

CONSTITUTIONAL COMPOSITION AND ALLELOPATHIC POTENTIAL OF JAMAN (*Syzygium cumini*) LEAVES AGAINST CANARY GRASS AND WHEAT

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ABSTRACT

*Excessive use of herbicides in the crop field threatens the environment by destroying flora and fauna. Allelopathic substances might be proved as a substitute of chemical herbicides to suppress the target plants. In this study the Jaman (*Syzygium cumini*) leaves were extracted with H₂O for its constitutional composition and then its allelopathic effects were investigated against pre- and post-germinated wheat and canary grass. By HPLC, total eight components were identified by comparing the chromatograms of the unknown with standard ones. The pre- and post-germinated bioassays were taken into consideration for wheat and canary grass separately. The results showed that over all 70% aqueous methanol extract of jaman leaves (2.5%-10%) increased the length of shoot and root linearly for both pre-germinated wheat bioassays (11.00-15.75 cm and 9.50-10.75) and post germinated wheat bioassays 14.50—17.00 cm and 10.35—12.75 cm, respectively) as compared with control ones. Similarly allelochemicals of jaman leaves depressed both the pre-germinated and post-germinated bioassays of canary grass as compared with control ones (6.75 - 1.75 cm and 4.25—0.90 cm for shoot and 4.00-0.45 cm and 1.85—0.30 cm for root, respectively). These results reveal that growth inhibition components may be present in the Jaman (*Syzygium cumini*) leaves and also certain components may be responsible for the allelopathic potential. Therefore, leaves of the titled plant may provide a possible material for the isolation and identification of allelopathic substances and may be helpful for the development of new natural herbicides*

Keywords: Allelopathy, identification, *Syzygium cumini*, test plant species, weedicides.

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INTRODUCTION

The extensive use of chemicals worked as a catalyst to shift the production frontier but the most critical factor of maintaining the environment clean has been totally ignored. It is observed that environmental effects of chemical herbicides and the limitations of their implementation have led to the increasing importance of non-chemical alternatives in the management of weeds (Challa and Ravindra, 1998). No doubt wheat production is the major factor of prosperity of most of the countries throughout world. Like other developing countries, wheat holds a major position in the economy of Pakistan. On average, 10% loss of production in Pakistan is due to the weeds that grow in wheat crop so weeds are thought to be undesirable and harmful (Iqbal *et al.*, 2010).

Throughout the world alternative means are being searched to protect the fauna and flora of the environment from the harmful effects of pesticides. Recently the allelopathic compounds are being utilized instead of weedicides in order to control the weeds throughout world. In this practice, the environment friendly components (allelochemicals) are released by the plants that affect other plants in their vicinity through stimulation or inhibition (Howell and Martens, 2000). These environment friendly components are found to be released to environment in sufficient quantities via root exudates, leaf leachates, roots and degrading plant residues which include a wide range of phenolic compounds such as benzoic and cinnamic acids, alkaloids, terpenoids and others. These allelochemicals are responsible to enhance the growth of certain plants while inhibiting the others (Faria *et al.*, 2011). Recently much attention has been paid to allelopathic effects of plants as a mean of biological control of weeds and some allelopathic plants have been identified to have herbicidal effects on some weeds (Cheema *et al.*, 2005; Wu, 2001) found that wheat allelopathy is prospective for the management of weeds, pests and diseases. Sorghum water extracts used in combination with ½ and ¼ suggested doses of fenoxaprop-*p*-ethyl, clodinafop-propargyl and carfentrazone + isoproturon reduced the weed dry weight similar to full dose of these weedicides (Cheema *et al.*, 2005).

So keeping in mind the herbicidal effects of plants constituents, the present study was carried out to identify and evaluate the allelopathic effects of Jaman (*Syzygium cumini*) leaves on canary grass and wheat.

