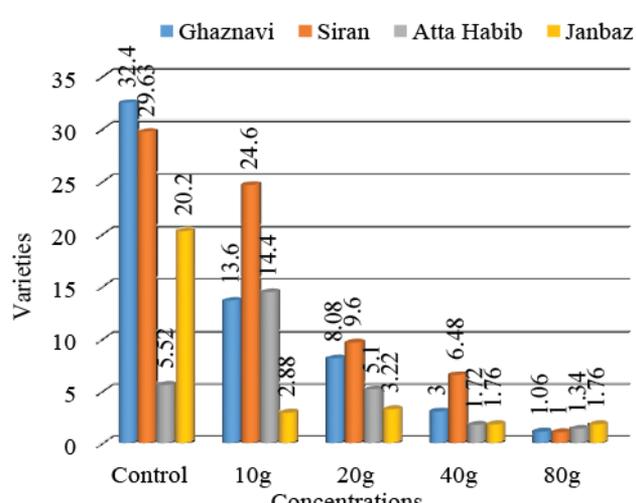


Fig. 6. Effect of hot water extract of *Populus nigra* leaves on plumule length of test species. Each value is a mean of 5 replicates, each with 10 seedlings.

**Plumule length (mm):** ANOVA for plumule length elucidated that there were significant differences among the mean values of V, C, and their interaction i.e. V x C at  $p < 0.05$  (Table 2). The highest plumule length (21.93mm) was recorded at the control level, followed by 10g (13.87mm), 20g (6.5mm), 40g (3.24mm) and 80g concentrations (1.29mm) respectively. Among varieties means minimum plumule length was recorded in variety *Atta Habib* (5.62mm) and *Janbaz* (5.96mm), while *Ghaznavi* (14.26mm) and *Siran* (11.63mm) exhibited highest plumule length. A gradual decrease was perceived in plumule length as concentration increased. The interactions between V and C showed maximum radicle length in *Ghaznavi* (32.40 mm) and *Atta Habib* (5.52 mm) respectively at the control level, while the minimum plumule length was shown by C 80g in variety *Siran* (1.0 mm) (Fig. 6).

#### Effect of methanol extracts

**Percent germination:** Analysis of variance for percent germination revealed significant differences among C means. Moreover, the expedient data for V and interactive effect V x C showed no significant difference at  $p < 0.05$  (Table 3). However, among sundry varieties maximum mean germination was shown in *Siran* (30.80%), followed by *Ghaznavi* (29.20%), *Atta Habib* (28.00%) and *Janbaz* (27.20%). According to C means, maximum percent germination (61.50%) was obtained in control condition, followed by 10g (28.50%), 20g (21.00%), 80g (19.00%) and 40g (14%) concentrations respectively. V x C means revealed that maximum percent germination (68%) was recorded in variety *Siran* at control level (Fig. 7).

**Radicle length (mm):** ANOVA for radicle length exhibited significant differences among the mean values of V, C, and interaction at  $p < 0.05$  (Table 3). The highest radicle length (21.35mm) was shown at the control level, followed by 10g (3.97mm), 20g (3.59mm), 80g (3.4mm) and 40g (1.63mm) concentrations respectively. Moreover, according to V means highest radicle length was shown by *Atta Habib* (9.09mm), followed by *Siran* (7.89mm), *Ghaznavi* (5.12mm) and *Janbaz* (5.09mm). According to V x C means maximum radicle length (33.92mm) was recorded in *Atta Habib* at the control level, while the minimum radicle length (0.83mm) was noted in *Janbaz* at 40g C (Fig. 8).

**Plumule length (mm):** ANOVA showed significant differences among the mean values of V, C and their interaction at  $p < 0.05$  (Table 3). According to C means highest plumule length (15.32mm) was recorded in control, followed by 10g (3.05mm), 80g (3.58mm), 20g (1.81mm) and 40g (1.13mm) respectively. V means showed the maximum plumule length in *Siran* (6.7mm), followed by *Atta Habib* (5.94mm), *Janbaz* (3.79mm) and *Ghaznavi* (3.47mm). V x C mean elucidated the maximum plumule length in *Ghaznavi* (22.50mm) at the control level, while minimum plumule length was recorded in *Siran* (0.60mm) at 40g C (Fig. 9).

