PHYTOTOXIC POTENTIAL OF WILD MEDICINAL PLANTS FROM DIFFERENT ALTITUDINAL GRADIENTS OF QUETTA BALOCHISTAN PAKISTAN ON Convolvulus arvensis L.

Shazia Saeed[1]*, Alia Ahmed[1], Syed Haider Shah[2], Sania Begum[3], Muhammad Zeeshan[1] and Waheed Khan[1]

https://doi.org/10.28941/24-4(2018)-2

ABSTRACT

Four wild medicinal and aromatic plants were studied for their allelopathic potential against Convolvulus arvensis. The wild plants were collected from different elevation zones of Quetta, Balochistan. Two species of genus Sophora viz. Sophora mollis (Royel) Baker. and S. alopecuroides L. were collected from Sra Ghurgai (Takatu mountain Range) and University of Balochistan Quetta campus, respectively. While Perovskia abrotanoides Karel. was collected from SraGhurgai and Zarghoon region of different elevation zones and Peganum harmala L. was collected from SraGhurgai (Takatu Mountain Range) and University of Balochistan Quetta Campus. Phytotoxic effect of the four plants was studied at various grades of aqueous extracts i.e 4 and 32% (w/v). To check germination impact and seedling growth of weed C. arvensis L. water-soluble phenolic content and its impact as inhibitory substance was also investigated along with the impact of altitudinal gradient on phenolic concentration and its effects on seed germination of C. arvensis. Water-soluble phenolic contents in the plant extracts were found. Total phenolic contents were higher i.e 0.122±0.08 in plants collected from higher altitudes which corresponded with the stronger inhibitory activity on C. arvensis. Total phenolic content was found as S. mollis>P.harmala>P.abrotanoides>S. alopecuroides. Final Germination Percentage of C. arvensis revealed that all tested wild plants leaf aqueous extracts significantly suppressed seed germination. Root and shoot length indicated that increased concentration of all plants extract showed significant reduction of C. arvensis Root and Shoot length. Our results revealed that these wild plants could prove as natural herbicide to control weeds like C. arvensis or serve as a template for synthetic herbicides.

Keywords: Allelopathy, aromatic plants, Convolvulus arvensis, phenolics, phytotoxicity, wild medicinal plants.

Citation: Saeed, S., A. Ahmed[1], S. H. Shah, S. Begum, M. Zeeshan and W.Khan.2018. Phytotoxic potential of wild plants from different altitudinal gradient of Quetta Balochistan on Convolvulus arvensis L. Pak. J. Weed Sci. Res., 24 (4): 323-334.

[1]Department of Botany, University of Balochistan, Quetta, Pakistan.
[2]Department of Statistics, University of Balochistan, Quetta, Pakistan.
[3]National Institute of Genomics and Advanced Biotechnology, NARC. Islamabad, Pakistan.
*Corresponding author's email: shazia_botany@yahoo.com

INTRODUCTION

Many plants are well-known to be phytotoxic. They release many allelochemicals and water soluble phytotoxins into their surroundings. The allelochemicals known as secondary metabolites and phytotoxic (Farooq et al., 2011). These compounds effect on early growth stages of plants, seed germination and seedling growth (Farooq et al., 2008; Jabran et al., 2010). These compounds also affect on the physiological and metabolic functions of plants (Einhelling, 2002). Allelopathic potential of many crop plants and weeds has been investigated against different crops (Kato-Noguchi and Tanaka, 2006). These allelochemicals have the potential to be used as natural herbicides, pharmaceuticals and biological control agents (Hirai, 2003; Cheema et al., 2004; Macias et al., 2007; Norton et al., 2008; Razzaq et al., 2010, 2012).