MATERIALS AND METHODS

Plant material

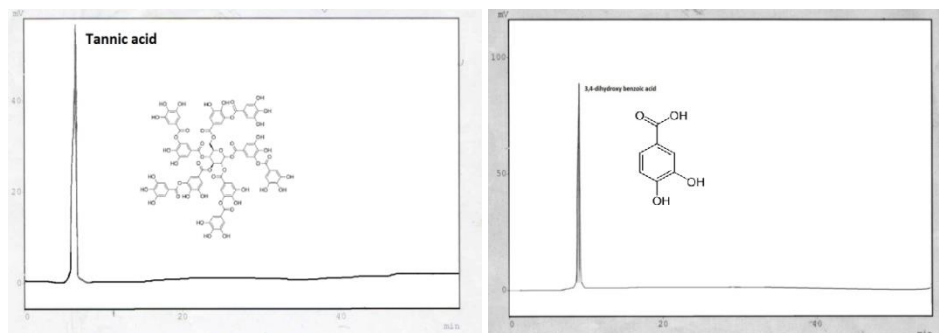
The leaves of the Jaman (*Syzygium cumini*) were collected in triplicate from three different trees from GC university Faisalabad new campus situated on Jhang Road Faisalabad Pakistan. The leaves were washed with distilled water, shade dried, ground into powder form in order to increase the surface area and then were stored in plastic bottle at low temperature before further use.

Seeds of the test plants

Healthy seeds of the wheat were purchased from the Faisalabad grain market. Similarly healthy seeds of the canary grass were obtained from Ayub Agriculture Research Institute Faisalabad, Pakistan. The canary grass was chosen as a test plant for bioassay along with wheat due to its existence as weed in the crop field throughout the world. Pre- and post- germinated bioassays of both of these test plants were studied in order to find out the allelopathic effects of the Jaman (*Syzygium cumini*) leaves. During pre- and post-germinated bioassays study of these test plants, shoot, root and fresh weight were taken into consideration.

Extraction

Ground leaves (50 g) of Jaman (*Syzygium cumini*) were extracted with 50ml of 70 % (V/V) aqueous methanol for two days. The extract was filtered under vacuum. The residue was re-extracted with 50 ml of same aqueous methanol for one day and was filtered again. The two filtrates were then combined and extracted with n-hexane to remove the less polar fraction. The original extract was then evaporated by rotary evaporator up to dryness at 40°C. The residue was re-dissolved into 2ml of HPLC grade methanol for analysis with reverse phase column and UV-visible detector at 278 nm. The components of the Jaman (*Syzygium cumini*) leaves were then identified qualitatively by comparing the retention time of the standard chromatograms with that of unknown one (Fig. 1).



