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Allelopathic effect of *Populus nigra* L. leaves on physiological parameters, photosynthetic pigment and proline content of common bread wheat (*Triticum aestivum* L.)

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Allelopathy is an important biological process which triggers both healthy and harmful interactions between plants. Understanding of the allelopathic interactions in managed agriculture can help in devising the cultivation of more appropriate crops in fields with respect to allelopathic plants. In Pakistan like other most countries, poplar (*Populus nigra* L.), a winter deciduous tree is either planted in rows in crop fields or as a boundary plantation. The current study was carried out to analyze the phytotoxic effect of *Populus nigra* L. leaves (PNLs) on physiological parameters, photosynthetic pigment and proline content of 4 wheat varieties (*Ghazanvi, Siran, Atta Habib and Janbaz*). Before applying *Populus nigra* L. leaves (PNLs) powder soil sample was analyzed for major and minor element i.e. pH, EC, Phosphorous, organic matter, total nitrogen, potassium, sodium and calcium + magnesium. The pots containing 10 seeds each of test species was separately treated with *Populus nigra* L. leaves dry powder taken in different concentration (10, 20, 40 and 80g). Results revealed that *Populus nigra* L. leaves have a significant effect on wheat species even at a lower concentration. The tested wheat varieties responded negatively at 10, 20, 40 and 80g leaves concentrations in terms of germination, growth, and yield parameters. Photosynthetic pigments and proline content of wheat were also affected by senescence poplar leaves even at lower concentration. GS-MS phytochemical screening revealed a total of 51 different bioactive compounds in leaves which were predominantly phenols. The study suggests that intercropping of poplar along with wheat may be chosen carefully as wheat is the major dietary staple and *Populus nigra* L. contain different allelopathic compounds with potential inhibitory effects.

Keywords: photosynthesis, chlorophyll pigments, allelochemicals, wheat, yield

INTRODUCTION

Allelopathy is generally perceived as the negative effect of chemicals released by one plant species (donor) on the growth and development of another (recipient) although in some cases the responses may be stimulatory (Inderjit and Callaway 2003). In case of a negative effect, it becomes biotic stress that can be termed as allelochemicals stress (Romero et al. 2002), where these allelochemicals negatively affect the growth and development of other plants. However, the mechanism of mode action of these allelochemicals to suppress the growth and development of other plants are still not clear. Allelopathy, in general terms, refers to any direct or indirect impact of plants on other plants through the release of allelochemicals and assumes an imperative role in many agro-ecosystems (Kohli et al. 1998), Singh et al. 2001) and Latif, et al. 2017)

Allelochemicals refer mostly to the secondary

metabolites produced by plants and are by-products of primary metabolic processes (Levin, (1976). They have an allelopathic effect on the growth and development of the same plant or neighboring plants. The term allelochemicals include, (a) plant biochemical that exert their physiological/toxicological action on plants (allelopathy, autotoxicity or phytotoxicity), (b) plant biochemicals that exert their physiological/toxicological action on microorganisms (allelopathy or phytotoxicity) and (c) microbial biochemicals that exert their physiological/toxicological action on plants (allelopathy and phytotoxicity). Secondary compounds are metabolically active in plants and microorganisms, their biosynthesis and biodegradation play an important role in the ecology and physiology of the organism in which they occur (Waller and Dermer (1981).Some of them are accumulated at various stages of growth, while accumulation of some compounds depends upon the time of day or season. According to Chon & Nelson (Chon and Nelson, (2010), allelopathy has a significant effect on weed management, crop establishment and protection. Latif et al. (2017) also asserted that allelopathy plays an important role in weed management, plants' defense and crop development.

Triticum aestivum L., common or bread wheat, is a member of Poaceae family is one of the top two cereal crops grown in the world for human consumption, and also significant portion of it is used for livestock forage and biofuel. Pakistan is an agricultural country and wheat is one of its major crops that was cultivated on an area of 8.0339 m ha during 2002-2003 with grain production of 19.183 m tons with average grain yield 2388 kg ha-1 (Anonymous,2008).

In Pakistan wheat is being grown in association with the boundary plantation of *Populus nigra* L. The genus *Populus* belongs to family Salicaceae which includes more than hundred species distributed in temperate and subtropical regions. This species have been grown as a boundary or block plantation that improves the physicochemical properties of soil through the addition of organic matter in the soil (Coleman et al. 2004 and Singh and Sharma, (2007).and provides an alternate source of income and employment (Balooni, K. (2003).and Puri, and Nair (2004).

However, some adverse effects have also been reported by various workers (Kohli et al. 1997 And Nandal and Dhillon, (2007). In our previous studies by Inayat et al. (2020) and those conducted by Khan et al. (2016) growth suppression of wheat and maize seedlings was observed when the plants were treated with extracts of *Populus nigra* L. A literature survey indicates that allelopathic effects of *Populus nigra* L. on physiological and biochemical attributes of wheat has not been carried out. Therefore, the objective of this study was to evaluate the effects of aqueous extracts of *Populus nigra* L. on some physiological and biochemical traits of four wheat cultivars in pot culture. Moreover, GC-MS screening was carried out to identify phytochemicals in the leaves of *Populus nigra* L.

MATERIALS AND METHODS

Seeds of 4 wheat (*Triticum aestivum* L.) varieties i.e. *Ghazanvi, Siran, Atta Habib and Janbaz* were obtained from The University of Agriculture Peshawar, Pakistan.

Donor Plant collection Site Introduction

Populus nigra L. (Fig. 1) leaves were collected from District Charsadda in October 2019. District Charssada covering an area of 96 sq km lies between 34-03′ and 34-28′ north latitudes and 71-28′ and 71-53′ east longitudes. The climate of Charsadda district is extreme. The summer season persists from May to September. June is extremely hot and dry when the temperature rises to over 10 Degree centigrade. July to September is the monsoon months. The months of July and August are hot and humid. The turn of season comes in October and up to mid-November it is cool and pleasant. There are two spells of rainy season in a year. The winter rainfall, due to western disturbances, shows a high record during the months of March and April. The highest summer rainfall is in the month of August. The average winter rainfall is higher than that of summer rainfall. Charsadda is being close to Peshawar. A table showing the mean maximum and minimum temperatures, precipitation and humidity recorded at Peshawar is given below:

Collected leaves were dried at room temperature (25±2ºC) for 2 weeks. The dry leaves were then crushed and powder was obtained. Hundred Pots of 58 x 28 cm were taken each filled with equal amount of loamy soil prepared by mixing clay, loam and farmyard manure in the ratio of 3:1:1. Before applying *Populus nigra* L. leaves (PNLs) powder soil sample was analyzed for major and minor element i.e. pH (6.14), EC (3.00 ds m^{-1}), Phosphorous (73.6 mg/kg), organic matter (3.48 %), total nitrogen (0.28%), potassium (1136 mg/kg), sodium (330 mg/L) and calcium + magnesium (27.5 mg/L).The powdered materials were applied to pots, watered in 1st week of November and was left for 2 days. Ten seeds each of wheat variety *Ghazanvi*, *Siran, Atta Habib and Janbaz* were equidistantly sow in pots after 2 days. Each treatment was replicated 5 times. The pots were kept under uniform open environment and irrigated with tape water when needed. Data for germination, vegetative, growth and yield parameters were collected analyzed.

Table1: Month wise temperature, Precipitation and Relative Humidity %

Mean Temperature (^{0}C)				
Month	**Maximum**	**Minimum**	**Precipitation (Millimeters)**	**Relative Humidity (%)**
January	18.3	4.0	26.0	58.6
February	19.5	6.3	42.7	57.5
March	23.7	11.2	78.4	58.4
April	30.0	16.4	48.9	51.7
May	35.9	21.3	27.0	37.3
June	40.4	25.7	27.7	36.2
July	37.7	26.6	42.3	55.0
August	35.7	25.7	67.7	64.6
September	35.0	22.7	17.9	58.7
October	31.2	16.1	9.7	54.9
November	25.6	9.6	12.3	60.1
December	20.1	4.9	23.3	63.7
Annual	**29.4**	**15.9**	**403.8**	**54.7**

Source: Data Processing Centre, Pakistan Meteorological Department, Karachi

Figure 1: Poplar-wheat based intercropping in District Charsadda, KPK, Pakistan

Figure 2: *Populus nigra* L.

Young leaves of Wheat species were collected for chlorophyll and proline test. The final data and harvesting of wheat was done in last week of April.

