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SCREENING OF ACTIVE FORMULATION FROM COMBINED SUNFLOWER (*HELIANTHUS ANNUUS*) AND NEEM (*AZADIRACHTA INDICA*) AQUEOUS EXTRACT TO CONTROL GROWTH OF LESSER CANARY GRASS (*PHALARIS MINOR*)

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Abstract

The aim of study was to find an effective way of managing growth of lesser canary grass (*Phalaris minor*) in wheat fields. Sixteen formulations (F1-F16) were prepared by combining the aqueous extracts of sunflower (*Helianthus annuus*) and neem (*Azadirachta indica*) in specific ratios (10-40%). Remarkable allelopathic potential was exhibited by improved growth of wheat and inhibition of the weed growth. The regulatory effects were determined on plant root and shoot lengths, free proline and total phenolic contents, and the antioxidant potential of the wheat and weed plants in pot experiment. The findings suggested 'F3' as the most effective formulation prepared by combining one part of aqueous extracts of *Helianthus annuus* with three parts of *Azadirachta indica* extract [S (10%) + N (30%)], as its application (10 times dilution) stimulated the shoot length of wheat and inhibited the root length of canary grass plants. So, we propose this combination as an effective allelopathic strategy to control growth of lesser canary grass in wheat fields.

Key words: Allelopathy, Wheat, Canary grass, Phytochemicals.

Introduction

Wheat *(Triticum aestivum)* is among the main cereal and principal food for world population (Su *et al.*, 2016). Wheat is the most important resource of carbohydrates and provides 68% calories for humans (Choudhary, 2016). Pakistan is among the top ten wheat producing countries in the world (Chhokar *et al.*, 2008), however, the total wheat production has declined due to various reasons such as drought, salinity, flood and rapid growth of population (Ho *et al.*, 2017). Weed infiltration has also become a significant reason of reduction of wheat yield (Gomes *et al.*, 2017). Weeds not only reduce the yield of crop but also deteriorate the quality by seed mixing with grains (Rice, 2012).

Numerous weeds invade wheat fields (Willis & Rosinska, 2016), of these, lesser canary grass (*Phalaris minor*, family: Poaceae) is found as challenging weed in wheat crops (Su *et al.*, 2016). Lesser canary grass is an obnoxious competitive weed of wheat fields; its intrusion into wheat crops poses significant loss in the wheat yield due to alterations in germination and growth patterns of crop (Ho *et al.*, 2017). For two decades, 'isoproturon' herbicide has been employed to control this weed. However, the regular use has resulted in development of insecticide resistance in lesser canary grass which reduces the crop yield (Ho *et al.*, 2017).

Various weed management strategies have been adopted to control weed production in wheat fields (Huang *et al.*, 2016). Allelopathy has gained the interest of scientists in recent years (Sithole *et al.*, 2016). Allelopathy refers to the biochemical interactions among the plants found in the same locality; secondary metabolites released by any plant induce their beneficial or harmful effect on the other plant. The allelochemicals released by a plant escape into the surroundings and influence the growth of nearby plants (Gomez-Macpherson *et al.*, 2016). Thus phytochemicals may be utilized as an effective alternative mean to control weeds (Palma-Tenango *et al.*, 2017). Allelochemicals affects the cell division and elongation phases by inhibiting the enzymes regulating the seed germination (Tauro, & Narwal, 1992). Today, extracts of several plants have been utilized for their effective allelopathic activity. Wheat itself shows allelopathy against weeds, the activity has been attributed to the presence of hydroxamic acids, related compounds, and the phenolic acids. It was noted that this effect on weed was concentration dependent (Peerzada *et al.*, 2017). Allelochemicals include; alkaloids, phenolics, terpinoids, and flavonoids (Jabran, 2017), and their release depends on their concentrations in different plant tissues; flowers, leaves, seeds, root and stem of living or decaying plant materials (Sithole *et al.*, 2016). Allelochemicals present in the water extracts of many plants act as natural herbicide (Cheng *et al.*, 2016). Sunflower and neem are well known for its allelopathic potential (Javaid, 2010). Sunflower release certain water soluble terpenes and phenols that are liberated into the surroundings after decomposition of plant deposits in the form of leaching and root exudation. Allelochemicals released from sunflower actually cause cellular injury and disturb cell membrane permeability of other plants by affecting its antioxidant system hence, reducing the target plant ability to grow (Liu *et al.*, 2016). Plant parts of neem have also been used for the allelopathic activity, especially during the germination and seedling growth stages. Neem leaves contain hundreds of chemicals exhibiting the phytotoxic effects. Various parts of neem plants have been conventionally used to control crop pests, pests in stored grains, domestic insects, and in human and livestock medicine. Neem leaves have inhibited considerably the seedling growth and germination percentage of targeted plant species (Miled *et al.*, 2017).