Phenolic compounds are defined as benzene ring containing compounds with one or more hydroxyl groups. Important phenolic compounds in plants have antioxiodant activity. These compounds play an important role in quality and nutritional value as colour, taste, aroma and flavor and also provide health beneficial effects (Maisuthisakul et al., 2007). There are 8000 identified phenolic compounds in plants, which are known as flavonoids, tannins, phenolic acids and coumarins (Howard et al., 2003). Flavonoids play important role in pigmentation of seeds and flowers. These also play a role in defense reactions against abiotic stresses like UV light or biotic stresses such as predator and pathogen attacks. Furthermore, it acts in fertility and reproduction of plants (Weisshaar and Jenkins, 1998; Shirley, 2001; Forkmann and Martens, 2001). It is also suggested that these are also involved in development and growth of plants (Murphy et al., 2000; Brown et al., 2001). The allelopathic effect of the following plants was investigated in our studies:

1. Sophora mollis (Royle) Baker.

A member of Family Fabaceae deciduous branched shrub upto 1.5 m tall found in rocks of dry valleys, 1200 - 2000 m (Fig. 1). Often gregarious on hillsides common in Balochistan. Yellow flowers in axillary racemes (Nasir and Rafiq, 1995)

2. S. alopecuroides L.

Herbaceous under shrub upto 1 m tall (Fig. 2). Creamish flowers on terminal spikes. Commonly found in Balochistan 400-2200 m elevations (Nasir and Rafiq, 1995).

3. Perovskia abrotanoides Karel.

Perovskia genus, Lamiaceae or mint family, has seven species such as P. abrotanoides, P. atriplicifolia and P. hybrida (Rechinger, 1982). P. abrotanoides is a shrub (Basher et al., 1997; 1997; Jassbi et al., 1999) Some of the pharmacological effects of plant such as anti-plasmodial, anti- inflammatory and cytotoxic effects have also been reported (Mohammadi, 2012). P. abrotanoides (Fig. 3) is used by local communities for treatment of typhoid, headache, gonorrhea, vomiting, motion, toothache, atherosclerosis, cardiovascular diseases, liver fibrosis, and cough, It has sedative, analgesic, antiseptic and cooling effect (Tareen et al., 2010)

4. Peganum harmala L.

It belongs to Family Zygophyllaceae, is a perennial herb (Figs. 4 and 5). It has a medicinal uses as anti-microbial, anti-inflammatory and analgesic properties. P. harmala L. is used in folk medicines. Its leaves are used in rheumatism treatment. Flowers creamish or white. Common plant 200-2500m wide spread from Sindh to Balochistan (Nasir and Rafiq, 1995)

Fig.6 Map of study area (Source Anon., 2012)

MATERIALS AND METHODS

Collection of plants and weed seeds

Two geographic sites selected for this research (Fig. 6). All these sites are rich in wild medicinal plants. Sites detail is given in Table-1 and Map as Fig. 6

Plants were collected from two adjacent areas, SraGhurgai (Takatu Mountain Range) and Zarghoonin the month of June and July 2013. Seeds of C. arvensis were collected from Botanical garden of UoB, Quetta. Gregarious growth of this weed was observed during the field survey and frequent visits of Botanical Garden. Therefore, this weed was selected for evaluating its susceptibility to aqueous extracts of wild test plants.

Preparation of aqueous extracts to check phytotoxicity

Leaves were dried in shade at room temperature. 16 g of plant material was soaked in 100 ml DDW and kept on orbital shaker (Edmand Bahler, VKS-75) for 24 h at room temperature (25±1.0) Extracted material was strained by two layers cheese cloth to remove solid materials. Then the extract was centrifuged at 5,000 rpm for 20 min. Then the filtrate was diluted to make different grades i.e. 4 and 32% (v/v). These extracts were kept at 4^0C for further experimental use. The pH was optimized at 6.8–7.4 (Macias et al., 2000; Singh et al., 2003).

Table-1. Ecological characteristics and habitats of study sites.