Estimation of Chlorophylls and Carotenoids

Chlorophyll and carotenoid contents of wheat leaves were determined spectrophotometrically. Pigments in sample were extracted with acetone-hexane (4:6). Absorbance of acetone extract of wheat leaves were recorded at 663nm, 645 nm, 505 nm and 453 nm for chlorophyll a, chlorophyll b, and carotenoids i.e. lycopene and β- carotene, respectively. From these values contents of chlorophyll a, chlorophyll b, and carotenoids were analyzed by via the anticipated equations:

Total chlorophyll content

Chl a content (mg_{chl}-g_{FW}^{-1}) = $A_{663} - A_{645}$

Chl b content (mg_{chl}-g_{FW}^{-1}) = $A_{663} + A_{645}$

Carotenoid content

Lycopene ($mg\mu g_{Lycop}.g_{FW}^{-1}$) = $A_{663} + A_{645} + A_{505} - A_{453}$

β- carotene ($mg\mu g_{Car}.g_{FW}^{-1}$) = $A_{663} - A_{645} - A_{505} + A_{453}$

(A=Absorbance at 663nm, 645 nm,505 nm and 453 nm)

Proline Content:

Proline content were measured using ninhydrin reagent. A 0.04g dry weight of samples was homogenized with 3% aqueous sulfosalicylic acid and placed at room temperature for 72hr to stimulate the release of proline. The sample was then centrifuged at 3000 ×g for 20 min. Supernatant were mixed with equal volume of acetic acid and ninhydrin boiled for 1hr and the absorbance at 520nm were determined by photo spectrometer. Proline content were measured using standard curve of Pure Proline

Statistical Analysis

The design of the experiment was randomized complete blocks, in five replications. All the results were statistically analyzed by using MSTATC program.

GC-MS Column Chromatography Fractionation

For column chromatography 1.0 kg of silica gel was dissolved in CH_3Cl and was put into the column (75 mm diameter). The column was washed with chloroform and the bubbles were removed. For slurry preparation 20 g of silica gel was uniformly mixed to a solution of 20 g of methanol fraction of *Populus nigra* L. leaves extract in 30 mL methanol, dried and powdered in mortar. The slurry was added to column and subjected to silica gel column chromatography using a chloroform/methanol (A- 100% chloroform, B- 98:2, C- 95:5, D- 85:15, E- 80:20, F- 75:25, G- 70:30, H- 65:35, I- 55:45, J- 45:55, K- 30:70 and M- 100% methanol (v/v) as the elution solvent. The resulting fractions (200 Ml) were applied to thin-layer chromatography (TLC). The chromatogram was visualized under ultraviolet (UV) light. The sub-fractions A (1-10), C (24-32), D (84-92) and M (232-257) showed noticeable bioactivity in the wheat germination and seedling growth and were chosen for further isolation. But no compound was isolated, and the fraction obtained were screened using GC-MS.

RESULTS

Allelopathic potential of *Populus nigra* L. leaves powder on wheat germination was studied with respect to wheat's physiological and biochemical parameters.

Physiological responses of wheat to allelopathic stress

At different concentration of *Populus nigra* L. plant material inhibition or reduction of the germination, growth and yield components of wheat occurred.

Percent germination

All the four wheat varieties exhibited varied inhibition ratio towards PNLs powder. Regarding to concentration means highest percent germination (81.0%) was observed in 80g concentration, followed by 10g powdered conc. (73.5%), 20g powdered conc. (72.0%), 40g powdered conc. (69.5%) and control level (68.5%). Maximum inhibition to seed percent germination was observed in variety *Ghaznavi* (64.0%), followed by *Atta Habib* (70.4), *Janbaz* (71.2) and *Siran* (86.0%) respectively (Fig 01).

Plant height

Plant height was significantly effected in all the varieties. The highest plant height was detected in 20g concentration (79.77 cm), followed by 10g concentrations (79.71cm), control (77.99cm), 80g concentrations (77.78 cm) and 40g powdered concentrations (75.19 cm). Maximum plant height was recorded in *Janbaz* (83.438 m) followed by *Ghaznavi* (79.328), *Atta habib* (76.934m) and *Siran* (72.65m) (Fig 02)

Number of tillers plant^{-1}

All treatments significantly affected the number of tillers per plant in all four varieties. The highest number of tillers were found in control (3.465**),** followed by 40g (3.445), 10g (3.1575), conc. 80g (3.05) and conc. 20g (2.78). Variety *Janbaz* (3.42) was less affected having maximum number of tiller followed by *Atta habib* (3.272), *Ghaznavi* (3.13) and *Siran* (2.896). (Fig 03).

Flag leaf area

All concentration significantly reduced flag leaf length in all the four varieties of wheat. Maximum flag leaf length was found in variety *Ghaznavi* (36.3), followed by *Janbaz* (35.566 cm), *Atta habib* (29.892 cm) and *Siran* (29.682 cm). However, according to

treatments maximum leaf area was found in conc. 10g (33.2775), followed by conc. 20g (32.785), conc. 40g (32.165) and conc. 80g (32.09) as compared to control i.e. (33.9825) (Fig 04).

Number of leaves per plant

Number of leaves plant^{-1} in all varieties was significantly affected by all concentration of *Populus nigra* L. leaves powder. The highest number of leaves plant^{-1} was observed in control level (Kohli et al. 1997 and Inayat et al. 2020) followed by 20g concentration, 80g concentration 10g concentration and 40g concentration (13.57, 13.26, 13.01 and 12.38 respectively). In accordance with varieties, the highest number of leaves plant^{-1} was viewed in *Atta Habib* with mean value 14.31, followed by *Janbaz* (Kohli et al. 1997and. Balooni, K. (2003). *Ghaznavi* (13.80) and *Siran* (10.86) (Fig 05). .

Reproductive Growth Parameters

Days leading to spike initiation

Significant effects were observed at all the concentrations in all the four varieties on days leading to spike emergence. The highest days leading to spike appearance was attended by 10g concentration (76.80), followed by 20g concentration (76.7), 40g concentration (76.15), control level (76.0) and 80g concentration concentrations (74.95) with the difference being significant. With the change in varieties, the days leading to spike appearance was also changing i.e. *Janbaz* with 77.36, *Ghaznavi* with 76.64, *Atta Habib* and *Siran* with 75.44 and 75.04 means values respectively. Day to spike appearance decreased when extract concentration increased. The result showed that 10g leaves powdered caused delay in spike appearance (Fig 06)

Days leading to spike completion

Extract concentrations had significant effects on days to spike completion in all varieties. Moreover, concentration means elucidated that maximum number of days taken to spike completion were showed in 80g concentrations (14.7), the minimum numbers of days taken to spike completion were seen in 20g concentration i.e. 12.2. Maximum numbers of days taken to spike completion were noticed for *Siran* (14.2), while minimum days were recorded for *Ghaznavi* (12.6) which was statistically similar to *Janbaz* (12.88). There was a significant increase in number of days taken to spike completion as concentration increased in different varieties (Fig 07).

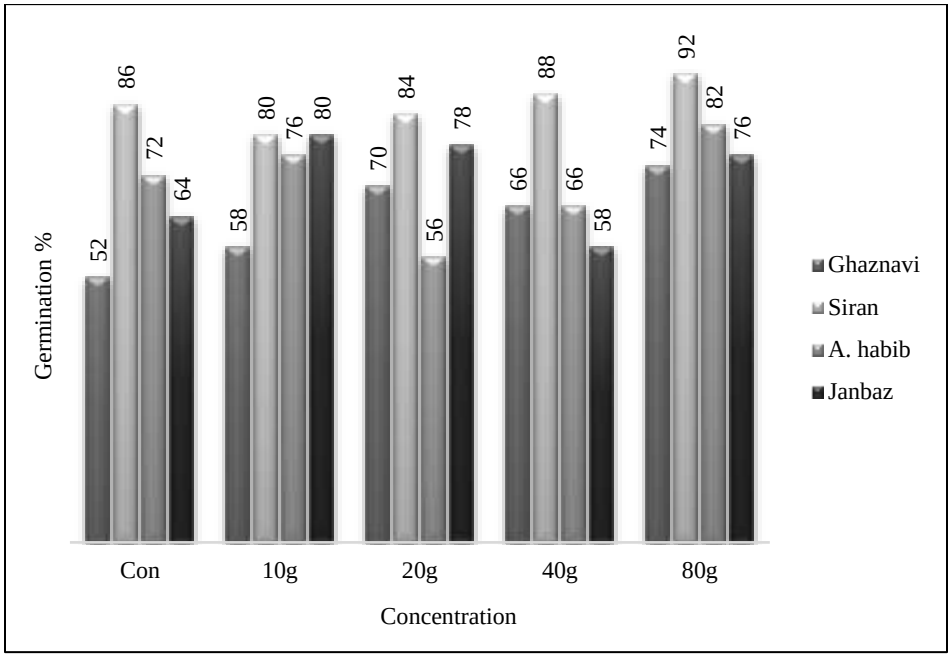

Figure 1: Effect of *P. nigra* leaves on germination percentage of test species. Each mean value is a result of 5 replicates.