#### Effect of litter

**Percent germination (%):** Result exhibited significant differences for C means, while V and Interactive effect of C x V were non-significant  $p < 0.05$  (Table 4). Germination % of all the four varieties was significantly inhibited by the litter of *P. nigraas* compared to controls. Maximum germination % was recorded in control (75%), followed by 5.0g, 10g, 2.5g and 7.5g (32%, 28%, 25% and 24% respectively). According to V mean highest germination % was noted in *Atta Habib* (41.2%), followed by *Siran* (39.6%), *Janbaz* (35.2%) and *Ghaznavi* (32%). Whereas, two-way interaction of V x C exhibited non-significant differences in wheat varieties with the highest germination percentage (92%) in *Siran* at control level (Fig. 10).

**Radicle length (mm):** Data for radicle length revealed that V and C means were significant, whereas interaction means were non-significant at  $p < 0.05$  (Table 4). Among the C means the phytotoxic influence of litter was minimum for control (23.92mm) and extreme for 5g concentration (3.47mm). The highest radicle length was that of variety *Siran* (15.09mm), followed by *Atta Habib*, *Janbaz*, and *Ghaznavi* (9.55mm, 8.56mm, and 6.3mm). The Interaction between V and C were non-significant with highest radicle length (31.98mm) in variety *Siran* at control level and lowest radicle length of 0.7mm in variety *Ghaznavi* at a 2.5g concentration (Fig. 11).

**Plumule length (mm):** Results obtained for plumule length showed a significant difference in C and interactive effect V x C means, while no significant difference was observed in V mean at  $p < 0.05$  (Table 4). However, mean values for concentrations showed that maximum plumule length was noted at control level (21.68mm), followed by 7.5g (5.17mm), 10g (3.71mm), 2.5g (3.65 mm) and 5.0g (2.85mm) respectively. Though, V means showed maximum plumule length in variety *Siran* (10.69 mm), followed by *Atta Habib* (39.60mm), *Janbaz* (35.20mm) and *Ghaznavi* (5.48 mm). V x C mean bestowed that highest radicle length was noted in variety *Siran* (34.2 mm) at the control level, while the lowest radicle length of (1.0 mm) was recorded in C 2.5g (Fig. 12).

#### Effect of mulching

**Percent germination (%):** According to analysis of variance for germination percentage means value for concentration and interaction between concentration and varieties were significant at  $\alpha 0.05$ . Whereas variety means were non-significant (Table 5). Means value for varieties showed that maximum germination was observed in *Atta Habib* (44%), followed by *Janbaz* (42.40%), *Ghaznavi* (42%) and *Siran* (40%) respectively. Concentration means exhibited the maximum germination percentage (54.50%) which was recorded at the control level, followed by 5.0g and 7.5g (44%) statistically similar, 2.5g (42.50%) and 10g (25%). Interaction means explicated that maximum germination % was seen in variety *Janbaz* (62%) at the control level, while the lowest germination percentage was observed in variety *Atta Habib* (10%) at concentration 10g (Fig. 13).

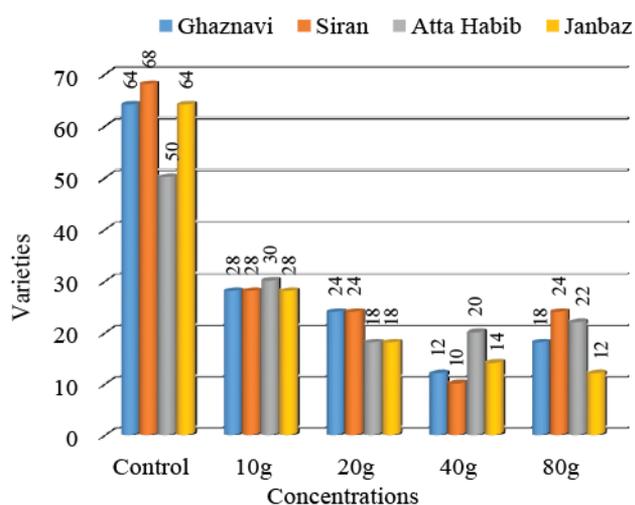


Fig. 7. Effect of Methanolic extract of *Populus nigra* leaves on germination percentage of wheat grains. Each value is a mean of 5 replicates, each with 10 seedlings.