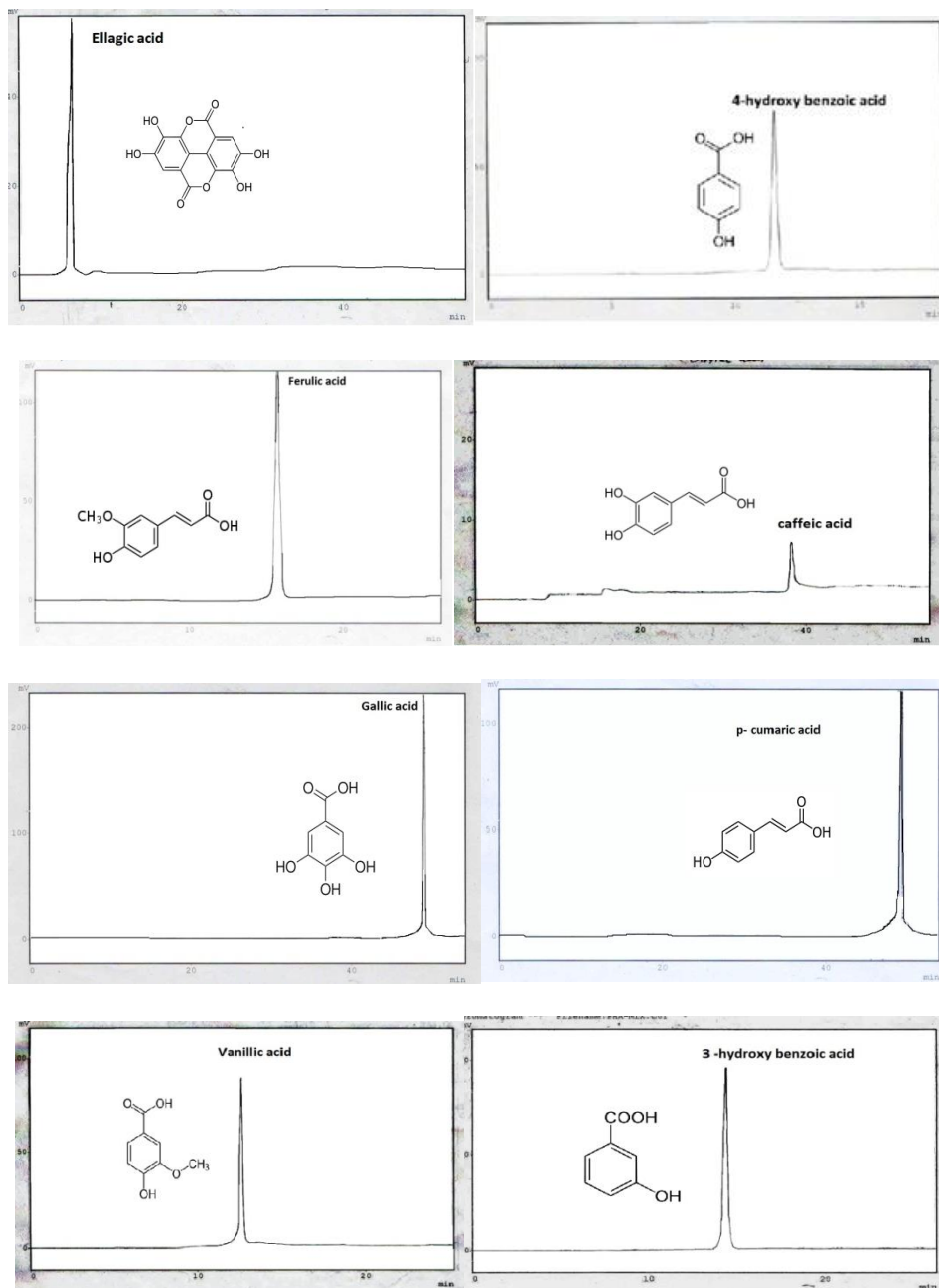
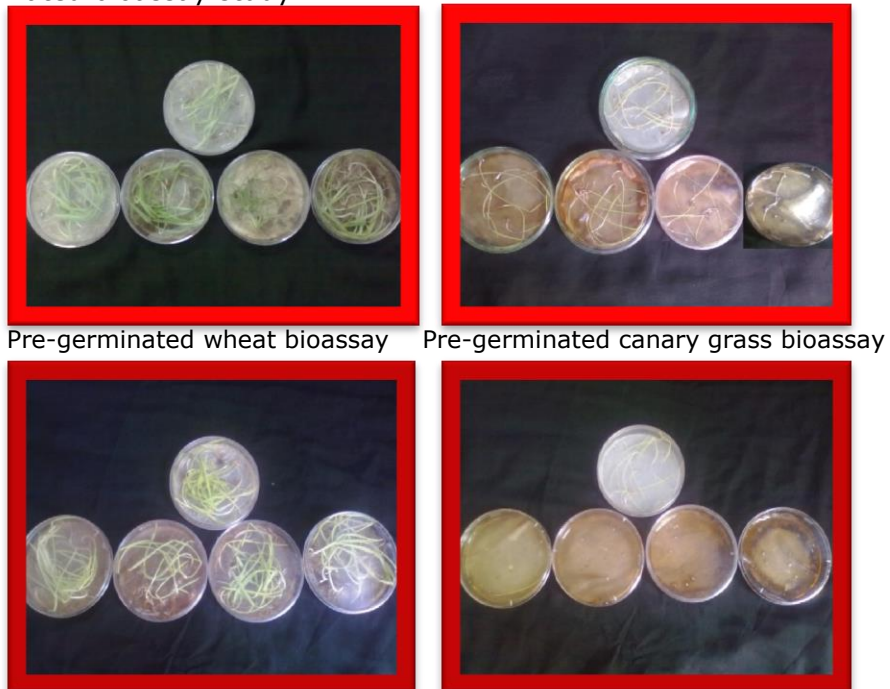