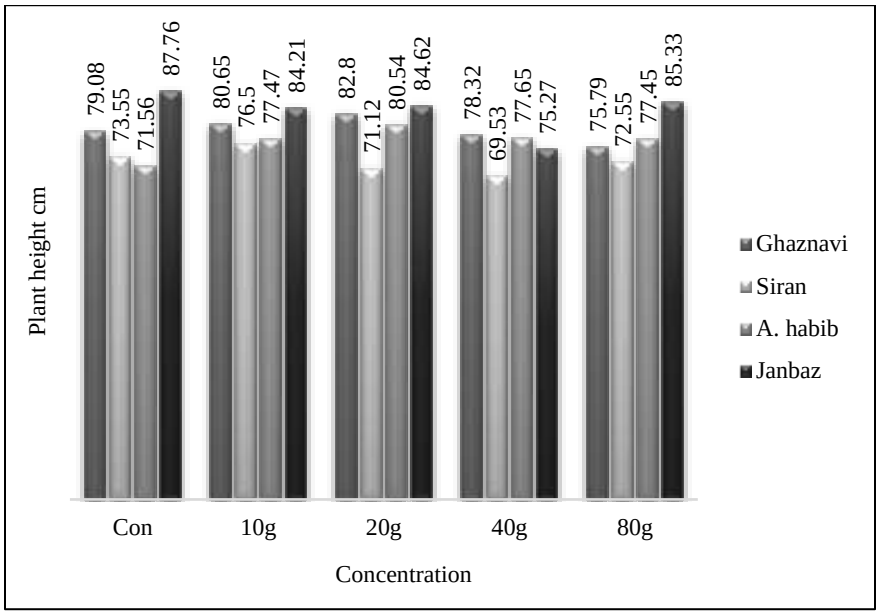

Figure 2: Effect of *P. nigra* leaves on plant height of test species. Each mean value is a result of 5 replicates.

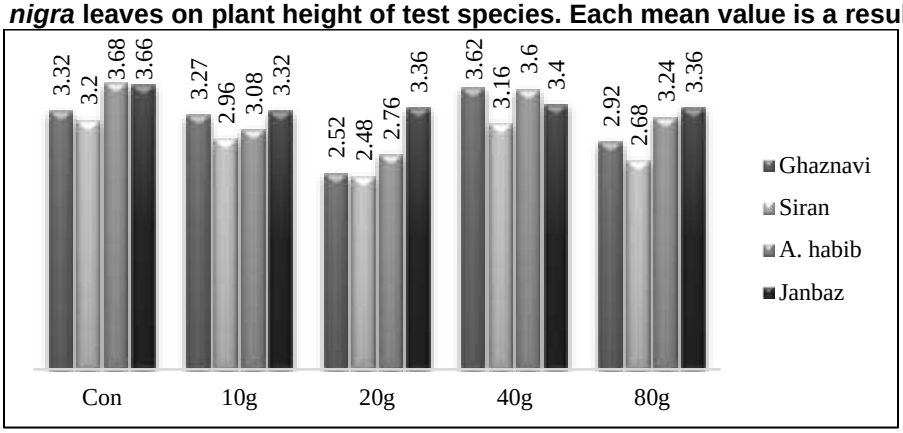

Figure 3: Effect of *P. nigra* leaves on number of tillers plant^{-1} of test species. Each mean value is a result of 5 replicates.

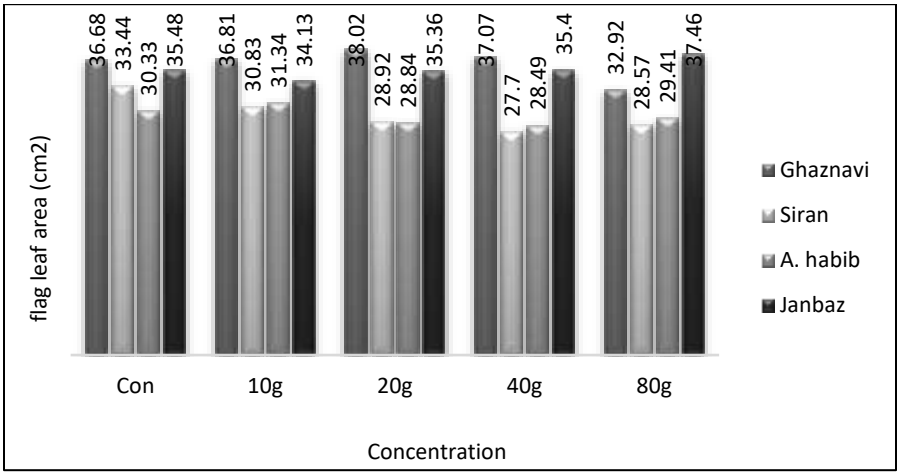

Figure 4: Effect of *P. nigra* leaves on flag leaf area (cm^2) of test species. Each mean value is a result of 5

replicates.

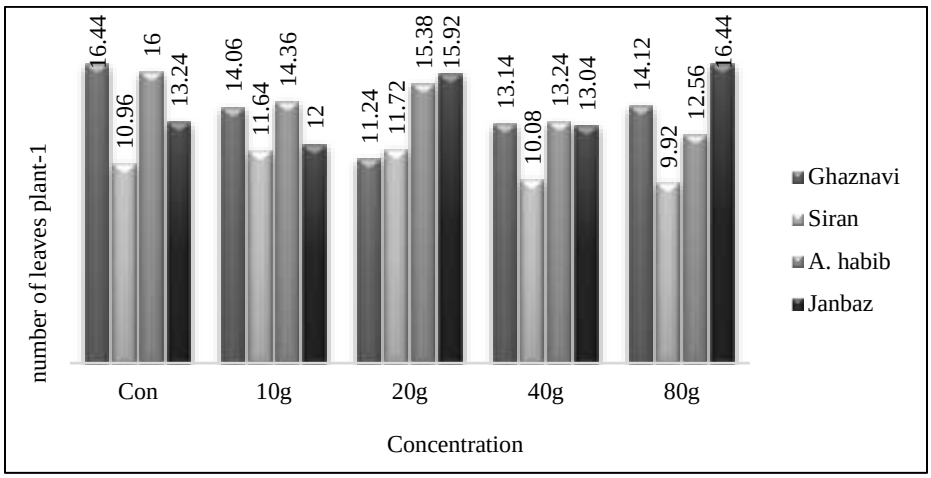

Figure 5: Effect of *P. nigra* leaves on number of leaves plant^{-1}of test species. Each mean value is a result of 5 replicates.

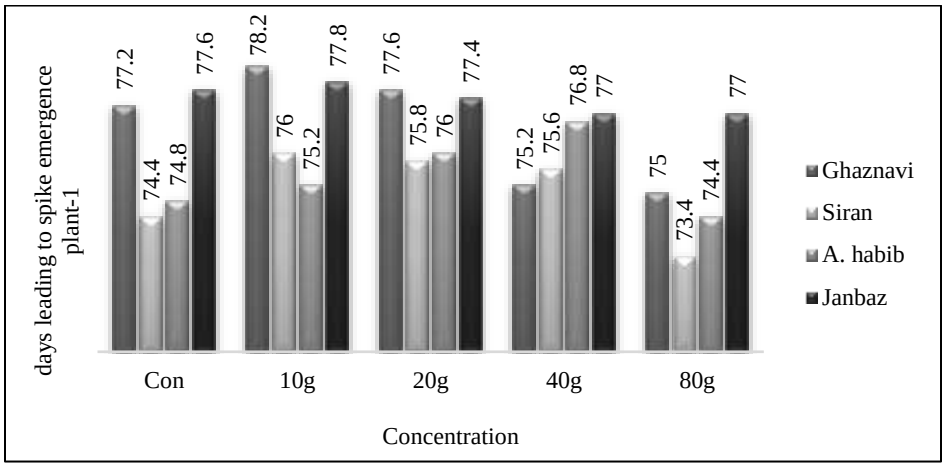

Figure 6: Effect of *P. nigra* leaves on days leading to spike emergence plant^{-1} of test species. Each mean value is a result of 5 replicates.

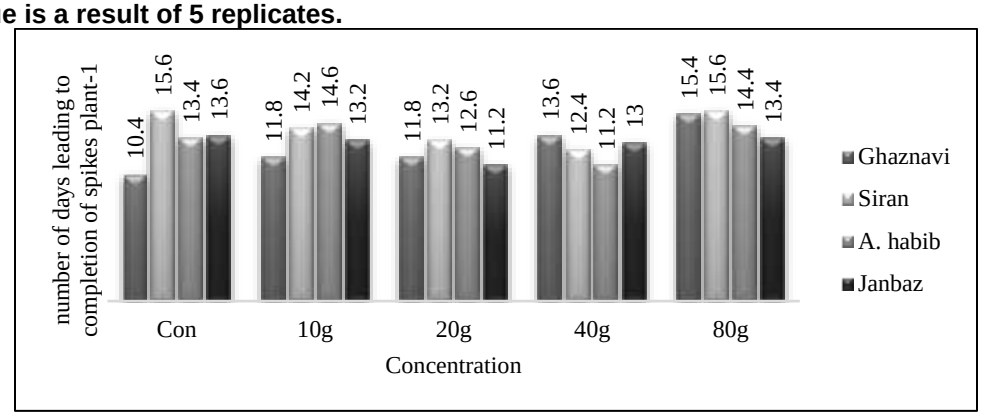

Figure 7: Effect of *P. nigra* leaves on number of days leading to completion of spikes plant^{-1}of test species. Each mean value is a result of 5 replicates.

