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Original Research Article

Phytochemical screening and allopathic evaluation of *Prunus. amygdaloides* cold and hot aqueous extracts on wheat

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Abstract

Allelochemicals are secondary metabolites products which emerge during various metabolic activities. However, these products are used to perform an important role in interactions of producers with receivers. The current study was conducted on the allelopathy of almond *(Prunus amygdaloides)* on wheat (*Triticum aestivum)* with respect of seed germination, seedling length and weight of seedling. The seed germination was stimulation in almond bark > almond fruit in respect to cold, hot conditions. The weight of seedling was also decrease in both conditions. The length of seedling were enhanced in fruit with compare to almond bark. The presence of allelochemicals such as terpenoid, tannin, flavonoid and saponin is present in bark and fruits while reducing sugar is present absent in fruit. The allopathic results showed that maximum inhibition was found in bark as compare to fruit. In the light of allelopathy phenomena the suppression of wheat plant growthdue to the presence of allelochemicals.

Keywords: Crop, Germination. Enhance, Allelochemicals, Terpene, Saponin

INTRODUCTION

Allelopathy is a natural marvel where plant stimulates the growth of another plant through the proclamation of allelochemicals, certain plants can enormously influence the development of different plants by filtering, decompositions, etc (Inayat et al., 2020). Plant allelopathy is utilized as a method for endurance in nature, decreasing competition from plants. Various compounds which released generally as a result of the plant's metabolism and microbes i.e. bacteria, viruses, and fungi can stimulus various procedures in the agroecosystem (Eichenberg et al., 2014; Chon and Nelson 2010). Allelochemicals are released from higher plants through leachates of decomposition residues, volatilization, and root exudates (Qian et al., 2018).

Appraisal of the allelopathic commotion of allelochemicals mostly depends on identify their impact on seed germination and length of seedling. Beneficial outcomes of allelochemicals have been accounted in previous studies. Even though they are more uncommon, notably, allelochemicals regularly repress seed germination, delay germination and increase seedling mortality (Friedman, 2017; Findura, et al., 2020). Allelochemicals, which suppress the growth and development of the same or various species at higher concentrations, can't impact the germination and development at a lower concentration of extracts and in reverse. Despite the noxious metabolites are disseminated in all plant tissues, the bark and leaves are the most powerful source (Qian et al., 2018); Ghafarbi et al., 2012). Mechanisms underlying those effects are multiple and they are associated with a disruption of normal cell metabolism, rather than with cell damage (Weston et al., 2013).

Trees are incredible instances of allelopathy in plants. For example, numerous trees use allelopathy to ensure their space by utilizing their roots to pull more water from the soil so other plants cannot thrive. Some utilization their allelochemicals to repress germination or reduced the growth of close by vegetation. Most allelopathic trees discharge these compounds through their leaves, bark which are toxic once consumed by other plants. Different trees and crops are produced in or around farming fields

under the traditional agricultural frameworks.

However, agriculture losses experienced by the inhabitants of different areas have been ascribed to the phytotoxic effects of most of the farm trees (Farooq et al., 2020; Mushtaq et al., 2020). Presently great efforts are underway to introduce herb, shrub, and tree of medicinal /aromatic value into the traditional agroforestry. But without knowing the compatibility of these medicinal plants with crops, it may not be feasible to do so. In view of the above-mentioned facts, the investigation was undertaken to analyze the response of hot and cold aqueous extracts of bark and fruit of medicinal/ economically important almond trees on the seed germination and seedling extension of wheat crops of the region.

Mostly farm trees of different areas that have been ascribed to phytotoxic effects, which causes the losses of agriculture (Andrew et al., 2015; Farooq et al., 2013). Presently incredible endeavors are in progress to make known to, herb, shrub, and tree of medicinal /aromatic value into the traditional agroforestry. But it may not be feasible to do so without knowing the compatibility of tree plants with crops. In the view of the previously mentioned studies, the investigation was undertaken to analyze the response of hot and cold aqueous extracts of bark and fruit of medicinal/economically important almond trees on the seed germination and seedling extension of wheat crops of the region.

MATERIAL AND METHODS

Sampling area

A fresh sample of bark and fruit of *P. amygdaloides* (Almond) and (*Triticum aestivum)* (local name kakari ghanum) were collected from Tehsil Mekhter district Loralai province Balochistan Pakistan. The bark and fruit were dried out in the oven at 80ºC for 3 days. The dry sample was crushed using pestle and mortar and preserved it in polythene bags with labeled data. Samples were used for different allelopathy tests.