Plant Name	Site Name	Code	Latitude (°N)	Longitude (°E)	Elevation (m)	Annual Rainfall (mm)	Temp. Range (ºC)
Perovskia abrotanoides	Sraghurgai	PSR	30.21	67.12	2200-3200	200-400	-16-34
P.abrotanoides	Zarghoon	PZR	30.21	69.44	1980-3350	300-350	-15-20
Sophoramollis	Sraghurgai	SSR	30.20	66.54	1700-2600	114-250	-12-32
S.alopecuroides:	Botanical garden UoB	SBG	30.21	67.12	2200-3200	200-400	-16-34
Peganum harmala	Sraghurgai	PHSR	30.21	67.12	2200-3200	200-400	-16-34
P. harmala	Botanical garden UoB	PHBG	30.20	66.54	1700-2600	114-250	-12-32

Seed germination test

Germination test was carried out to with the treatment of different concentrations i.e 4% and 32%. Seeds of Convolvulus. arvensis (Fig. 7) were surface-sterilized for 2 min with 5% sodium hypochlorite aqueous solution (Oueslati, 2003). Placed 9 cm Whatman No. 1 filter paper in petri dishes. Arranged Ten seeds of weed C. arvensis in it. Poured 5ml of extracts at given concentrations and distilled water as control. Three replicates of every treatment were used. Petri dishes covered with para film to incubate at 25 ± 1^0C. Germinated seeds were counted daily and germinated seeds were discarded after counting. When radical of seed reached to 2 mm length then seeds were counted as germinated. After 7 days, calculated final germination percentage (FGP %) following Bewley and Black (1986) as: FGP % = n/N x100 where n = No. of seeds germinated and N represents number of seeds used in the test.

Growth of C. arvensis

Growth rate were estimated on the seedling growth of C. arvensis seeds. Seeds were placed in Petri dishes at 25 ± 1^0C temp and 16/8 h light–dark photoperiod for 7 days. Measured root and shoot length in cm (RL and SL respectively) by using a ruler (±0.1 cm).

Estimation of Total Phenolic Content (TPC)

Total phenolic content (TPC) of plants extracts were estimated by using Folin–Ciocalteu reagent according to Swain and Hillis (1959). 1 ml extract was mixed with 1 ml reagent (Folin–Ciocalteu reagent) to calculate total phenolic concentration. Add 1 ml of Na_2CO_3 (20%) in mixture after 3 min. Mix solution immediately and kept for 30 min in the dark. Prepared Blank with distilled water instead of extract. Absorbance was determined spectrophotometrically by using Shaimadzu Spectrophotometer 1601 S at 700 nm. Total Phenolic content (TPC) were measured against ferulic acid as a standard.

Statistical Analysis

Data were collected form three replicates. Mean and standard deviation of average of three were taken for recording of results. Data were analyzed by using Minitab Computer Software.

RESULTS

Total phenolic content

Total Phenolic content in aqueous extract of selected plants showed

variation among different species (Table-2) that might be due to difference in ecological characteristics of sites (Table-1). Results revealed that Phenolic amount was the maximum in S. mollis with a Mean and SD values of 1.78±0.04 (32% extract), while the minimum phenolic content was recorded in P. abrotanoides amounting to 0.134±0.31 (in 32 % extract solution) The amount of phenolics was increased significantly as the concentration of extract was increased.

Table-2. Mean±SE values of Total Phenolic content (mg/g).

Species	4% Extract	32% Extract
Perovskia abrotanoides	0.077±0.42	0.134±0.31
P. abrotanoides	0.122±0.08	0.171±0.11
Sophora mollis	0.089±0.04	1.78±0.04
S. alopecuroides	0.092±0.01	0.192±0.01
Peganum harmala	0.118±0.003	1.23± 0.01
P. harmala	0.157±0.28	1.11± 0.27

Fig. 1. Sophora mollison from the Hills of SraGurgai (Takatu Mountain Range), Balochistan, Pakistan.

Fig. 2. S.alopecuroides from Balochistan University Campus Quetta, Pakistan.

Fig. 3. Perovskia. Abrotanoides from Zarghoon, Balochistan, Pakistan.

Fig. 4. Pegnum hermala from Sragurgai (Takatu Mountain Range), Balochistan, Pakistan.

Fig. 5. Peganum harmala from different areas of Quetta City, Pakistan.