Number of grains per spike

Number of grains per spike depicted that there were significant effect of the different concentrations in all varieties on number of grains spike^{-1} . Control and 10g concentration demonstrated same and highest means values i.e. 18.04. However, the lowest number of grains spike^{-1} was observed in 80g concentrations (11.52), followed by 40g powdered concentrations (12.78) and 20g (12.84) respectively.. Varieties means also varied to a significant extent i.e. *Ghaznavi* (16.79), *Atta Habib* (14.35), *Siran* (14.19) and *Janbaz* (13.47) grains spike^{-1} respectively. It revealed that by increasing the concentration of powdered portion, the grains spike^{-1} reduced accordingly (Fig 08).

Spike length (cm)

Spike length varied significantly among different varieties at various concentrations. The maximum spike length was achieved at concentration 10g (9.19 cm), which was statistically similar to control (9.14 cm), followed by 20g (8.74 cm), 40g (8.73 cm) and 80g (8.7 cm). Maximum spike length was shown by *Janbaz* (9.22 cm) and minimum by *Siran* and *Atta Habib* (8.74 cm) respectively which were statistically similar (Fig 09).

Thousand grains weight

Thousands grains weight was recorded to be affected by various concentration to varying degree in all varieties. The highest thousand grains weight was observed in control level (35.64 g) followed by 10g (35.07), 40g (32.39 g), 20g concentrations (31.50 g) and 80g concentrations (31.30 g). However, among varieties means, maximum thousand grains weight was observed in *Ghaznavi* (35.74 g), followed by *Siran*, *Janbaz* and *Atta Habib* (33.54 g, 32.19 g and 30.52 g respectively) (Fig 10).

Biomass Parameter

Shoot fresh weight (g)

All the four varieties showed significantly varied shoot fresh weight at various concentration. However, fresh weight of tested plant seedlings generally reduced in various concentrations as compared to control (9.12 g), 20g (8.9 g), 10g and 40g (8.5 g) and 80g (7.64 g). However, the inhibition was related to test species. Maximum shoot fresh weight was noticed in *Ghaznavi* and *Janbaz* (9.72 g and 9.64 g), followed by *Atta Habib* and *Siran* (8.81g and 6.07 g) respectively (Fig 11).

Shoot dry weight (g)

Shoot dry weight disclosed significant differences between different varieties. Concentration means exhibited that maximum shoot dry weight was found in control (4.85 g) which was statistically similar to 10g (4.58 g), followed by 20g (4.77 g), 40g (4.69 g) and 80g (4.27). Varieties means indicated that shoot dry weight was maximum in *Janbaz* (5.44 g) which was statistically similar to *Ghaznavi* (5.08 g). Varieties *Atta Habib* & *Siran* had shoot dry weight 4.44 g & 3.59 g respectively (Fig 12).

Moisture contents (%)

Moisture content was significantly affected by all concentration in all varieties. Concentration means exhibited that maximum moisture content was found in concentration 10g (13.03 %), followed by 40g and control (12.40 & 12.24 respectively) which were statistically similar, 40g (10.61 %), and 80g (9.48 %). Varieties means clarified that shoot moisture contents was maximum in *Ghaznavi* (13.81 %) and minimum in *Siran* (8.76 %). Varieties *Atta Habib* & *Janbaz* had moisture contents values 12.87 % & 10.78% respectively (Fig 13).

Biochemical Changes

Photosynthetic Pigments

Chlorophyll *a* and Chlorophyll b content were also measured for wheat in the presence of the poplar leaves extract. Statistical analysis showed significant reduction ($P < 0.05$) in chlorophyll content after treatment with PNLs at each concentration (Fig 14). Maximum chlorophyll *a* content was observed in control (0.288mg$_{chl}$-g $_{FW}^{-1}$). Initially, chlorophyll content was significantly ($P < 0.05$) reduced to 0.075mg$_{chl}$-g $_{FW}^{-1}$ concentration but increased thereafter at conc. 20g (0.15mg$_{chl}$-g $_{FW}^{-1}$), 40g (0.15mg$_{chl}$-g $_{FW}^{-1}$) and 80g (0.19mg$_{chl}$-g $_{FW}^{-1}$) respectively. Varietal response showed that variety *Atta habib* was mostly effected variety (0.11mg/100ml), however variety *Ghaznavi* showed maximum resistance (0.23mg$_{chl}$-g $_{FW}^{-1}$) to PNLs extract. Chlorophyll *b* was found more sensitive to phytochemicals and decreased significantly with increase in concentration. Maximum chlorophyll content was observed in control (3.61mg$_{chl}$-g $_{FW}^{-1}$), followed by 10g (3.53mg$_{chl}$-g $_{FW}^{-1}$), 20g (3.51mg$_{chl}$-g $_{FW}^{-1}$), 40g (3.40mg$_{chl}$-g $_{FW}^{-1}$) and 80g (3.43mg$_{chl}$-g $_{FW}^{-1}$). Varietal response showed maximum chlorophyll content (3.65mg$_{chl}$-g $_{FW}^{-1}$) in variety *Siran* (Fig 14).

β-carotene content initially declined from (0.246 mg/100ml) in control level, (0.218μg$_{Car}$.g$_{FW}^{-1}$) 10g, (0.24μg$_{Car}$.g$_{FW}^{-1}$) 20g, and (0.15μg$_{Car}$.g$_{FW}^{-1}$) 40g conc. However, at higher concentration 80g chlorophyll content increases to (0,48mg/100ml). Variety that was more effected by PNLs extracts was *Ghaznavi* (0.19μg$_{Car}$.g$_{FW}^{-1}$), while *Atta habib* (0.39μg$_{Car}$.g$_{FW}^{-1}$) was observed to be less effected (Fig 15).

In lycopene content a gradual decrease was seen with increase in concentration of PNLs concentration. Maximum content was found at control level (3.60μg$_{Lycop}$.g$_{FW}^{-1}$), followed by 10g (3.51μg$_{Lycop}$.g$_{FW}^{-1}$), 20g (3.53μg$_{Lycop}$.g$_{FW}^{-1}$), 80g (3.37μg$_{Lycop}$.g$_{FW}^{-1}$) and 40g (3.08μg$_{Lycop}$.g$_{FW}^{-1}$). According to varietal response variety

Ghaznavi contained more lycopene content ($3.55\mu g_{Lycop}.g_{FW}^{-1}$), than *Atta habib* which revealed the least lycopene content ($2.88\mu g_{Lycop}.g_{FW}^{-1}$) (Fig 16).

Proline Content

Proline content of wheat leaves decreased from $0.33\mu g_{Prol}.g_{FW}^{-1}$ to $0.27\mu g_{Prol}.g_{FW}^{-1}$ with an increase in PNL concentration from control level to 80g concentrations, respectively. Varietal response showed that proline content was maximum ($0.322\mu g_{Prol}.g_{FW}^{-1}$) in *Ghaznavi and Janbaz* (Fig 17).

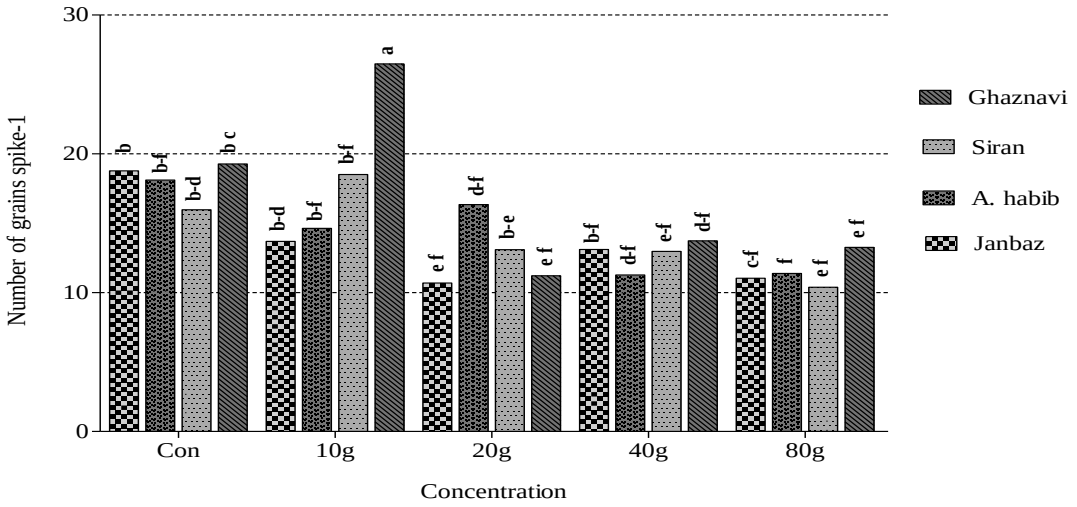

Figure 8: Effect of *P. nigra* leaves on number of grains spike^{-1} of test species. Each mean value is a result of 5 replicates.