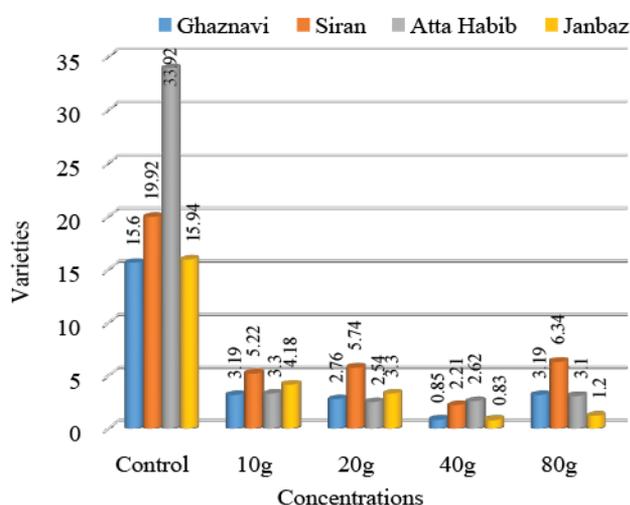


Fig. 8. Effect of Methanolic extract of *Populus nigra* leaves on radicle length of wheat seeds. Each value is a mean of 5 replicates, each with 10 seedlings.

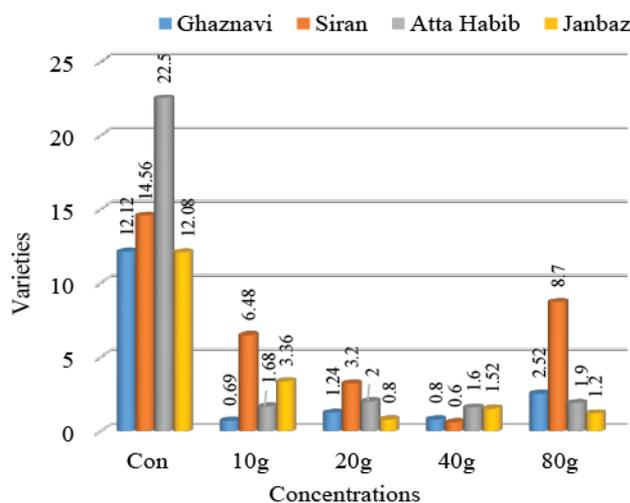


Fig. 9. Effect of Methanolic extract of *Populus nigra* leaves on plumule length of wheat grains. Each value is a mean of 5 replicates, each with 10 seedlings.

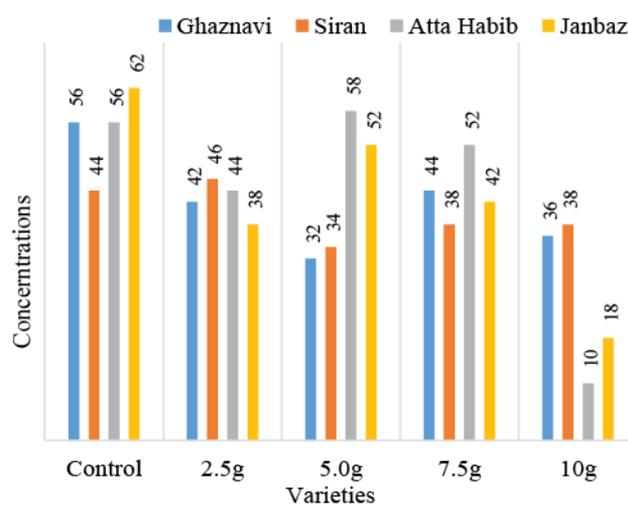


Fig. 10. Effect of Mulching of *Populus nigra* leaves on germination (%) of test species. Each value is a mean of 5 replicates, each with 10 seedlings.

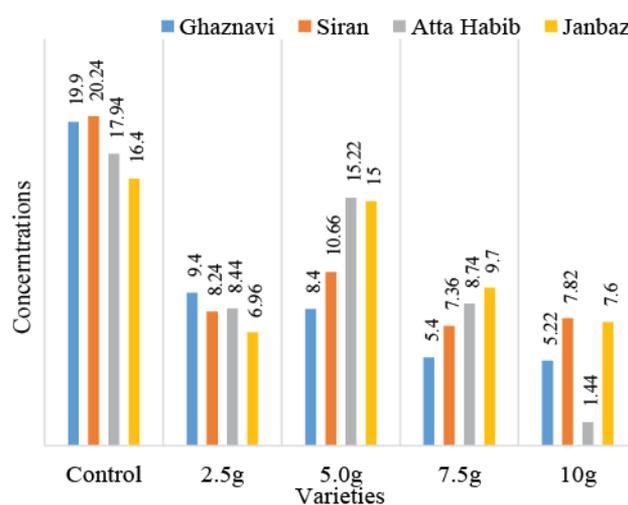


Fig. 11. Effect of Mulching of *Populus nigra* leaves on radicle length of test species. Each value is a mean of 5 replicates, each with 10 seedlings.