Fig. 7. Convolvulus arvensis gregariously growing in a field near Quetta city, Pakistan.

SEEDLING GROWTH

Effect of extracts on shoot length (SL)

Results recorded for the shoot length indicated that increased concentration of all plants extract showed significant reduction of C. arvensis Shoot length (SL). 32% extract solution of P. abrotanoides of Sites1 & 2 decreased the SL with mean value 2.44±0.58 and 2.14±0.61 respectively, while 4% extract solution with mean value 4.23±0.76 and 3.15±0.86 respectively as compared to control 8.13±0.81 (Fig. 8). At 32% extract of S. mollis and S. alopecuroides SL decreased with mean value of 2.50± 0.46 and 2.27±0.31 respectively as compared to control 8.13±0.81(Fig. 8). In 4% extract SL at S. mollis and S. alopecuroids decreased to 5.17 ±0.76 and 4.87±1.64, respectively as compared to control 8.13±0.81. 32% extract of P.harmala decreased the SL with mean value of 4±0.14 and 1.21±0.66, respectively as compared to control 8.41±0.55. The 4% extract SL of P.harmala exhibited 6.21±0.06 and 3.58±0.84, respectively as compared to control 8.41±0.55.

Effect of extracts on root length (RL)

Root length (RL) of C. arvensis was also inhibited significantly as the concentration of extract was increased. 32% extract solution of P. abrotanoides of Site 1 & Site 2 decreased the RL with mean value of 2.87±0.63 and 2.14±0.44 respectively also at 4% extract solution decrease the RL with mean value of 6.48±0.55 and 7.42±0.64, respectively (Fig. 9) as compared to control 9.27±0.31. Similarly decrease in RL of C. arvensis is greater in 32% in both species of S. mollis and S. alopecuroides decreases with mean value of 2.37± 0.55 and 2.20±0.72 respectively. On 4% extract RL decreased with mean value of 8.51 ±0.57 and 7.50±0.56 respectively (Fig. 9) as compared to control 9.27±0.31. At 32% extract of P.harmala leaves decreases RL with mean value 2.37±0.09 and 1.41±0.55 respectively, while the concentration of 4% extract decreased RL 8.11±0.18 and 6.14±1.21, respectively as compared to control 8.27±0.45 (Fig. 9).

Final Germination Percentage (FGP)

Data for the Final Germination Percentage of C. arvensis revealed that all tested wild plants leaves aqueous extracts significantly suppressed seed germination. At 4% concentration of S.mollis, P.hermala and P.abrotanoides (Site 2) shows 70% inhibition of seed germination. Whereas, P. abrotanoides (Site 1) and S. lopecuroides inhibited 60% seed germination. An increased dose of 32% concentration of leaves extracts of S.mollis and P.hermala suppressed the seed germination vigorously to 90%. Whereas S. alopecuroides and P. brotanoides suppressed 80 % germination (Fig. 10)

DISCUSSION

Our findings are supported by the previously reported work that the amount of total phenolics were increased significantly as extract concentrations increased (Sadoaeizadah et al., 2009). Gilani et al. (2010a, 2010b) also communicated similar findings while working on different plants. They were of the view that toxicity of leaf leachates increased with increasing concentrations from 10 mg to 50 mg. Allelopathic activities of different parts of plants were earlier studied to check inhibited growth of test species. Ageratum conyzoides L. was studied to check phytotoxic potential and inhibited germination and growth of radish (Raphanus sativus L.).Different plant parts of black mustard (Brassica nigra L.) were used to check strong weed-suppressive potential against germination and growth of wild barley (Hordeum spontaneum Koch.) [Xuan et al., 2004; Tawaha and Turk, 2003; Kadioglue et al., 2005: Mahboubi et al., 2009; Moallem and Niapour, 2008].