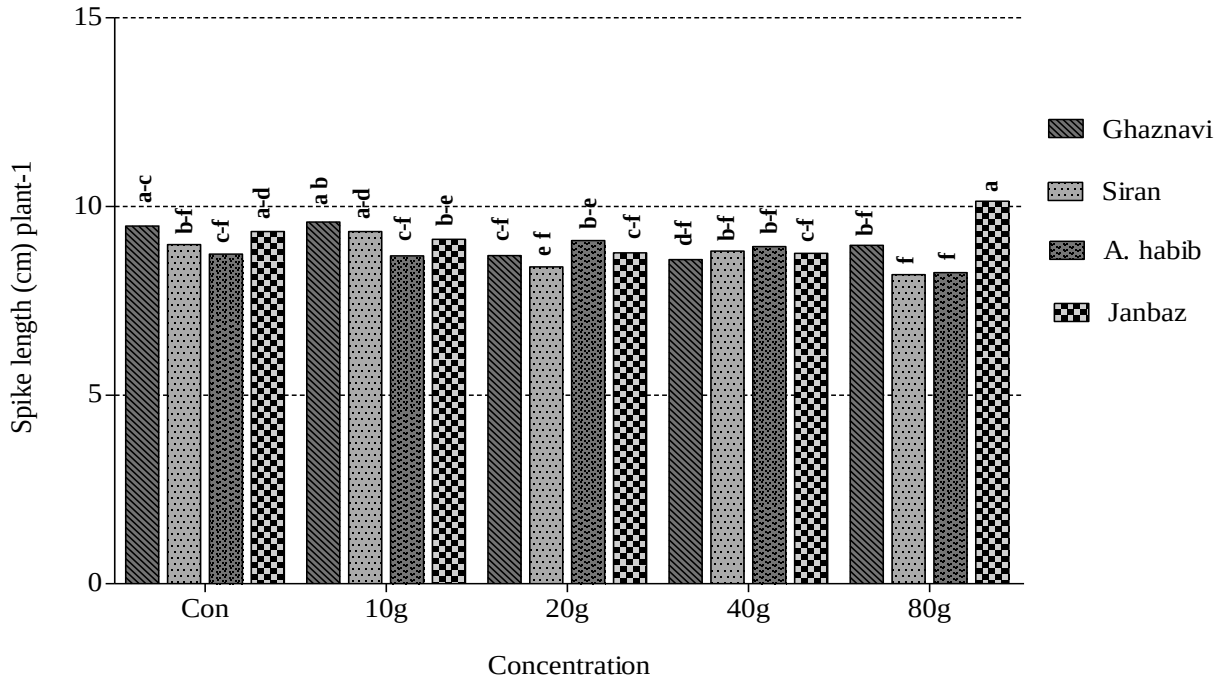

Figure 9: Effect of *P. nigra* leaves on spike length (cm) plant^{-1} of test species. Each mean value is a result of 5 replicates.

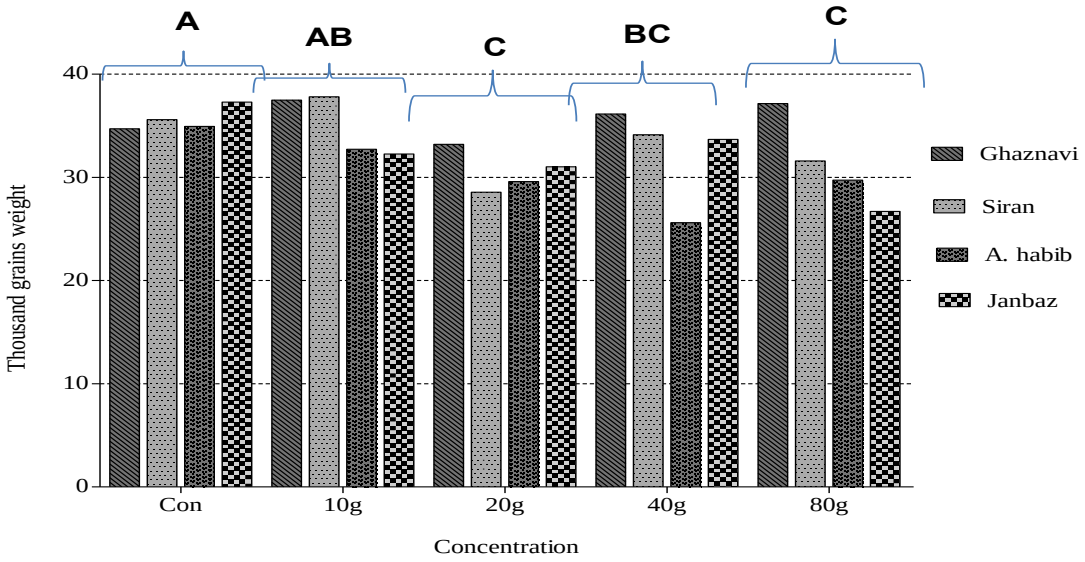

Figure 10: Effect of *P. nigra* leaves on thousand grain weight of test species. Each mean value is a result of 5 replicates.

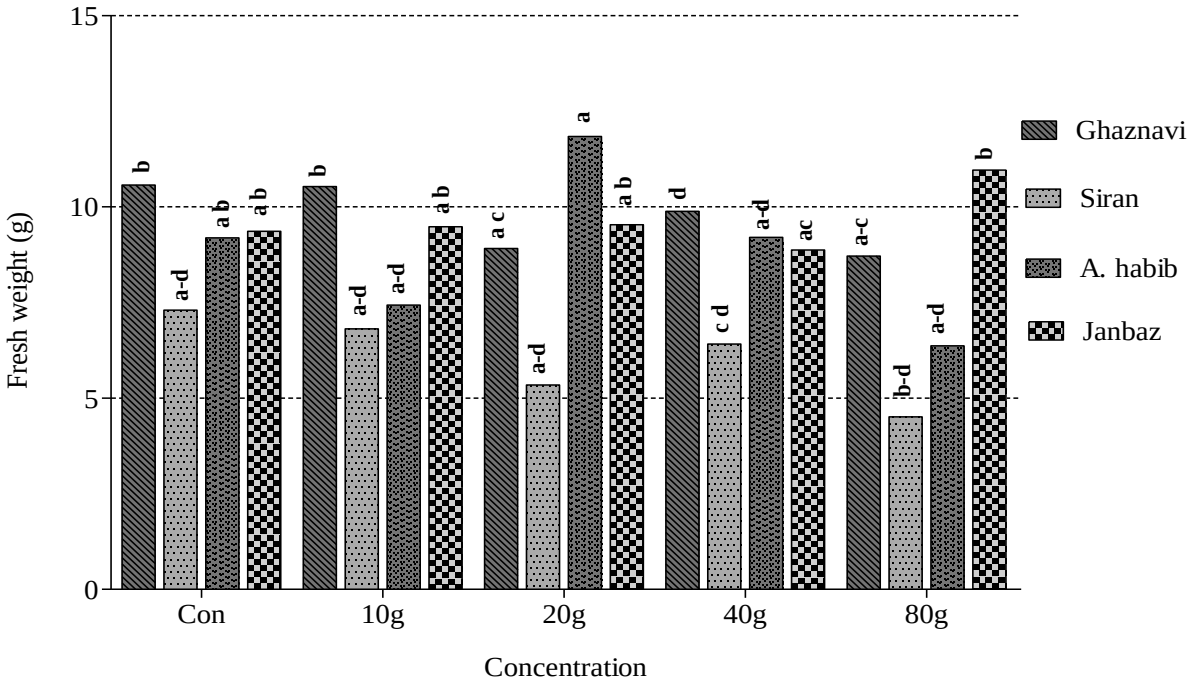

Figure 11: Effect of *P. nigra* leaves on fresh weight (g) of test species. Each mean value is a result of 5 replicates.

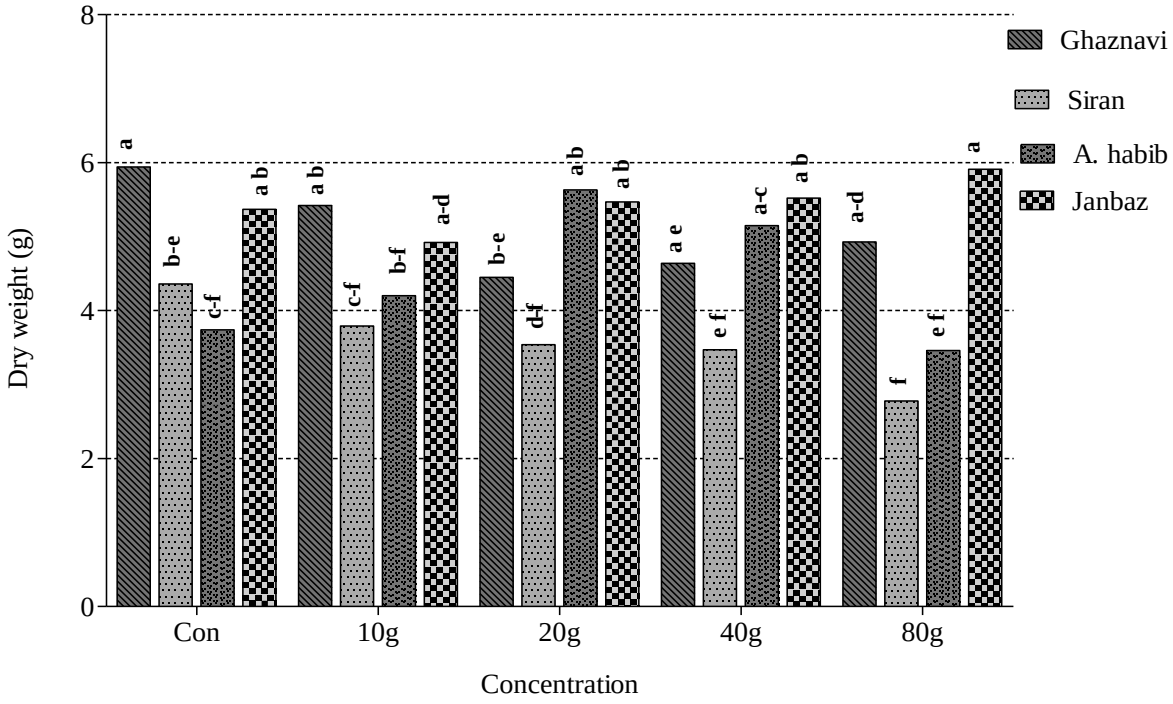

Figure 12: Effect of *P. nigra* leaves on dry weight (g) of test species. Each mean value is a result of 5 replicates.