Extraction of almond bark and fruit

The extracts were prepared in 100ml of hot and cold water with different concentrations 0.5, 1.0, and 1.5g for 24, 48, and 72hr respectively. Extracts were obtained by filtration weight (Gulzar and Siddiqui, 2014).

Phytochemical Screening tests

The cold and hot solution was extracted from the bark and fruit of *P. amygdaloides* (section 2.2). For the detection of reducing sugar. Five ml of boiling Fehling solution A and B were added in one ml of a hot and cold solution. Fehling solution A was prepared by dissolving 6.93g $CuSO_4$ in 100ml H2O and Fehling solution B was Dissolve 34.6g KNaCu+10gNaOH in 100ml H_2O (Johar et al., 2015). For the flavonoid the individually1ml of hot and cold aqueous extracts was added into 5ml H_2O, 5ml dilute NH_3, and 1ml conc. H_2SO_4 (Johar et al., 2015). For the analysis of terpene add one ml of hot and cold solution in, 2ml of $CHCl_3$ and 3ml of conc. H_2SO_4 (Johar et al., 2015). For saponin 1ml of hot and cold extracts were added in 5ml H_2O with few drops of olive oil then shake it slightly However for the tannin, 1ml crude hot and cold extracts were added in boiled 10ml H_2O with few drops of $FeCl_3$ (Johar et al., 2015).

Seed germination of wheat

Selected seeds of local variety of wheat were sterilized with10% hydrogen peroxide and seeds were thoroughly washed three times with distilled water and air-dried for 30 minutes (Daud et al., 2012). Furthermore, dried seeds were imbibed overnight for 12 hours in total darkness. For germination ability testing five seeds/plate was soaked in different concentrations at various period aqueous extracts. Distilled water was used for control. Seeds germination take place in germinator with 22% of humidity and temperature was 25ºC. The experiment was performed in three replicates. The data were recorded in seed germination rate and seedling length and biomass weight (Gulzar and Siddiqui, 2014).

RESULTS

Phytochemical screening of almond

The hot and cold extracts of bark and fruit were investigated to determine phytochemical compounds in the extracts. The common phytochemistry content from the plant such as reducing sugar, flavonoid, saponin, terpenoid, and, tannin has identified (Table. 3). For reducing sugar test dark green color appeared in almond bark cold and hot condition and blue color in almond fruit in both conditions. The result showed that sugar is present in bark and absent in fruit solution. The yellow color was observed in almond fruit in both conditions which indicates the presence of flavonoid. While in bark crud extract there was no reaction. For the saponin test, oil emulsion was formed in the top of the almond bark and fruit extracts. Similarly for the detection of terpenoid reddish-brown color appeared in bark and fruit extract which was denoted the presence of high concentration in cold condition and low concentration in hot condition Tannin was found in bark and fruit by the detected by brown color in both conditions (Johar et al., 2015).

Table 1. Effect of cold extracts of bark and fruit on seed germination percentage with % of control. Values are means of three replicates ± SE.

Treatment	Germination%	% of control	Treatment	Germination%	% of control
	Bark			**Fruit**	
Control	98		Control	98	
	0.5g			0.5g	
24h	90±0.024	91.83	24h	93±0.023	94.89
48h	87±0.022	88.77	48h	90±0.017	91.83
72h	75±0.024	76.53	72h	79±0.018	80.61
1.0g			1.0g		
24h	88±0.026	89.79	24h	90±0.013	91.83
48h	84±0.012	85.71	48h	87±0.015	88.77
72h	84±0.011	85.71	72h	88±0.011	89.79
1.5g			1.5g		
24h	83±0.013	84.69	24h	86±0.023	87.75
48h	80±0.021	81.63	48h	86±0.221	87.75
72h	75±0.014	76.53	72h	80±0.036	81.63

Table 2. Effect of hot extracts of bark and fruit on seed germination percentage with % of control. Values are means of three replicates ± SE.

Treatment	Germination%	% of control	Treatment	Germination%	% of control
Bark				**Fruit**	
Control	98		Control	98	
0.5g				0.5g	
24h	85±0.011	86.73	24h	88±0.020	89.79
48h	83±0.011	84.69	48h	87±0.016	88.77
72h	79±0.012	72.44	72h	76±0.013	77.55
1.0g				1.0g	
24h	84±0.013	85.71	24h	87±0.017	88.77
48h	80±0.012	83.67	48h	85±0.014	86.73
72h	75±0.013	76.53	72h	80±0.012	81.63
0.5g				0.5g	
24h	79±0.010	80.61	24h	84±0.012	85.71
48h	75±0.012	76.53	48h	78±0.014	79.59
72h	70±0.013	71.42	72h	74±0.011	75.51

Test Species Verses different extract

Triticum aestivum wheat (local name kakari ghanum)used as trial species against hot and cold aqueous extracts of bark and fruit with various concentrations and periods of *(Prunus amygdaloides)* almond.