The present study is therefore conducted to evaluate the allelopathic potential sunflower (*Helianthus annuus*) and neem (*Azadirachta indica*) aqueous extracts combination on growth of wheat and lesser canary grass *(Phalaris minor)* to suggest some effective bioherbicide alternative to synthetic herbicides to control canary grass growth.

Materials and Methods: Experiments were conducted from September 2015 to Februay 2016, at the Plant Nursery of University College of Agriculture (UCA), University of Sargodha, Pakistan. Leaves of *Helianthus annuus* (sunflower) and *Azadirachta indica* (neem) were obtained from agricultural resources UCA and identified at Department of Biological Science, University of Sargodha. Leaves were thoroughly washed with tap water and rinsed with distilled water. All leaves were air dried and ground to fine powder by electrical grinder and finely powdered by mortar and pestle. The ground powder was kept in polythene bags.

Extraction: Powdered leaves of *Helianthus annuus* (sunflower) and *Azadirachta indica* (neem) were added to the distilled water in a ratio 1:10, separately and placed on arbitrary shaker for 24 hours (Optima^R OS-752). Aqueous extracts from plant samples were filtered through Whatman filter paper No. 1, and the filtrates were preserved at 4^oC in refrigerator.

Preparation of plant formulations: Filtrates of both plants extracts were used as stock solutions (10% w/v). Stock solution of each plant aqueous extract was diluted to four dissolutions *i.e.* 10, 20 30 and 40% (v/v). The individual plant dissolutions were combined in different proportions to produce sixteen different formulations (F1-F16) (Table 1).

Table 1. Composition of different formulations.

Formulations	Plant combinations	
	Sunflower	Neem
F1	10%	10%
F2	10%	20%
F3	10%	30%
F4	10%	40%
F5	20%	10%
F6	20%	20%
F7	20%	30%
F8	20%	40%
F9	30%	10%
F10	30%	20%
F11	30%	30%
F12	30%	40%
F13	40%	10%
F14	40%	20%
F15	40%	30%
F16	40%	40%
Control	Water	

Determination of allelopathic effects: The allelopathic effects of all the formulations (F1-F16) were determined on growth attributes of wheat and lesser canary grass in a pot experiment. During experiment the average temperature was 30^oC. Assay was performed in polythene pots (8 inch height, 6 inch diameter) filled in with soil. In each pot 10 seeds of each plant were sown for each formulation with three replicates. Pots were moistened by water and monitored every day. Ten days after sowing, the plants of each pot were irrigated with 10 times diluted formulations and the experiment was terminated at 20^{th} day after irrigation with formulations. The effects of formulations on root length and shoot length of wheat and its weed were recorded with measuring tape.

Biochemical tests

Total phenolic contents: Total phenolic content was estimated according to Folin-Ciocalteu reagent method (Folin & Ciocalteu, 1927). Fresh leaves (50 mg) of wheat and lesser canary grass were homogenized by adding 1 mL of 80% acetone using mortar and pestle. After centrifugation for 10 minutes at 10,000 *g*, the supernatant was separated. To the supernatant (100 µL), distilled water (2.0 mL) and Folin–Ciocalteau's phenol reagent (1 mL) was added. Then 20% Na_2CO_3 solution was added and the final volume was made up to 10 mL with distilled water. Thoroughly mixed the solution and absorbance was recorded at 720 nm on spectrophotometer (Shimadzu, Japan). Gallic acid was used as standard for phenolics and thus total phenol content was calculated as milligrams of Gallic acid equivalents (GAE)/g fresh weight.