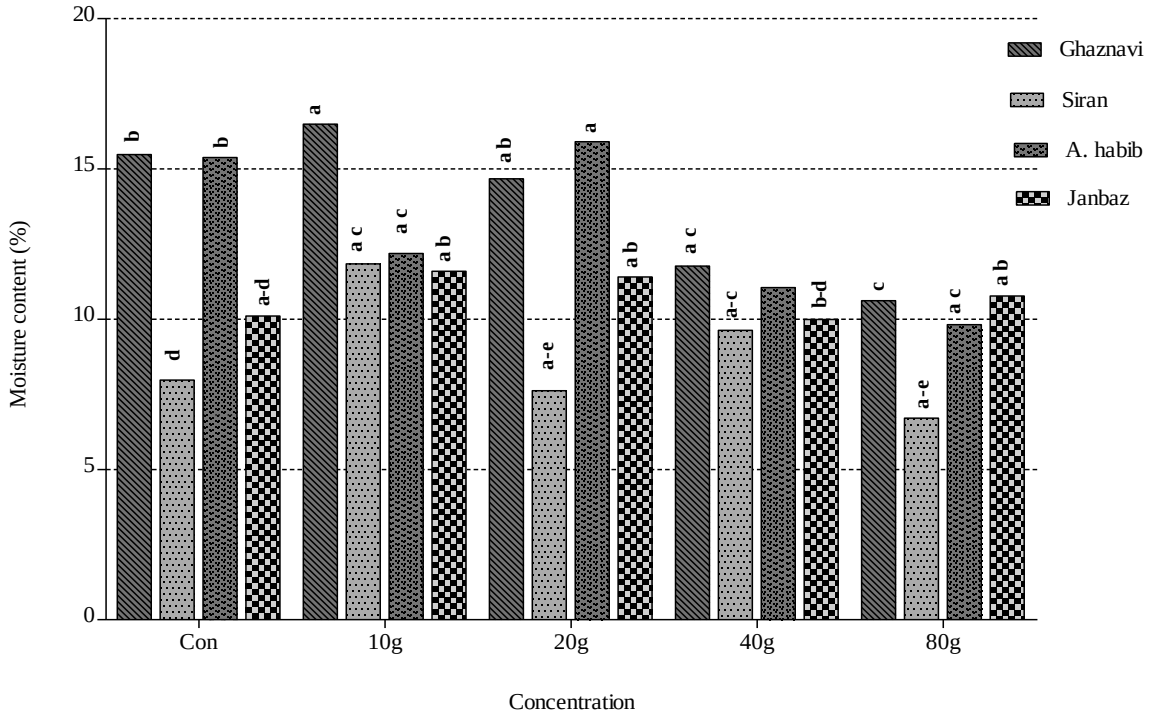

Figure 13: Effect of *P. nigra* leaves on moisture content (%) of test species. Each mean value is a result of 5 replicates.

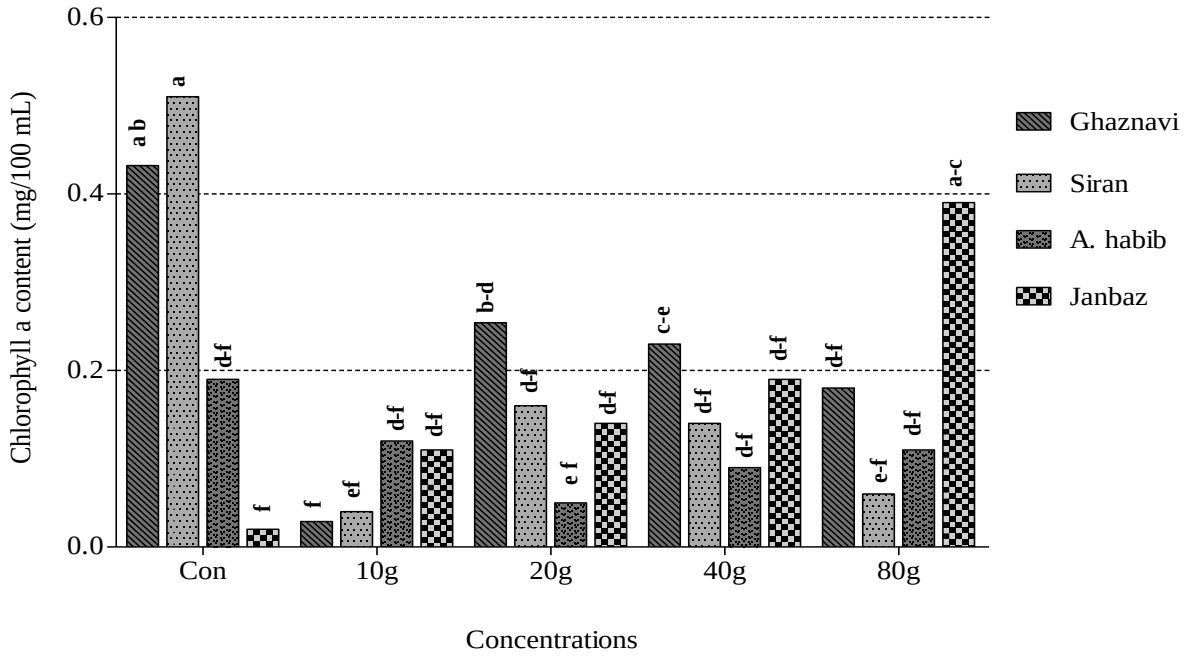

Figure 14;Effect of *P. nigra* leaves on chlorophyll (a) content (mg/100mL) of test species. Each mean value is a result of 3 replicates.

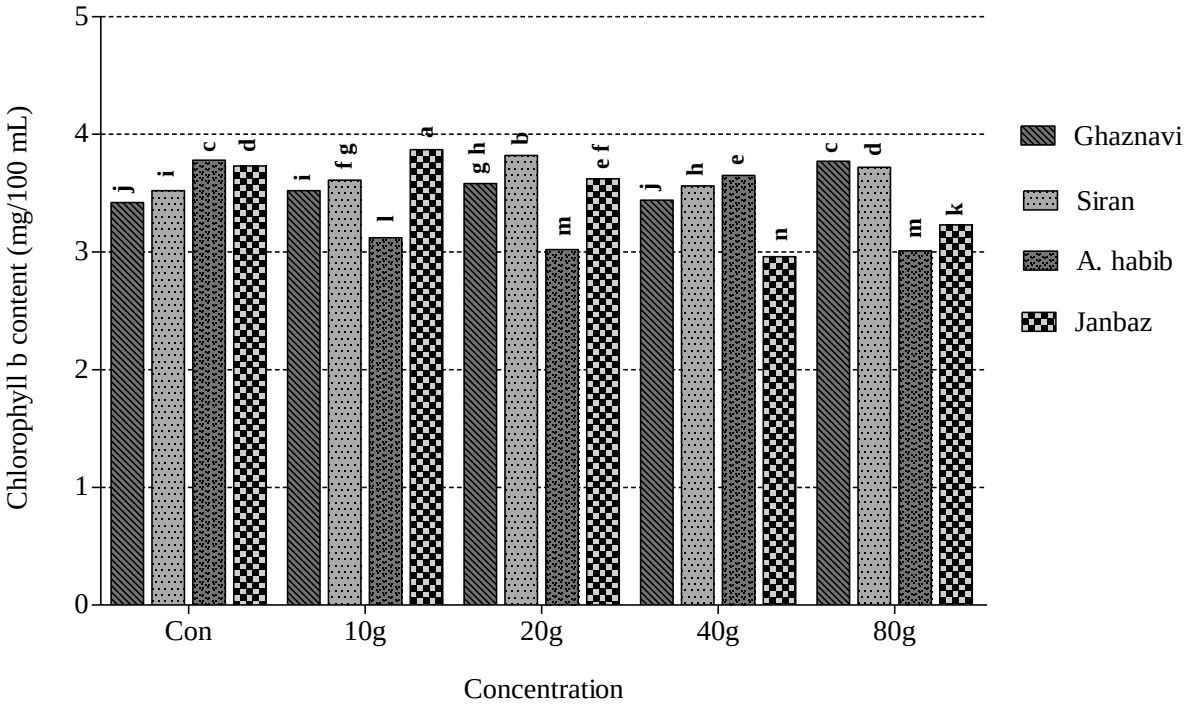

Figure 15: Effect of *P. nigra* leaves on chlorophyll (b) content (mg/100mL) of test species.Each mean value is a result of 3 replicates.