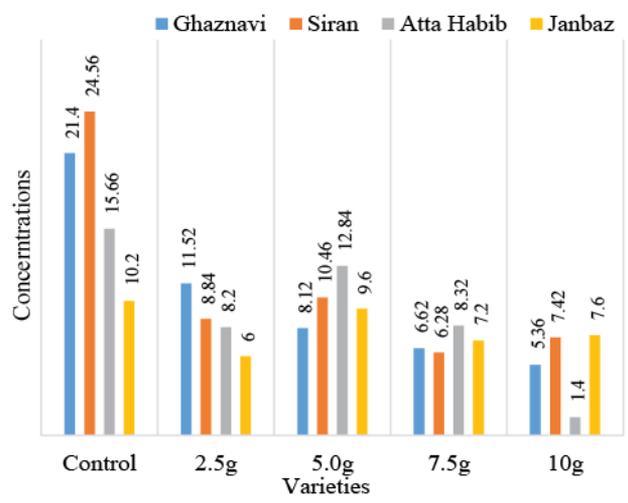


Fig. 12. Effect of Mulching of *Populus nigra* leaves on plumule length of test species. Each value is a mean of 5 replicates, each with 10 seedlings.

**Table 1. Mean squares of the ANOVA for germination (%), radicle and plumule growth (mm) of rain leachates.**

Sources	d.f.	Germination (%)	Plumule growth (mm)	Radicle growth (mm)
Varieties (V)	3	351.667*	8.964*	6.605*
Treatments (T)	4	841.667*	10.840*	14.607*
V×T	12	311.667 <sup>NS</sup>	0.867*	1.133 <sup>NS</sup>
Error	80	120.833	1.721	2.060
Coefficient of variation (%)		14.31%	42.28%	33.13%

d.f. = Degree of freedom, \* = Significant and NS= Non-significant

**Table 2. Mean squares of the ANOVA for germination (%), radicle and plumule growth (mm) of hot water extracts.**

Sources	d.f.	Germination (%)	Plumule growth (mm)	Radicle growth (mm)
Varieties (V)	3	2009.000*	455.540*	141.396 <sup>NS</sup>
Concentrations (C)	4	3769.000*	1445.519*	1797.769*
V×C	12	392.333*	184.964*	197.184*
Error	80	201.000	58.244	75.096
Coefficient of variation (%)		28.19%	81.48%	63.30%

d.f. = Degree of freedom, \* = Significant and NS= Non-significant

**Table 3. Mean squares of the ANOVA for germination (%), radicle and plumule growth (mm) of methanol extract.**

Sources	d.f.	Germination (%)	Plumule growth (mm)	Radicle growth (mm)
Varieties (V)	3	61.333 <sup>NS</sup>	63.151*	101.379*
Concentrations (C)	4	7226.500*	686.785*	1339.035*
V×C	12	137.167 <sup>NS</sup>	39.240*	77.791*
Error	80	274.000	10.211	18.446
Coefficient of variation (%)		57.48%	64.20%	63.18%

d.f. = Degree of freedom, \* = Significant and NS= Non-significant

**Table 4. Mean squares of the ANOVA for germination (%), radicle and plumule growth (mm) of litter.**

Sources	d.f.	Germination (%)	Plumule growth (mm)	Radicle growth (mm)
Varieties (V)	3	438.667 <sup>NS</sup>	130.299*	347.924*
Concentrations (C)	4	9207.500*	1286.130*	1391.995*
V×C	12	302.833 <sup>NS</sup>	85.642*	48.287 <sup>NS</sup>
Error	80	211.500	20.963	58.710
Coefficient of variation (%)		39.31 %	61.81 %	77.55 %

d.f. = Degree of freedom, \* = Significant and NS= Non-significant

**Table 5. Mean squares of the ANOVA for germination (%), radicle and plumule growth (mm) of mulching.**

Sources	d.f.	Germination (%)	Plumule growth (mm)	Radicle growth (mm)
Varieties (V)	3	67.667 <sup>NS</sup>	55.337 <sup>NS</sup>	10.425 <sup>NS</sup>
Concentrations (C)	4	2183.500*	471.268*	531.800*
V×C	12	556.833*	59.260 <sup>NS</sup>	32.058 <sup>NS</sup>
Error	80	225.500	48.354	53.854
Coefficient of variation (%)		35.67%	61.81%	69.86%