Figure 1. Chromatograms of allelochemicals expected in Jaman leaves.

Pre-germinated bioassays

Two hundreds wheat seeds were sown in 6 petri-dishes (10 cm diameter) between the two layers of filter paper Whatman No. 1. When almost all seeds were germinated, 10 germinated seeds were transferred into each petri-dish, put them at equal distances and were not covered. Each extract (4 ml) was applied in each petri dish except control and petri dishes were placed in an incubator at $24 \pm 2^\circ\text{C}$. Equal volume of distilled water was served as control. Later distilled water was applied in all the treatments to provide the required moisture, if needed. Three replicates were arranged in a randomized complete block design under the experimental conditions mentioned above. Shoot, root lengths and fresh weight of each plant in each petri-dish were noted 15 days after sowing. Then dry weight of all plants in each petri-dish was noted after oven drying at 40°C till constant weight (Gowri and Vasantha, 2010). Same procedure was adopted for canary grass. All the concentrations were tabulated in ascending order (2.5%, 5.0%, 7.5% and 10.0%). Similarly for post germinated bioassays ten seeds of wheat and ten seeds of canary grass were sown separately in the sterilized petri dishes between the two layers of filter paper Whatman No.1. The remaining procedure was same as for the pre-germinated bioassay study.



Pre-germinated wheat bioassay

Pre-germinated canary grass bioassay

Post-germinated wheat bioassay

Post-germinated canarygrass bioassay

Figure 2. Pre & post germinated bioassays of wheat and canary grass

RESULTS AND DISCUSSION

After extracting the Jaman (*Syzygium cumini*) leaves with 70% aqueous methanol, less polar fraction was separated with n-hexane. The polar methanol fraction was then evaporated up to dryness under vacuum. The residue was re-dissolved in 2ml of methanol and then was filtered through syringe filter prior to inject for HPLC analysis. 20 μ l was then injected for analysis by using methanol/water (3:1) mobile, reverse phase column and uv-visible detector (278 nm). The allelochemicals of the extract were identified qualitatively by comparing the retention time of the standard chromatograms (Fig. 1) with unknown chromatogram of the jaman (*Syzygium cumini*) leaves extract. Total 16 allelochemicals were eluted from the Jaman leaves (Fig. 3 a-p) but among them only 7 were identified (a-b, e, h-i, k, n and o) while allelochemicals that were eluted at retention time 12.00, 13.50, 16.00, 17.00, 36.00, 41.50, 45.50 and 52.00 minutes (d, f, h, j, l-m and p) were not identified due to unavailability of respective standards. Among the identified components the first allelochemical to be eluted was ellagic acid (5.5 min) while the last one to be eluted was p-cumaric acid (49.50 min). All others were eluted with in this time limit (Table-2). After identification the same extract was used in different concentrations (2.5%, 5%, 7.5% and 10%) to study the allelopathic effect of the titled plant against pre-germinated and post-germinated wheat and canary grass seeds.