Cold aqueous extracts of bark and fruit

Bark extracts of 0.5g, 1g, 1.5g

Seeds were treated with cold aqueous extracts of bark (0.5, 1, 1.5g at 24, 48, 72hrs) for seven days. The germination was higher 98% as compared with treatment (75-90%). The length of seedling was inhibit (13.5- 14.74 cm) with a comparison of control (14.92 cm). The weight of seedling (1.33-1.49 g) was decreased when compared with control (1.53 g). Treated seed with 1gm, the germination was (84-88%) as compared with control. The seedling length was decreased (12.67- 13.97cm) with the comparison of control (14.92 cm). Similarly, seedling weight (1.21-1.46 g) was decreased when compared to control (1.53 g). The germination was inhibit (75-83%) as compared to control. The length and weight of seedling was decreased (9.85- 12cm), (0.77-0.82 g) with a comparison of control (14.92 cm) (1.53 g) respectively in 1.5g extract (Table 1), (Table 3), (Figure 1).

Fruit extracts of 0.5g, 1g, 1.5g

In the case of 0.5g of fruit, extracts were showed (93-79%). The seedling length was less (13.92- 14.61cm) with the comparison of control (14.92 cm). The weight of seedling (1.09-1.51 g) was decreased when compared with control (1.53 g). While in (1gm) the germination was

Table 3. Effect of cold extracts of bark and fruit on seedling weight with % of control. Values are means of three replicates ± SE.

Treatment	Seedling weight (g)	% of control	Treatment	Seedling weight (g)	% of control
Bark				**Fruit**	
Control	1.53		Control	1.53	
0.5g				0.5g	
24h	1.49±0.050	97.38	24h	1.51±0.043	98.69
48h	1.43±0.045	93.46	48h	1.43±0.041	91.5
72h	1.33±0.048	86.92	72h	1.09±0.047	71.24
1.0g				1.0g	
24h	1.46±0.044	95.42	24h	1.22±0.041	79.73
48h	1.34±0.026	87.58	48h	1.13±0.042	73.85
72h	1.21±0.026	79.08	72h	1.06±0.037	69.28
1.5g				1.5g	
24h	0.82±0.044	53.59	24h	1.38±0.042	90.19
48h	0.81±0.045	52.24	48h	1.1±0.037	71.89
72h	0.77±0.026	50.32	72h	0.57±0.022	37.25

Table 4. Effect of hot extracts of bark and fruit on seedling weight with % of control. Values are means of three replicates ± SE.

Treatment	Seedling weight (g)	% of control	Treatment	Seedling weight(g)	% of control
Bark				**Fruit**	
Control	1.08		Control	1.08	
0.5g				0.5g	
24h	1.05±0.042	97.22	24h	1.14±0.035	105.55
48h	0.93±0.032	86.11	48h	1.09±0.025	100.92
72h	0.92±0.0310	85.18	72h	0.95±0.042	83.33
1.0g				1.0g	
24h	0.86±0.039	97.62	24h	1.06±0.033	98.14
48h	0.73±0.027	67.59	48h	0.64±0.023	59.25
72h	0.61±0.024	56.48	72h	0.65±0.033	29.62
0.5g				0.5g	
24h	0.54±0.012	37.03	24h	1.08±0.040	96.43
48h	0.45±0.011	37.03	48h	0.57±0.027	52.77
72h	0.38±0.013	35.18	72h	0.46±0.015	32.4

slowdown (87-90%) as compared with control The length of seedling was inhibit (10.79-13.87cm) with a comparison of control (14.92 cm). The weight of seedling (1.06-1.22 g) was impede when compared with control (1.53 g). Similarly in 1.5g, the germination was slightly inhibited(80-86%) as compared with control. The length of seedling was (7.74-14.36cm) which is decrease to control (14.92 cm). Seedling weight (0.57-1.38 g) was less as compared to control (1.53 g). (Table. 1), (Table 3), (Figure 1)