Proline estimation: Free proline contents were determined following method by Bates (Bates *et al.*, 1973). Each sample (500 mg) of fresh leaves of both plant species were homogenized with 3% sulfuric acid, and then centrifuged at 15000 rpm for 15 minutes under 4^oC. The supernatant (2 mL) was mixed with acid ninhydrin (2 mL) and 2 ml glacial acetic acid (1.25 g ninhydrin + 20 ml orthophosphoric acid + 30 ml glacial acetic acid). Samples were heated in a water bath at 100^oC for 1 hour, cooled, and added toluene (4 mL). Vortex for 20 minutes to get two distinct layers. The upper layer containing proline, was separated by separating funnel and discarded the lower one. The absorbance at 520 nm was noted on spectrophotometer. Proline contents of the samples were calculated using the standard curve from following formula;

$$\text{Proline (µmoles/g fresh weight)} = \frac{(\text{µg proline/mL} \times \text{mL of toluene})}{(115.5\ \text{µg/umole})\big/(\text{g sample}/5)}$$

Antioxidant potential: Antioxidant potential of the plant samples was determined by radical scavenging activity by the method described by Blois (2002). Each plant extract (3 mL) was added to 0.2 mM DPPH (1,1-diphenyl-2-picrylhydrazyl) methanolic solution (1 mL) as the free radical source, and the mixture was shaken and kept for 30 min at room temperature. The decrease in solution absorbance (517 nm) due to proton donating activity of plant components, indicate the higher free radical scavenging activity. Gallic acid was used as the positive control. The DPPH radical scavenging activity was calculated using the following formula;

$$\text{DPPH radical scavenging activity (\%)} = [(A_0\text{-}A_1)/A_0 \times 100]$$

where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract or standard sample.

Table 2. Effects of formulations on growth attributes of wheat and lesser canary grass: the effects on growth parameters are measured and the most affected attributes are underlined and represented in bold form.

Formulations	Growth attributes of wheat					Growth attributes of lesser canary grass				
	Proline contents (µmoll/ g.f.wt.)	Phenolic contents (mg)	Antioxidant potential (%)	Root length (cm)	Shoot length (cm)	Proline contents (µmoll/ g.f.wt.)	Phenolic contents (mg)	Antioxidant potential (%)	Root length (cm)	Shoot length (cm)
Standard	-	-	98.2	-	-	-	-	98.2	-	-
Control	0.051	5	94	9	36	0.18	5.8	94	6	6.5
F1	0.045	3	**93.9**	9	30	**0.02**	2.6	91.5	3	6
F2	0.05	3.3	91.3	8	30	0.08	5.2	90	2.75	5
F3	0.05	4	91.7	6	**45**	0.04	4.8	88.6	**2**	2.3
F4	0.05	4.4	92.3	7	39	0.04	4.4	88.8	3	4
F5	0.043	3.9	88.7	9	36	0.03	4.6	90.8	3	2
F6	0.044	4	90.9	**12**	40	0.03	4.7	**83.7**	2.5	4
F7	0.048	4.4	89.8	9	38	0.05	3.9	86.4	**2**	**1.5**
F8	0.041	4.3	92.8	6	38	0.07	4.5	88.3	3	4
F9	**0.07**	4.4	91.8	8	40	0.09	4.2	88.6	2.5	**1.5**
F10	0.06	4.4	89.2	8	38	0.098	2.4	87.8	**2**	2
F11	0.02	4.6	86	9	40	0.1	**2.4**	85.5	**2**	2
F12	0.05	5.1	91.9	7	40	0.1	2.8	88.8	**2**	3
F13	0.05	**5.4**	90	6.5	36	0.11	3.2	87.5	4	3
F14	0.051	4.5	91	7	40	0.06	4.7	89.1	3	4
F15	**0.07**	4.6	92.4	10	37	0.07	4.7	87.4	2.5	2.5
F16	0.05	4.7	91.2	**12**	42	0.14	4.3	87.8	3	2

Statistical analysis: The experiment was conducted using Completely Randomized Design (CRD) with three replications and data were subjected to one-way ANOVA and means were compared by Tukey's test at alpha = 0.05. The percentages of growth parameters were scaled so that control was 100%.

Results

Different fractions of combined aqueous extracts of sunflower and neem were evaluated for its effects on growth of wheat and lesser canary grass. Growth attributes like shoot length, root length, antioxidant activity, and proline and phenolic contents were studied (Table 2).