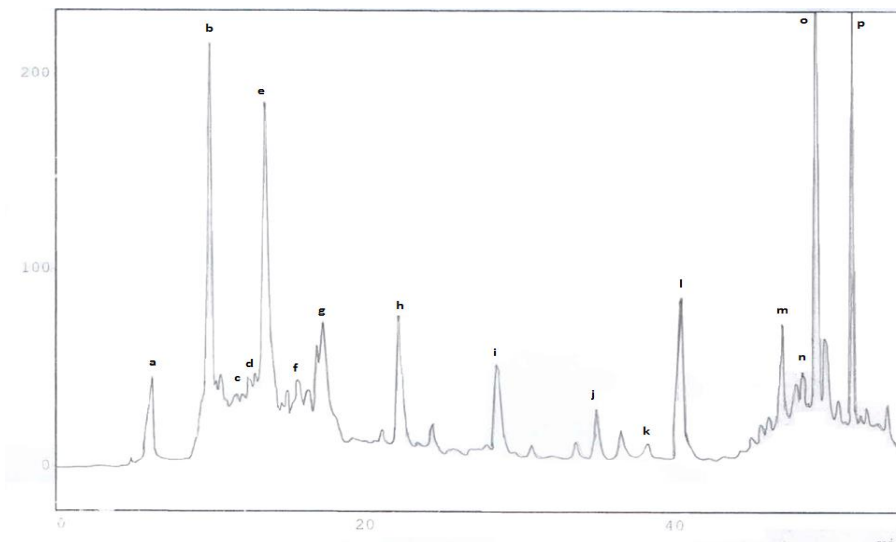


Figure 3. HPLC Chromatogram of various allelochemicals of jaman

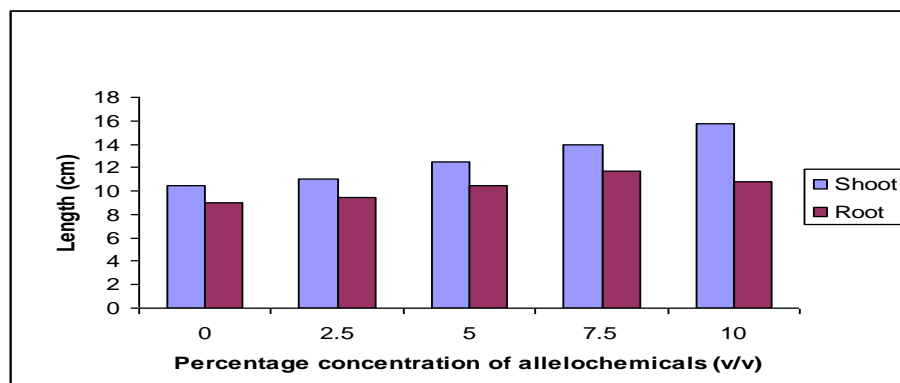
The bioassay study of the pre-germinated wheat and canary grass (Table-2 and -3) explained that in case of wheat allelochemicals/phenolic compounds of Jaman leaves linearly enhanced the length of shoot as well as root, as the concentrations of these phenolic compounds was increased (11.00 cm at 2.5% and 15.75 cm when the concentration of the allelochemicals was 10% for shoot and 2.5%/9.50 cm, 5%/10.00 cm, 7.5%/10.5 cm and 10%/10.75 cm for root, respectively) as compared with the control ones (10.50 cm & 9.00 cm) while the effect of these allelochemicals/phenolic compounds was observed inhibitory for both the development of shoot and root of canary grass (6.75 cm/2.5%, 6.00 cm/5%, 3.00 cm/7.5% and 1.75 cm/10%) and 5.75 cm/2.5%, 4.00 cm/5%, 2.75 cm/7.5% and 0.45 cm/10%, respectively) as compared with control (7.50 cm and 5.75 cm) ones. The fresh weight of the wheat was increased (2.75 mg-3.50 mg) and canary grass was decreased (0.65-0.1 mg) linearly as the concentration was increased. The results can be visualized in Fig. 4 and 5.