Also the geographical locations are taken into consideration. These differences may possibly be related to the natural climatic differences which occur over a particular geographical area as influenced by several climatic factors. It was previously reported comprising the recording of climatic data and agricultural practices at different geographical locations incorporating

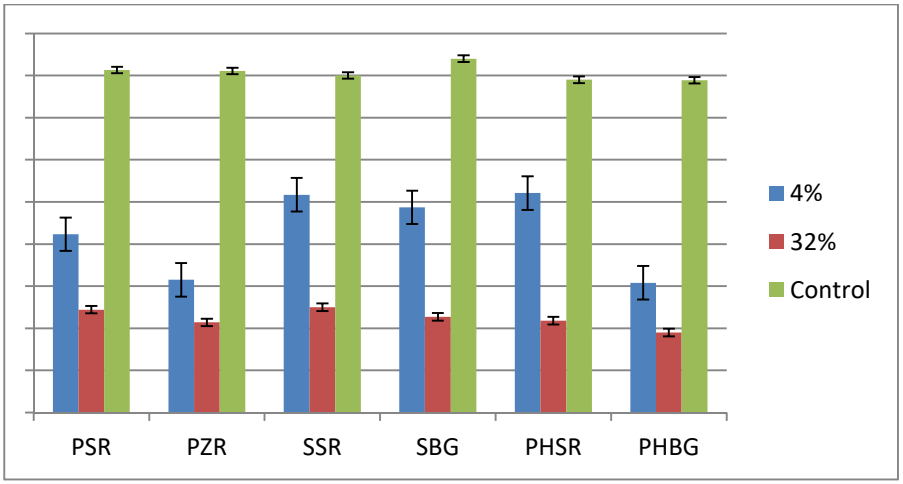

Fig. 8. Effect of different concentrations of aqueous extracts on shoot length.

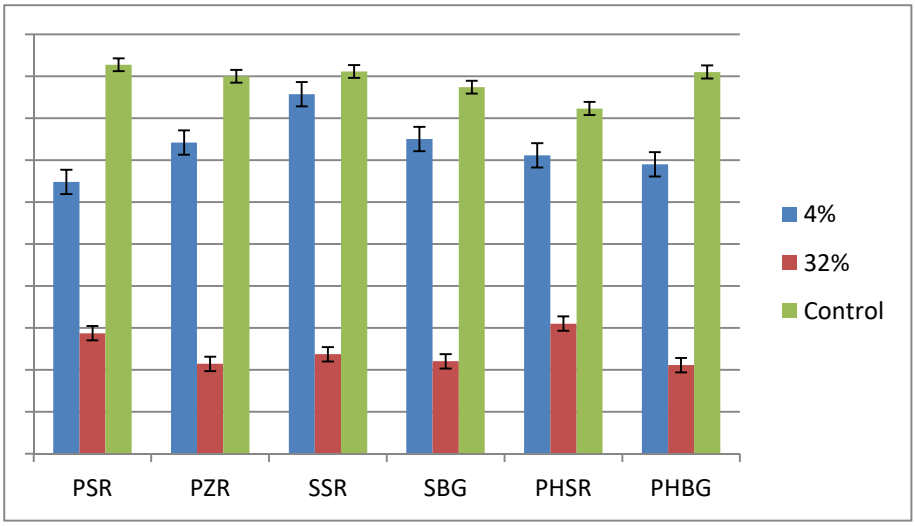

Fig. 9. Effect of different concentrations of aqueous extracts on root length.