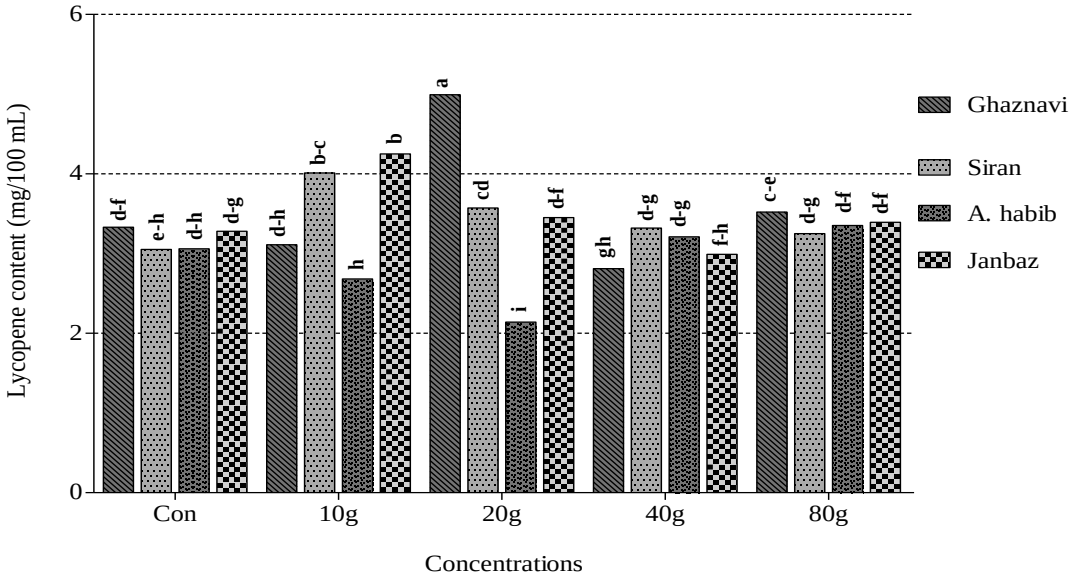

Figure 16: Effect of *P. nigra* leaves on lycopene content (mg/100mL) of test species. Each mean value is a result of 3 replicates.

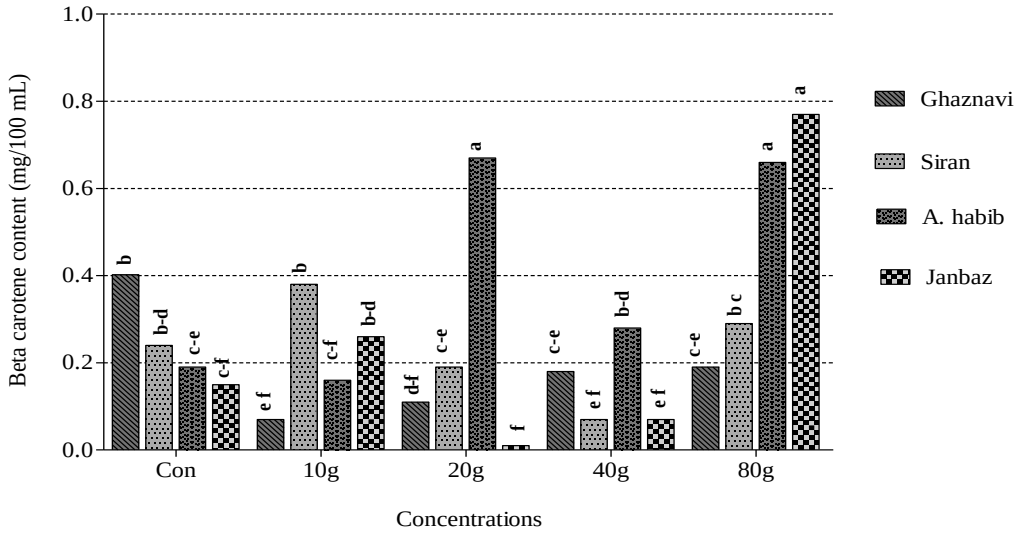

Figure 17: Effect of *P. nigra* leaves on β- carotene content (mg/100mL) of test species. Each mean value is a result of 3 replicates.

Phytochemicals identification through GC-MS screening

Chromatographic screening of D (84-92) and M (232-257) provided an additional insight into phytotoxin-mediated suppression of the tested varieties by determining the qualitative and quantitative presence of different compounds. GC-MS analysis provide peaks of 32 different compounds in D (84-92) and 19 compounds in M (232-257). Sub- fraction D (84-92) revealed the existence of Octadecane in highest concentration 25.67 % with retention time 22.203 min, followed by Heneicosane (18.12%), Hexadecane (9.28 %) and 2-Methylnaphthalene (7.90 %) with retention time 26.212 min, 17.797 min and 10.524 min respectively. Meanwhile, sub-fractions M (232-257) showed the existance of 3-Hydroxydihydro-2(3H)-furanone in highest concentration of 29.55% with retention time 4.062 min, followed by Butyl(dimethyl) Sailyloxycyclopentane (15.58 %) and 5- (Hydroxymethyl)2(5H)- furanone (7.40%) with retention time 14.893 min and 7.462 min respectively. Name of the identical/similar compounds, their retention times, % of peak area, molecular weight and their possible functional activity was described in Table 01 and 02.

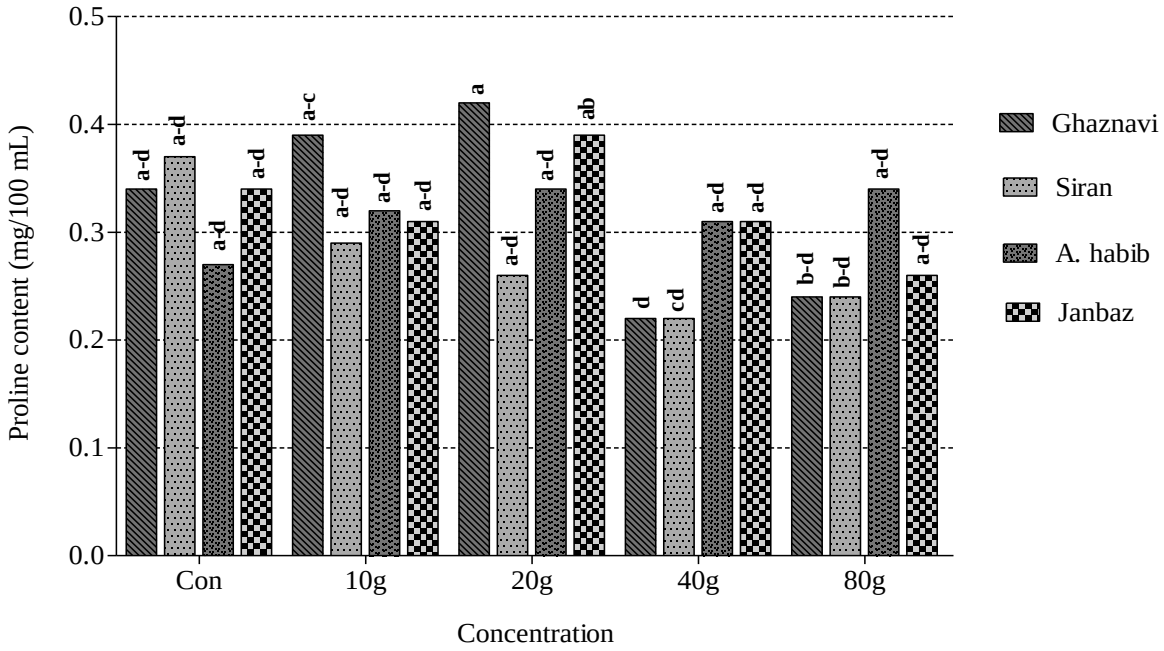

Figure 18: Effect of *P. nigra* leaves on proline content (mg/100mL) of test species. Each mean value is a result of 3 replicates.

Table 1: GC-MS analysis of chemical composition of *Populus nigra L.* leaves D (84-92) column fraction.