Table-1. Allelochemicals of the jamen (*Syzgium cumini*) leaves extract

Sr. no.	Allelochemicals	Retention time (minutes)
A	Ellagic acid	5.5
B	3,4- dihydroxy benzoic acid	10.55
C	Unknown	12.00
D	Unknown	13.50
E	3- hydroxy benzoic acid	14.00
F	Unknown	16.00
G	Unknown	17.00
H	Unknown	22.00
I	Ferullic acid	25.50
J	Unknown	36.00
K	Galucic acid	39.00
L	Unknown	41.50
M	Unknown	45.50
N	Gallic acid	47.50
O	p-cumaric acid	49.50
P	Unknown	52.00

Table-2. Effect of allelochemicals concentrations on bioassay of pre-germinated wheat

Sr.no.	Volume of extract in p. plate	Shoot length (cm)	Root length (cm)	Fresh weight (mg)
Control	4 ml	10.50	9.00.00	2.5
2.5%	4 ml	11.00	9.50	2.75
5%	4 ml	12.50	10.50	1.75
7.5%	4 ml	14.00	11.75	3.50
10%	4 ml	15.75	10.75	3.50

**Figure 4.** Effect of allelochemicals concentration on bioassay of pre-germinated wheat**Table-3.** Effect of allelochemicals concentrations on bioassay of pre-germinated canary grass

S. No.	Vol. of extract in p. plate	Shoot length (cm)	Root length (cm)	Fresh weight (mg)
Control	4 ml	7.5	5.75	1.15
2.5%	4 ml	6.75	4.00	0.65
5%	4 ml	6.00	2.75	0.60
7.5%	4 ml	3.00	1.30	0.07
10%	4 ml	1.75	0.45	0.10

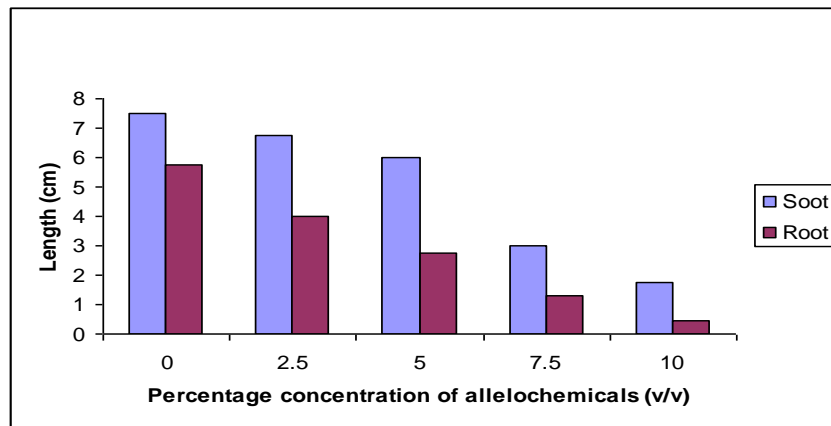


Figure 5. Effect of allelochemicals concentration on bioassay of pre-germinated canary grass.

Similarly, the results of the post-germinated wheat and canary grass seeds explain (Table-4 and-5) that the effect of the allelochemicals/phenolic compounds on the development of shoot as well as root at 4 concentrations of the allelochemicals/phenolic compounds (2.5% , 5%, 7.5% and 10%) was about similar to that pre-germinated one. The shoot length of the wheat was enhanced at all the concentrations as compared with the control (12.5 cm) being maximum (17.00 cm) at 10% concentration and minimum (14.5cm) at 2.5%. The root development of wheat showed somewhat irregular results (10.35 cm/2.5%, 11.80 cm/5%, 10.75 cm/7.5% and 12.75 cm/10%) as compared with the control one (9.50 cm). Both the shoot and root of the canary grass showed negative response at all the concentrations of the allelochemicals/phenolic compounds being minimum at 10% concentration (0.90cm and 0.30 cm) as compared with control (6.25 cm and 2.85cm) ones. In case of wheat the fresh weight was increased (2.75–4.50 mg) while in case of canary grass it was decreased (0.075–0.00 mg) linearly as compared with control ones.