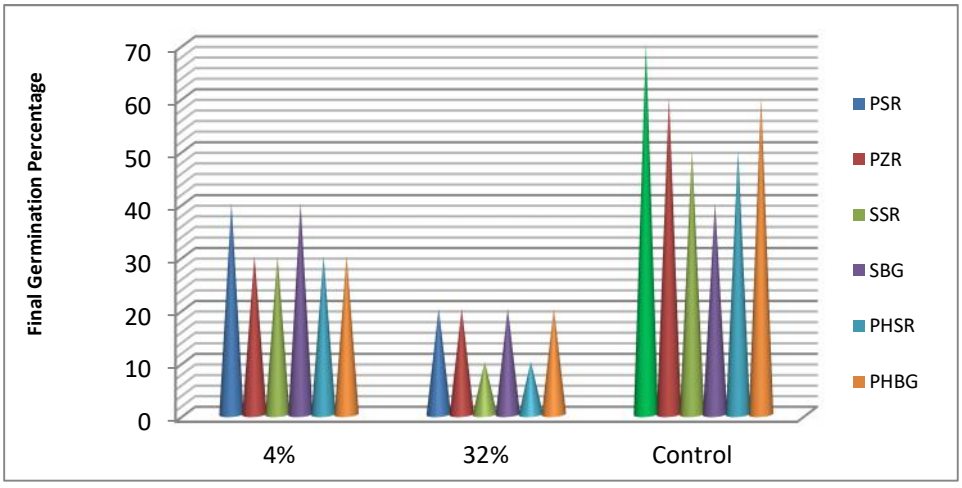

Fig. 10. Leaf Extracts effect on Final Germination Percentage.

seasonal patterns, climatic incidences, temperature, biotic and abiotic stresses. It changes secondary metabolites production in plants. Biotic and abiotic stresses, may influence the production of stress induced phenyl propanoid compounds. Nutritional stress like low iron levels cause increased release of phenolic acids, presumably to help solubilize metals and thereby facilitate their uptake (Marschner, 1991; Banerjee and Bonde, 2011). Climatic conditions have phenomenal influence in the content of camptothecin in Nothapodytes nimmoniana (Namdeo et al., 2010)

CONCLUSIONS

Our experimental findings show that the aqueous leaf extracts of selected plants collected from different elevations inhibited seeds germination and seedling growth of C. arvensis. It may be due to water soluble phenolic compounds present in extracts. The phytotoxic potential also showed considerable difference in impeding seed germination and growth of the studied weed, by different test species. Increased concentration resulted in the reduction of the weed growth and germination and vice versa. However, other factors such as topography, climatic and seasonal variation may affect the phenolic content. Overall, the findings exhibit the synthesis and use of natural herbicides or by incorporating the studied wild plants in agricultural systems for weed control. Other aromatic medicinal plants may also be selected for further phytotoxic analysis from different altitudinal zones of the province. Furthermore water-soluble phenolic compound must be identified by GC/MS or HPLC.

ACKNOWLEDGEMENT

Authors are obliged to Mr. Taj Muhammad Rind, Chief Conservator of Forest Balochistan, for plants collection.

REFERENCES CITED

Anonymous., 2012. Cameos Consultants. Engineering Firm. Chaman Housing Society, Quetta, Pakistan.

Basher, K.H.C., T. Ozek, B. Demirchakmak, B.Y.Abduganiev, K. R. Nuriddinov and K.N. Aripov. 1997. Essential oils of Perovskia angustifolia from Kyrgyzystan. ChemNat Comp., 33: 296 -98.

Banerjee, S. and C. G. Bonde. 2011. Total phenolic content and antioxidant activity of extracts of Bridelia retusa Spreng Bark: Impact of dielectric constant and geographical location. J. Med. Plant Res., 5(5):817-822.

Batish, D.R., K. Lavanya, H.P. Singh and R.K. Kohli. 2007. Root mediated allelopathic interference of Nettle-leaved Goosefoot (Chenopodium murale) on wheat (Triticum aestivum L.). J. Agron. Crop Sci., 193: 37-44.

Bewley , J.D. and M. Black. 1986. Seeds, physiology of development and germination, 2nd Ed. Plenum Press, New York.

Brown, D., A.M. Rashotte , AS. Murphy, J. Normanly, B.W. Tague, W. A. Peer, L. Taiz and G.K. Muday. 2001. Flavonoids act as negative regulators of auxin transport in vivo in Arabidopsis. Plant Physiol., 126, 524-535.

Cheema, Z.A., A. Khaliq and S. Saeed. 2004. Weed control in maize (Zea mays L.) through sorghum allelopathy. J. Sustain. Agric., 23: 73-86.ber 2007

Einhelling, F.A.2002. The physiology of allelochemical action: clues and views. In M.J. Reigosa and N. Pedrol. (eds.) Allelopathy from Molecules to Ecosystem. Science Publishers, Enfield, New Hampshire, USA.