Hot aqueous extracts of bark and fruit

Bark extracts of 0.5g, 1g, 1.5g

The (77-85%) germination was observed in all treated seed and control in hot aqueous extracts of bark, (0.5gm at 24, 48, 72hrs). The length of seedling was stimulated (10.70- 12.31 cm) with a comparison of control (11.987 cm). The weight of seedling (1.33-1.49 g) decreased with the comparison of control (1.08 g). In 1g the germination was less (77-87%) as compared to control. The length of seedling was slowdown (4.84-9.78cm) with the comparison of control (11.987 cm). The weight of seedling (0.77-0.82 g) was decreased when compared with control (1.08 g). The maximum (70-79%)germination was found with seedling length and weight (4.70-5.24cm), (0.38-0.54 g) as compared to comparison control (11.987 cm), (1.08 g) respectively in 1.5g extract. (Table 2), (Table 4), (Figure 2).

Fruit extracts of 0.5g, 1g, 1.5g

The germination was inhibited (76-88%) as compared to control. The inhibition (6.56- 12.442 cm) was observed when compared to control (11.987 cm). The weight of seedling (0.95-1.14g) was decreased when compared with control (1.08 g). 1n 1g and 1.5g the germination was (80-87%), (74-84%) was observed respectively that is less than to control. Similarly in both concentration, the length of seedling was inhibited (4.68-12.40cm), (3.17-13.25 cm) with the comparison of control (11.987 cm).

Table 5. Phyto-screening test of Almond bark and fruit in cold and hot aqueous extracts

Test	Almond Bark		Almond fruit		Result
	Cold	Hot	Cold	Hot	
Reducing sugar.	+ Dark green color.	+ Dark green color	- Blue color.	- Blue color.	High conc. In bark while absent in fruit
Terpenoid.	+ Reddish brown color.	+ Reddish brown color	- No Reddish brown.	- No Reddish brown.	High conc. In bark cold, while low conc. in bark hot. Absent in fruit
Flavinoid.	+No Yellow color.	+No Yellow color	+Yellow reaction	-No reaction	High conc. In bark hot absent in bark cold while no reaction in fruit.
Tannin.	+ Brown color	+Black Brownish color	+Brown color	+Brown color	Tannin is present in both bark and fruit.
Saponins.	+ Oil emulsion	+ Oil emulsion	+ Oil emulsion.	+ Oil emulsion.	High conc. In bark while low in fruit.