The whole results are further illustrated in figure 6 & 7. Earlier the effect of weed allelopathic of sorghum (*Sorghum halepense*) on germination and seedling growth of wheat (Alvand cultivar) was observed. Aqueous extract of leaves, stems, seeds and roots of sorghum had a significant deterrent effect on wheat seeds germination. Allelopathic effect of leaves and stems of this plant had a significant deterrent effect on the growth of wheat seedling length although the roots extract increased partial longitudinal growth of wheat seedling. Extracts of different tissues of sorghum reduced also

the wheat seedling fresh weight (Hamid *et al.*, 2012). Similarly, it was found that allelopathic effect of Neem (*Azadirachta indica* A. Juss) against the germination of certain weeds was found inhibitory. Germination, root and shoot growth of all the tested plant species were inhibited at concentration greater than 0.001g dry weight equivalent extract/ml. It was further observed that the allelopathic effect was much higher on the root development than on the shoot growth of the test plants (Abdus-Salam and Hisashi, 2010). In another study allelopathic effects of Yarrow (*Achillam illefalium*) on the weeds of corn (*Zea mays* L.) were studied. Results showed that with increasing concentration (1.25-20%) of yarrow extracts, their effects on reducing seed germination and seedling growth of the test plants were more sever (Shafique *et al.*, 2007).

Table-4. Effect of allelochemicals concentrations on bioassay of post germinated wheat.

Sr.no.	Volume of extract in p. plate	Shoot length (cm)	Root length (cm)	Fresh weight (mg)
Control	4ml	12.5	9.50	2.60
2.5%	4ml	14.50	10.35	2.75
5%	4ml	14.90	11.80	3.00
7.5%	4ml	13.50	10.75	3.75
10%	4ml	17.00	12.75	4.50

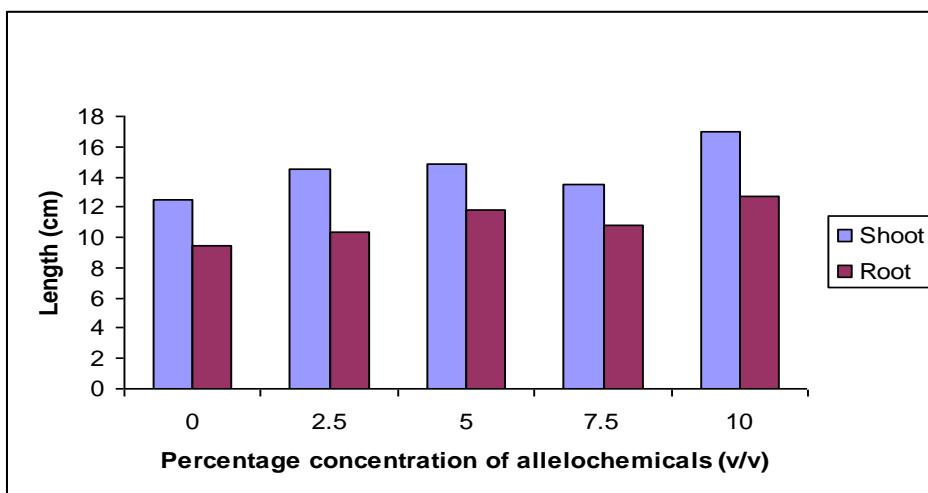
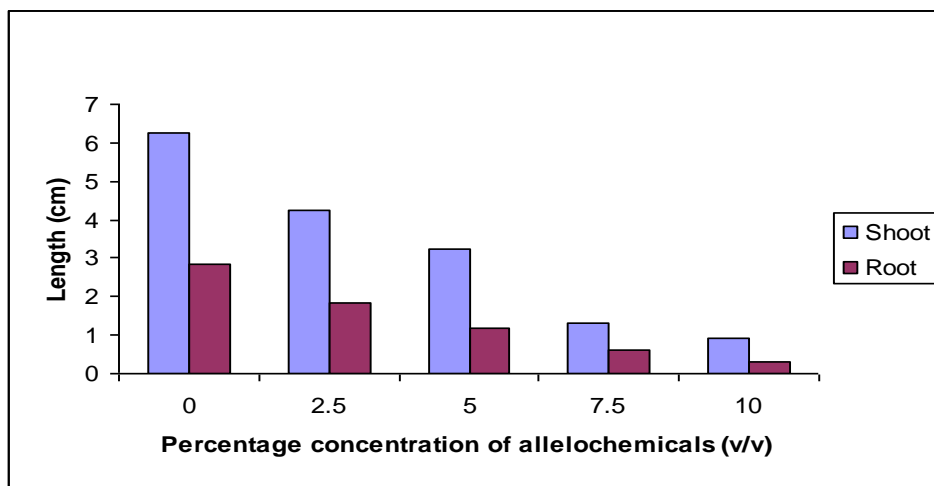