ID	Name	R. Time	Area	Conc. (%)
1	Phenylcarbamic chloride	3.768	55307	0.33
2	Ethylhexanol	4.730	47135	0.28
3	Prehnitene	6.466	62118	0.37
4	Durene	6.566	158530	0.93
5	1-Ethyl-3,5-dimethylbenzene	7.242	99400	0.58
6	Azulene	7.952	498354	2.93
7	1-Ethyl-2,4, 5-trimethylbenzene	8.505	65392	0.38
8	Pentamethylbenzene	9.104	37159	0.22
9	4,7-Dimethylindan	9.460	100600	0.59
10	1, 6-Dimethylindane	9.786	69001	0.41
11	Pentamethylbenzene	10.198	217339	1.28
12	2-Methylnaphthalene	10.524	1342918	7.90
13	1-Methylnaphthalene	10.936	713836	4.20
14	Biphenyl	12.551	34001	0.20
15	2-Ethylnaphthalene	12.925	118187	0.70
16	2,9-Dimethylundecane	13.189	245021	1.44
17	2,6-Dimethylnaphthalene	13.188	312999	1.84
18	2,3-Dimethylnaphthalene	13.546	265877	1.56
19	1, 7-Dimethylnaphthalene	13.626	152264	0.90
20	Diphenylmethane	13.843	43008	0.25
21	3-Methylbiphenyl	14.018	91312	0.54

22	1, 2-Dimethylnaphthalene	14.395	66793	0.39
23	1,1'-Biphenyl, 3-methyl	15.104	42400	0.25
24	Pentadecane	15.451	61077	0.36
25	beta-Isopropylnaphthalene	15.575	68521	0.40
26	2,4-Di-tert-butylphenol	16.702	129145	0.76
27	1, 4, 6-trimethylnaphthalene	16201	459321	0.27
28	P- Benzyltoluene	16.392	40400	0.24
29	9-Octadecene, (E)-	17.616	131782	0.78
30	Hexadecane	17.797	1576443	9.28
31	Biphenyl-2, 2,5,5-tetramethyl	20.277	235081	1.38
32	Heptadecane, 3-methyl-	21.579	183754	1.08
33	9-Eicosene, E-	22.040	570418	3.36
34	Octadecane	22.203	4361890	25.67
35	Heptadecane, 2,6,10,15-tetramethyl-	24.239	277722	1.63
36	1-Heptadecene	26.078	588778	3.46
37	Heneicosane	26.212	3078799	18.12
38	1-Hexadecanol	27.745	805138	4.74

Table 2: GC-MS analysis of chemical composition of *Populus nigra L.* leaves M (232-257) column fraction.

ID	Name	R. Time	Area	Conc. (%)
1	3-Hydroxydihydro-2(3H)-furanone	4.062	495849	29.55
2	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	6.925	36834	2.20
3	4-Hydroxydihydro-2(3H)-furanone	7.001	100754	6.00
4	5-(Hydroxymethyl)2(5H)-furanone	7.462	124145	7.40
5	Ethriol	10.042	68152	4.06
6	8-Hydrazinoquinoline	12.942	46861	2.79
7	Tridecane	13.034	88107	5.25
8	Butyl(dimethyl)Sailyloxycyclopentane	14.893	261439	15.58
9	Pentadecane	15.455	81406	4.85
10	Hexadecane	20.040	64283	3.83
11	4-Hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-2-cyclohexen-1-one	21.922	58676	3.50
12	Bisomel	22.713	28291	1.69
13	Heneicosane	24.240	42392	2.53
14	Hexadecanoic acid, methyl ester	24.735	58863	3.51
15	Pentadecanoic acid	25.353	11063	0.66
16	1-Heptadecanol	27.742	6587	0.39
17	9,12-Octadecadienoic acid, methyl ester	27.961	4700	0.28
18	Tetratetracontane	28.080	65823	3.92
19	Octadecanoic acid methyl ester	28.577	38349	2.29

DISCUSSION

The current research exhibited that the powders form of *Populus nigra* L. have stimulatory and inhibitory effects on growth of wheat. Our results show negative effect of PNLs powder on germination percentage of wheat varieties but there is no significant difference among various concentration applied. The present study follows the same pattern as Samreen et al. (2009) reported that aqueous extracts from leaves and stems of *Calotropis procera* (Ait.) Ait. significantly inhibited the germination in *Pennisetum americanum* L. and *Setaria italic* L. Similarly, (Al -Sobhi et al. 2006)found that germination % was reduced with increase in concentration of extract. Both of these findings are opposite to our findings that there was none significant effect of concentrations on seed percent germination. Likewise, grain yield in the current research was observed to be concentration dependent. At lower concentration stimulatory effects on grain yield were observed though at higher concentrations reduction in grain yield was found. Our result coincides with (Majeed et al. 2012) who reported the concentration-dependent effect of *Chenopodium album* L. on wheat. However, the results are in contradiction to the investigations of (Mohsin and Ram, (2020)who reported decreased growth and yield of wheat plants when they were intercropped with *P. deltoids* Marsh. Our study is partially supported by the findings of (Shang et al. 2012) which revealed that leaf litter extracts of P. tomentosa Carr. promoted germination, height and dry weight of cucumber. A study conducted by Sher et al. (2011) revealed that aqueous extract of different parts of *P. euphratica* Oliv. Completely inhibited germination and seedling growth of wheat which does not support our findings. These differences in results may be due to different experimental procedures for aqueous extracts preparation and wheat varieties used in the studies. The *Populus nigra* L. leaves powder showed significant reduction in plant height, number of tillers per plant, number of spikes per plant, number of grains per spike and 1000-grains with increase in concentration of powder. This might be due to lowered water and minerals uptake and their translocation from rhizosphere to other parts and reduction of other physiological processes such as photosynthesis and respiration. The results of various researchers are in accordance to our results that shows decrease in growth and yield parameters of test species with increase of plant extract concentrations (Samreen et al. 2009, Majeed et al. 2012, El-Khatib et al. 2004, Ullah et al. 2010, Hadi et al. 2013 and Hadi et al. 2014)

There are numerous information that chlorophyll content of leaves reduced under stressful conditions. The findings declare that chlorophyll content decline in wheat varieties are in accordance with those of Al-Sobhi *et al.* (28) and Jaleel *et al*. (29). This decrease in chlorophyll concentrations might be due to the inhibitory impact of the ions accumulation (30). Reduction in chlorophyll content is also supported by earlier observations by Singh *et al.* (31). This Decline in chlorophyll and carotenoids may also be associated with senescence. Breakdown of chlorophylls may be one of the earliest symptoms of senescence. The chlorophyll a/b ratio declined with the advancement of senescence (Siffel et al. 2001 and Hidema et al. 1992)

probably due to nonsynchronous dismantling of lamellae and grana thylakoids and the asymmetrical dispersal of photosystems amid them. Carotenoids are lost at a much lower rate than chlorophylls Young et al. 1990 and

Scheumann et al. 1999) observed that in senescence leaves of barley chlorophyll b reduction was the first and requisite step of chlorophyll b breakdown that was passed out by chlorophyll b reductase, a thylakoidal enzymatic activity which peaks earlier(day2) than chlorophyllase (day4) during dark-induced leaf senescence. Our phytochemical screening revealed the presence of 51 different compounds in leaves of *Populus nigra* L. which are closely relevant to the findings of Kis *et al*. (36) who reported about 48 compounds like phenols, phenolic acids and phenylpropanoids, terpenoids, flavanones, caffeic/ferulic acids and their derivatives. Thus, the allelopathic activity of the leaves of *Populus nigra* L might be attributed to the presence of a diverse range of phytochemicals. Proline a water soluble amino acid is supposed to play a vital part in osmotic adjustment with esteem to decrease of osmotic potential due to solutes accumulation (Raggi, (1994) and *Chutipaijit,* et al. 2009)

Plants accumulate osmolytes like proline in response to abiotic stress condition to protect the macromolecules of cells (Hong et al. 2000, Kishore et al. 2005) and Chutipaijit et al. 2009)

Osmolytes accumulation might help plants to abide stress conditions by maintaining osmotic balance within the cell Apse and Blumwald (2002) and Kumar et al. 2003)

Existing finding displayed that proline accumulation reduced significantly in wheat leaves in retort to the application of PNLs powder. Abiotic stresses induced accretion of many compounds such as glutathione, proline, ascorbate, α-tocopherol, betaine, and other amino acids, quaternary ammonium compounds, polyamines, oligosaccharides, sucrose, polyols in the affected plant (Smith et al. 2001) .Proline content is positively correlated up to a significant level with stress severity which may be either due to inhibition of protein oxidation or due to breakdown of protein from its precursors (45). Proline is also involved in intracellular osmotic adjustment (Subbarao, (2001) and D'Souza, and Devaraj, (2010).

CONCLUSIONS

From the present study it is concluded that *Populus nigra* L. leaves have inhibitory effect on physiological and biochemical parameters of wheat-an important cereal crop. In general, the inhibitory effects on the studied attributes were proportional to the extract concentrations. The inhibitory effects of leaf extract on growth, yield, physiological and biochemical attributes of wheat may be due to the presence of strong allelopathic substances present in the plant. We isolated 51 different bioactive compounds, predominantly phenols which might be the causal factor for suppressing the studied parameters of wheat. Based on the suppressive effects of *Populus nigra* L., it is concluded that the tree has strong allelopathic potentials and intercropping of wheat with this tree is not recommended.

Supplementary materials

The supplementary material / supporting for this article can be found online and downloaded at: https://www.isisn.org/article/

Author contributions

Dr. Naila Inayat the corresponding author did all the research work both in Lab. And field
Dr. Zahir Muhammad did statistical analysis of the data through Mstatc software
Dr. Rasool Khan did GCMS analysis of the samples and the chemistry portion work was done by him
Dr. Abdul Majeed and Amir Hasan did paper write-up and setting
Dr, Muhammad Adil correct the grammatical mistake

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Conflict of interest

The authors declared that present study was performed in absence of any conflict of interest.

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