Farooq, M., K. Jabran, H. Rehman and M. Hussain. 2008. Allelopathic effects of rice on seedling development in wheat, oat, barley and berseem. Allelopath. J., 22: 385-390.

Farooq, M., K. Jabran, Z.A. Cheema, A. Wahid and H.M. Siddique. 2011. The role of allelopathy in agricultural pest management. Pest Manage. Sci., 67: 493-506.

Forkmann, G. and S. Martens. 2001. Metabolic engineering and applications of flavonoids. Curr. Opin. Biotechnol., 12: 155-160.

Gilani, S.A., M. Adnan, A. Kikuchi, Y. Fujii, S. K. Shinwari, N. Kazuo and K. N. Watanabe. 2010a. Phytotoxic studies of medicinal plant species of Pakistan. Pak. J. Bot., 42(2), 987-996.

Gilani, S. A., Y. Fujii, Z. K. Shinwari, M. Adnan, A. Kikuchi and K. N. Watanabe. 2010b. Phytotoxic studies of medicinal plant species of Pakistan. Pak. J. Bot., 42(2): 987-996.

Grant, M. 2007. RHS Trial of Perovskia. RHS Plant Trials and Assessments, 23(6):447-916.

Hirai, N. 2003. Application of allelochemicals to agriculture. Biol. Sci. Space, 17:4-5.

Howard, L.R., J.R. Clark and C. Brownmiller. 2003. Antioxidant capacity and phenolic content in blueberries as affected by genotype and growing season. J. Sci. Food Agric., 83: 1238–1247.

Jabran, K., M. Farooq, M. Hussain, H. Rehman and M.A. Ali. 2010. Wild oat (Avena fatua L.) and canary grass (Phalaris minor Retz.) management through allelopathy. J. Plant Prot.Res., 50: 32-35.

Jassbi, A,R., V.R.Ahmad and R.B. Tareen. 1999. Constituents of the essential oil of Perovskia

atriplicifolia Benth. Flavour Fragrance J., 14: 38 – 40.

Kadioglue, I., Y. Yanar and U. Asav. 2005. Allelopathic effect of weed leachates against seed germination of some plants. J. Environ. Biol., 26: 169-173.

Kato-Noguchi, H. and Y. Tanaka. 2006. Abscisic acid-glucose ester as an allelopathy agent from citrus fruit. Acta Physiolgiae Plantatrum, 28: 635-639.

Macias, F.A., D. Castellano and J.M.G Molinillo. 2000. Search for a standard phytotoxic bioassay for allelochemicals. Selection of target species. J Agric. Food Chem., 48:2512–2521.

Macias, F.A., J. M. G. Molinillo, R. M. Varela and J. C. G. Galindo. 2007. Allelopathy: A natural alternative for weed control. Pest Manage. Sci., 63: 327-348.

Mahboubi, M and N. Kazempour. 2009. The antimicrobial activity of essential oil from Perovskia abrotanoides Karel and its main components. Ind. J. Pharma Sci.,71: 343–347.

Maisuthisakul, P., M. Suttajit and Pongsawatmanit. 2007. Assessment of phenolic content and radical scavenging capacity of some Thai indigenous plants. Food Chem.,100:1409-1418.

Moallem, S.A. and M. Niapour. 2008. Study of embryotoxicity of Perovskia abrotanoides, an adulterant in folk medicine, during organogenesis in mice. J Ethnopharma., 117: 108 –114.

Mohammadi, B. 2012. The evaluation of medicinal properties of Perovskiaa brotanoides Karel. Middle-East J. Sci. Res., 11: 189–193.

Murphy, A., W. A. Peer and L. Taiz. 2000. Regulation of auxin transport by aminopeptidases and endogenous flavonoids. Planta, 211: 315-324.