Figure 6. Effect of allelochemicals concentration bioassay of post germinated wheat

Table-5. Effect of allelochemicals concentrations on bioassay of post-germinated canary grass

S. No.	Vol. of extract in p. plate	Shoot length (cm)	Root length (cm)	Fresh weight (mg)
Control	4 ml	6.25	2.85	0.15
2.5%	4 ml	4.25	1.85	0.075
5%	4 ml	3.25	1.20	0.06
7.5%	4 ml	1.30	0.60	0.00
10%	4 ml	0.90	0.30	0.00

**Figure 7.** Effect of allelochemicals concentration on bioassay of post-germinated canary grass**CONCLUSION**

The chemical as well as HPLC analysis of the leaves of the Jaman (*Syzygium cumini*) justify that the title plant contains reasonable number of phenolic compounds/allelochemicals. Hence, this plant may be used as a substitute of the weedicides against various types of the weeds that direct or indirect affect the yield of the cash crop without polluting the environment and indirectly may improve the health standard of animals.

REFERENCES CITED

- Abdus-Salam, M. and K. Hisashi. 2010. Evaluation of allelopathic potential of Neem (*Azadirachta indica* A. Juss) against seed germination and seedling growth of different test plant species. Int. J. Sust. Agric. 2(2): 20-25.

- Challa, P. and V. Ravindra. 1998. Allelochemical composition of the herbal plants. *Allelopathy J.* 5: 89-92.
- Cheema, Z.A., A. Khaliq and I. Nadeem. 2005. Use of allelopathy in field crops in Pakistan. *J. Estab. Sci. Base.* 4: 550-553.
- Faria, A.F., M.C. Marques and D.Z. Mercadante. 2011. Identification of bioactive compounds from jambolao (*Syzygium cumini*) and antioxidant capacity evaluation in different pH conditions. *J. Food Chem.* 126(4): 1571-1578.
- Gowri, S.S. and K. Vasantha. 2010. Phytochemical screening and antibacterial activity of *Syzygium cumini* L. leaves extracts. *Intl. J. Pham. Tech. Res.* 2(2): 1569-1573.
- Howell, M. and K. Martens. 2000. Cultural weed control methods, controlling weed populations before they become a problem. *J. Eco-Agricul.* 30(8): 13-18.
- Iqbal, J., F. Karim and S. Hussain. 2010. Response of wheat (*Triticum aestivum* L.) crop and its weeds to allelopathic crop water extracts in combination with reduced herbicide rates. *Pak. J. Agric. Sci.* 47(93): 309-316.
- Shafique, S., A. Javaid and R. Bajwa. 2007. Effect of aqueous leaf extracts of allelopathic trees on germination and seed borne mycoflora of wheat. *Pak. J. Bot.* 39(7): 2619-2624.
- Wu, H., J. Pratley, D. Lemerle and T. Haig. 2001. Allelopathy in wheat (*Triticum aestivum*). *Annls. Appld. Biol.* 139(1): 1-9.