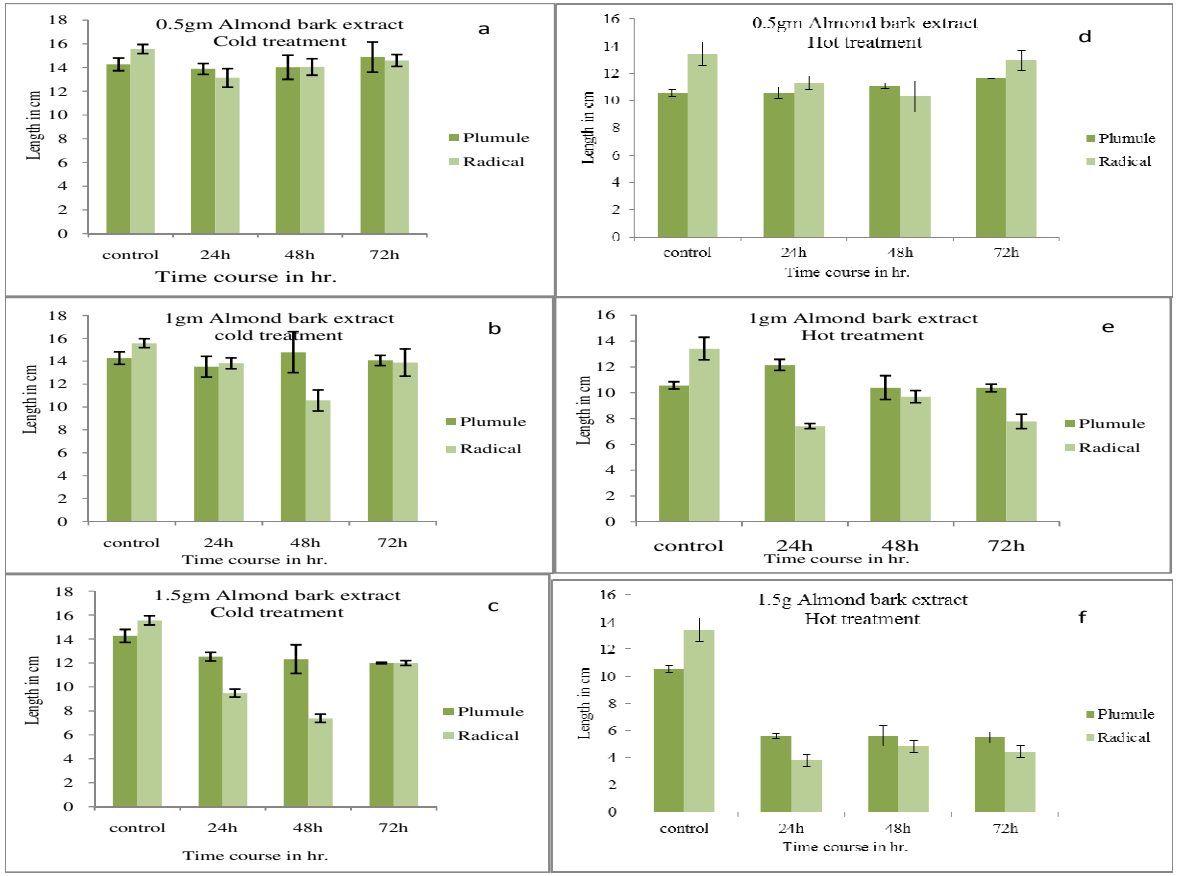

Figure 1. Length of seedling of wheat varieties Left panel (Cold extracts of bark, a, b c), Right panel (Hot extracts of bark, d, e, f). values are means of three replicates and vertical bars represent the standard error.