d.f. = Degree of freedom, \* = Significant and NS= Non-significant

**Radicle length (mm):** Data for radicle length elucidated that concentration means were significant. On the other hand varieties and Interaction between varieties x concentration means were non-significant (Table 5). According to varieties means all the four varieties responded differently to different concentration of *P. nigra* leaves. A Maximum radicle length (11.13mm) was recorded in *Janbaz*, followed by *Atta Habib* (10.86mm), *Siran* (10.36mm) and *Ghaznavi* (9.66mm) respectively. Different concentrations of *P. nigra* leaves reduced the radicle length of all the four varieties. The Maximum radicle length was observed in control (18.62mm), followed by 5.0g (12.32mm), 2.5g (8.26mm), 7.5g (7.80mm) and 10g (5.52mm). Interaction means exhibited a maximum radicle length (20.24mm) in control in variety *Siran* and a minimum

radicle length was observed in *Atta Habib* (1.44mm) at concentration 10g (Fig. 14).

**Plumule length (mm):** Plumule length showed that concentration means were significant ( $p < 0.05$ ), while varieties and interaction between Varieties x Concentration means were non-significant (Table 5). According to varieties means a maximum plumule length (11.51mm) was recorded in *Siran*, followed by *Ghaznavi* (10.61mm), *Atta Habib* (9.28mm) and *Janbaz* (8.12mm). Maximum Plumule length was perceived in control (17.96mm), followed by 5.0g (10.26mm), 2.5g (8.64mm), 7.5g (7.11mm) and 10g (5.45mm). Interaction means conferred that maximum radicle length (24.56mm) was noted in control in variety *Siran* and minimum radicle length was observed in *Atta Habib* (1.40mm) at concentration 10g (Fig. 15).

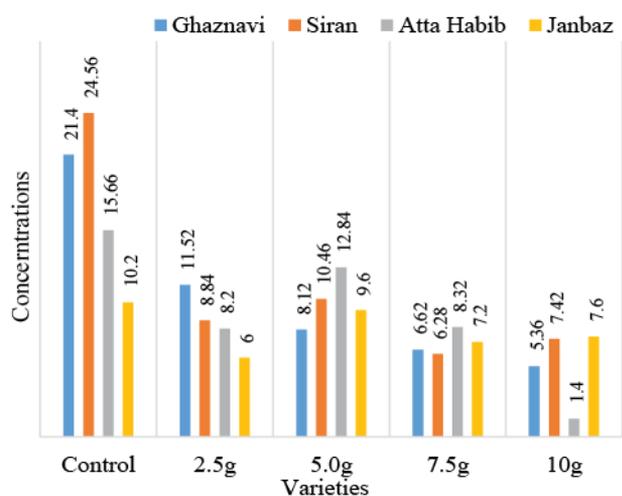


Fig. 13. Effect of litter of *Populus nigra* leaves on germination (%) of test varieties. Each value is a mean of 5 replicates, each with 10 seedlings.

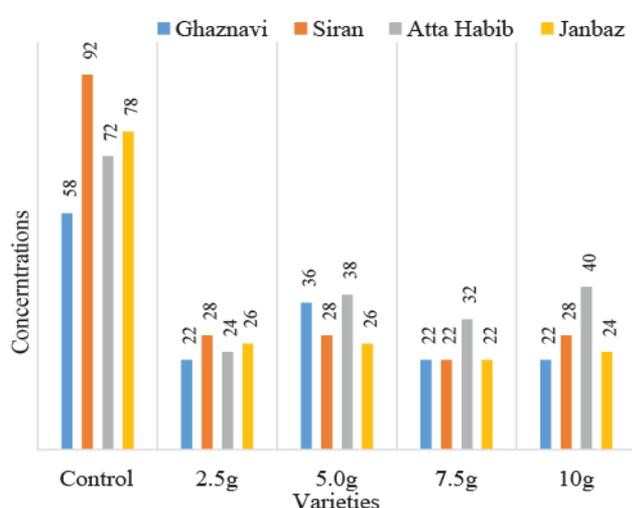


Fig. 14. Effect of litter of *Populus nigra* leaves on radicle length of test species. Each value is a mean of 5 replicates, each with 10 seedlings.