Namdeo, A.G., A.B.Sharma, D. P. Fulzele and K. R. Mahadik. 2010. Influence of geographical and climatic conditions on camptothecin content of Nothapodytes nimmoniana. Rec. Nat. Prod., 4 (1): 64-71.

Nasir Y.J .and R.A Rafiq. 1995. Wild flowers of Pakistan. Elite publishers limited Pakistan.

Norton, A.P., A.C. Blair, J. Hardin, S.J. Nissen and G.R. Brunk. 2008. Herbivory and novel weapons: No evidence for enhanced competitive ability or allelopathy induction of Centurea diffusa by biological control. Biol. Inv.,10: 79-88.

Oueslati, O. 2003.Allelopathy in two durum wheat (Triticum durum L.) varieties. Agric. Ecosyst. Environ., 96:161–163.

Razzaq, A., Z.A. Cheema, K. Jabran, M. Farooq, A. Khaliq, G. Haider and S.M.A. Basra. 2010. Weed management in wheat through combination of allelopathic water extracts with reduced doses of herbicides. Pak. J. Weed Sci. Res., 16: 247-256.

Razzaq, A., Z.A. Cheema, K. Jabran, M. Hussain, M. Farooq and M. Zafar. 2012. Reduced herbicide doses used together with allelopathic sorghum and sunflower water extracts for weed control in wheat. J. Plant Prot. Res., 52: 281-285.

Rechinger, K.H. 1982. Flora Iranica. Graz-Austria. Akademiche Druck-U verlagsantalt,150: 477.

Sadoaeizadah,H.,M. Rafieiolhossaini, J. Havlik and P.V.Damme. 2009. Allelopathic activity of different plant parts of Peganum hermala L. and identification of their growth inhibitors substances. Plant Growth Regul., 59:236.

https://doi.org/10.1007/s10725-009-9408-6.

Saeed, S., M.Y.K. Barozai, A. Ahmed and S.H.Shah.2014.Impact OF Altitude On Soil Physical and Chemical Properties in SraGhurgai [Takatu mountain range] Quetta, Balochistan. Int.l J. Scient. Engg. Res.,5(3):730-735.

Shirley, W. B. 2001. Flavonoid Biosynthesis. A Colorful Model for Genetics, Biochemistry, Cell Biology, and Biotechnology. Plant Physiol.,126: 485-493.

Singh, H.P., D.R. Batish, S. Kaur and R.K. Kohli. 2003. Phytotoxic interference of Ageratum conyzoides with wheat (Triticum aestivum). J. Agron. Crop Sci., 189 (3):341–343.

Singh, H.P., D. R. Batish, K. Shalinder, R. K. Kohli and K. S. Dogra. 2005. Allelopathic interference of Ageratum conyzoides L. against some crop plants. Weeds management: balancing people, planet, profit. Proc. 14th Aus. Weeds Conf., Wagga Wagga, New South Wales, Australia, 6-9: 558.

Swain, T and W. E. Hillis. 1959. The phenolic constituents of Prunus domestica I.. The quantitative analysis of phenolic constituents. J.Sci. Food Agric., 10:63–6.

Tareen, R. B., T. Bibi, M.A. Khan, M. Ahmad and M. Zafar. 2010. Indigenous knowledge of folk medicine by the women of Kalat and Khuzdar Regions of Balochistan, Pakistan. Pak J Bot., 42:1465–1485.

Tawaha, A. M. and M. A. Turk. 2003. Allelopathic effects of Black Mustard (Brassica nigra) on germination and growth of Wild Barley (Hordeum spontaneum). J. Agron. Crop Sci., 189:298–303

Weisshaar, B and G. Jenkins. 1998. Phenylpropanoid biosynthesis and its regulation. Curr. Opin. Plant Biol., 1: 251-257.

Xuan, T. D., T. Shinkichi, N.H. Hong, T.D. Khanhand and C.I. Min. 2004. Assessment of phytotoxic action of Ageratum conyzoides L. (billy goat weed) on weeds. Crop Prot., 23:915–922.