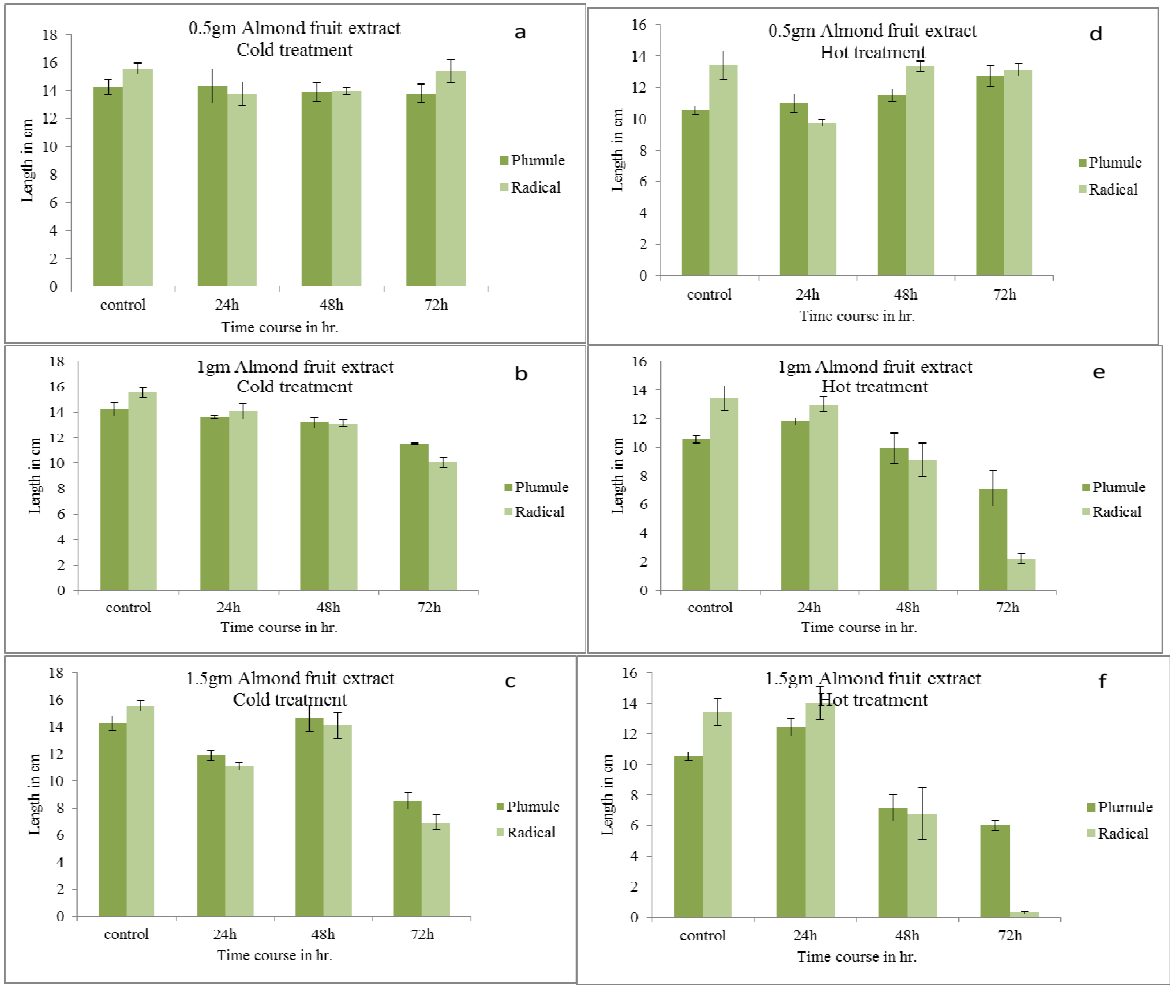

Figure 2. Length of seedling of wheat varieties Left panel (Cold extracts of fruit, a, b c), Right panel (Hot extracts of fruit, d, e, f). Values are means of three replicates and vertical bars represent the standard error

The same trend was observed in seedling weight (0.65-1.06g), (0.46-1.08g). (Table 2), (Table 4), (Figure 2).

DISCUSSION

Allelopathy is a science that has multidisciplinary aspects, and their persuasions are noted in agriculture and forestry sectors. However, in a few cases, a suitable information of released alleloch-

emical is used for the appropriate applications. It has been accounted for that metabolites, for example alkaloids, fatty acid, phenols, hydroxamic corrosive, terpenoids, indoles, Tannins, saponins and steroid, etc are some significant allelochemicals.

The presence of phenolic compounds might interfere with the activities of respiratory enzymes in seed germination thus causing inhibitory effect on its germination. Enzymes that mostly affected by phenolic compounds, an enzyme involved in the first step of oxidative pentose phosphate pathway (Inayat et al., 2020). Our results generally agree with previous studies conducted on the allelopathic activities of several plants on wheat germination and growth (Khan et al., 2016).This study is focused on the role of secondary metabolites as allelochemicals and their effects on crops.

The allelochemicals can influence physiology of plant of neighbouring plants like, seed germination rate, respiration, rate of photosynthesis, ion uptake, content of enzymes, transpiration, stomatal opening, hormone levels (Muscolo et al., 2001). Moreover, they can influence cell cycle and differentiation, cell signaling and plant eliciators and gene expression, suppression and cell membrane permeability (Kong et al., 2019).Various enzymatic activities affected by allelochemicals such seed germination inhibit due to phenolic compounds, that is involve in respiration mechanism. Flavonoids, phenolics that are engaged in oxidative pathway (Cheng etal.,2015). Our study outcomes by and large concur with past research conducted on the allelochemicals of several plants on wheat germination and growth (Todaria et al., 2005; Khan et al., 2016) .This study is concentrated on the role of secondary metabolites as allelochemicals and their effects on crops.

Agriculture management point of view, categrized local tree crops with a least accumulation of phtotoxins in the soil. Phytotoxic comebacks of bark, leaf, and fruit extracts of several agriculture trees crops on germination and elongation of seedling in field have been reported (Weston et al., 2013; Khan et al., 2016). Most published work (Todaria et al., 2005; Khan et al., 2016) indicates that vegetative parts of almond plants are powerful sources of phytotoxic and their toxic effects are inverse on wheat crop. Our study revealed inhibitory effects of bark and fruit extracts on germination, seedling growth, and biomass of wheat.

Number of environmental factors like temperature, light, humidity, water table that are modified higher plants allelochemicals especially trees. Our study determines that hot extracts were showed more inhibitory effects than cold extracts. A similar study observed (Ehsan et al., 2011). In fruit and bark the allelochemicals, such as terpenoids, Tannin, saponin reducing sugar, flavonoid and saponin are present. Our research, that in bark extract were minimum germination activity as compares to fruit. Zuo et al., (2005) also reported that the inhibitory effect.

CONCLUSION

The current study put forward that *(Prunus amygdaloides)* is an allelopathic plant, that has capabity to inhibit the the seed germination of local wheat cultivars. Bark and fruit crude extracts contain different bioactive compounds e.g., alkaloids, flavonoids, steroids, terpenoids, saponins, tannins, reducing sugars, terpenes, which might be responsible for negative allelopathy effect. The reason is behind that these compounds act as growth inhibitor, which impacts on plant physiology.

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