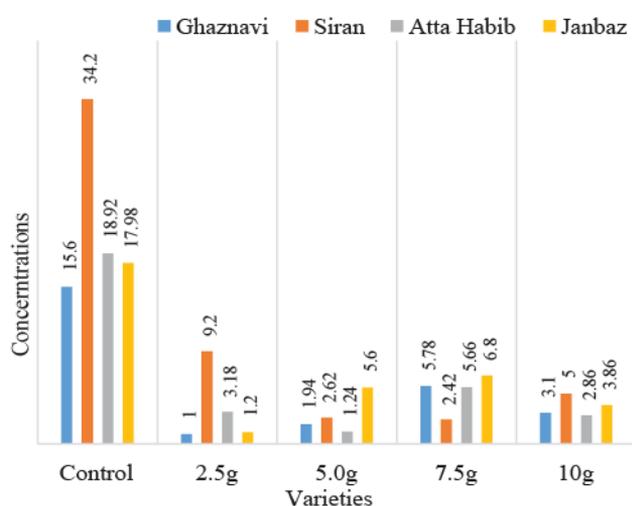


Fig. 15. Effect of litter of *Populus nigra* leaves on plumule length of test species. Each value is a mean of 5 replicates, each with 10 seedlings.

## Discussion

The present study revealed that natural rain leachates suppressed the early seedling growth of wheat varieties *viz.* Ghaznavi, Siran, Atta Habib, and Janbaz, however, the most affected variety was Janbaz. Furthermore, germination and seedling growth of the four above mentioned wheat varieties in DwC and RwC remained unaffected with virtually homogeneous results attesting that rainwater had no inhibitory effects. These findings agree with Hussain (2011), who recorded the inhibitory effects of natural rain leachates from other plants. Rain leachates might be inhibitory to all four varieties of wheat due to the presence of some water-soluble phytotoxins, which are strong inhibitors of germination and seedling growth of vulnerable plants (Hussain *et al.*, 2011). Moreover, these toxins are presumed to delay and retard the division and elongation of meristematic cells (Hussain & Ilahi, 2009) thus, might be responsible for the retarded seedling growth of all wheat varieties in our study.

Hot water extracts from leaves of *P. nigra* significantly reduced growth parameter of tested cultivars. The suppression effect was found dependent on the extract concentration. Findings of Kenany and El-Darier, (2013), Barkatullah *et al.*, (2010) and those reported by Sultana & Asaduzzama (2012) were also in agreement with our results. It was withal discerned that hot water extracts, especially from leaves, had a more inhibitory effect than methanolic and rain leachates extracts. These inhibitory substances are perceived to affect physiological, biochemical and enzymatic activities of the target plants (Majeed *et al.*, 2017; Siyar *et al.*, 2019). It is suggested that allelopathic stress corresponds to the production of reactive oxygen species (ROS) which in turn could modify the antioxidant mechanisms like catalase, superoxide dismutase, and peroxidases and in target plants with subsequent effects on membrane permeability and cell damage (Weir *et al.*, 2004; Cai *et al.*, 2011).

Methanolic extracts also inhibited the test varieties. However, the inhibition was less compared to hot water extracts. This suggested that the toxins present in *P. nigra* leaves were better extractable in hot water than in Methanol and that the sundry toxins were differentially extractable in hot water and alcohol. Our findings are close to those of Kadioglu & Yanar (2004) who reported the inhibition of test species by methanolic extracts. This reduction of inhibitory capability was probably due to a degradation and/or transformation of some allelochemicals phytotoxic compounds (Macías *et al.*, 2008). Mulching effect of *P. nigra* showed significant inhibition on four different wheat varieties. It may be asserted that allelochemicals produced by donor plants gather in rhizosphere to physiologically dynamic compositions that might be responsible for the inhibition effect of wheat (Samreen *et al.*, 2009). In early work, Barkatullah *et al.*, (2010) reported that different concentration of mulch of *D. viscosa* had inhibitory effects on seedling growth of some plants thus confirming our results. Mechanism of allelopathic activity triggers modifications in different processes such as water and nutrient uptake potentials, respiration rate and photosynthesis which may accordingly correspond to

either growth abnormalities or improvement of plants (Gniazdowska & Bogatek 2005). These results were in line with Barkatullah *et al.*, (2010), and Kaul & Bansal (2002), who reported the effect of a litter of some plants as inhibitory to the growth of others.

## Conclusion

From the above results, it might be concluded that *P. nigra* leaves were responsible for the suppressive effect on germination and growth of wheat. Phytotoxic activity of the guided-bioassay was observed in the following order hot water (HwE) > methanolic extracts (MeE) > rain leachate extracts (RI). Further study is needed for identification of phytotoxic substances and it may serve as a potential agent for weed control.

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