Effects on morphological attributes: In the present study, the prepared formulations (F1-F16) were tested for the growth (root length and shoot lengths) stimulatory effects on wheat and inhibitory effects on lesser canary grass in comparison to the control plants that were irrigated with distilled water only. Among all the formulations, we found that F6 showed the significant ($p<0.05$) stimulation of root length for wheat plants (33%) and also inhibited the root length of the lesser canary grass plants (-59%). The formulation F3 showed the significant shoot growth stimulatory effect on wheat (25%) and inhibitory effect on lesser canary grass (-65%) plants The morphological effects of formulation treatments on wheat and canary grass plants are presented in table 2.

Effects on biochemical attributes: We compared the effects of combined water extract formulations (F1-F16) on the amount of free proline; the major osmoregulator in the plants, and on the antioxidant potential of the weed and wheat plants by determining the amount of total phenolic contents and the radical scavenging activity of the plants. Among all the formulations, we found that the application of F9 formulation significantly enhanced the proline contents of wheat plants (37%), while F1 formulation reduced the proline contents of lesser canary grass plants (-12%). The formulation F13 showed the significant increase in TPC (8%) of wheat plants, while formulations F10 and F11 reduced the TPC (-59%) in lesser canary grass plants. Sunflower and neem combined formulation-F1 showed better free radical scavenging activity in wheat (93.9%), while F6 formulation poorly scavenged the free radicals (83.7%) in weed, indicating the increased amount of generated radicals in the plant tissues. The biochemical effects of the formulation treatments are presented in table 2.

Table 3. Effects of sunflower and neem aqueous extracts (alone/ combined) on wheat and weed growth.

	Plant extracts	Effects on wheat	Effects on weed	References
1.	Sunflower+ Neem	Root length 33% ↑ Shoot Length 25% ↑ Proline 37%↑ TPC 8%↑ Antioxidant activity 93.9%↑	Canary Grass Seed Root Length 67% ↓ Shoot Length 77%↓ Proline 12% ↓ TPC 59% ↓ Antioxidant activity 83.7%↓	Findings of present study
2.	Sunflower	Yield ↑	Canary Grass Seed Germination ↓ 30% Shoot Length ↓ 9% Root Length ↓ 66%	Hamad, 2017
3.	Sunflower	Yield 50%↑	Canary Grass Weed Density↓ Weed Biomass↓	Khan *et al*., 2015
4.	Sunflower + Sorghum	Grain Yield ↑ 89%	Canary Grass Dry Matter ↓ 36-55%	Jamil *et al*., 2009
5.	Sunflower + Sorghum+ Mulberry	Unaffected	Canary Grass Density ↓ 34-42%	Khaliq *et al*., 2012
6.	Sunflower	Yield ↑ 100%	Canary Grass Weed Density ↓52%	Khan *et al*., 2017
7.	Neem	Root Length ↑ Shoot Length ↑	Mung, Cow pea and Jowar Germination rate ↓	Kasarkar *et al*., 2016

Discussion

Allelopathy is an emerging field in agriculture and attracted farmers as being natural, non toxic and easy way to eradicate weeds. Allelopathic extracts may be applied together with the lower herbicide dose to achieve effective weed control. The work on allelopathy was initiated in Pakistan in early 1970s with screening of local flora for allelopathic potential in laboratory bioassays, while field studies were taken up during the early 1980s. Water extracts of allelopathic plants have been found effective mostly because the allelopathic activity may be attributed to the presence of suppressive allelochemicals in these extracts. It has been noted that in comparison to the single source, the mixtures of allelopathic water extracts showed more effective allelopathic activity (Cheema *et al.,* 2013). Recently a field trail was carried out by Khan *et al*., (2018) to determine the allelopathic potential of mulberry and sorghum water extracts against weeds of wheat; *Phalaris minor*. Retz, *Chenopodium album* L., *Avena fatua* L. and *Convolvulus arvensis* L. Application of combined sorghum water extract (SWE) and mulberry water extract (MWE) at the rate of 18 L ha^{-1} each exhibited better weed management (51-55%), improving the grain yield (28%) also as compared to control.

The novelty of present study is the application of mixture of neem and sunflower water extracts for use in allelopathy, as the individual and/ or combination of either neem or sunflower with other plant extracts have been reported previously (Table 3). We observed the significant effects of all the formulations on root and shoot lengths of wheat and weed seedlings. Among all the test formulations, F6 was found to be most effective in stimulating the root length of wheat and inhibiting the root length of weed plants. The formulations; F3, F7, F10, F11, and F12 showed the significant reduction of root length of weed seedlings as compared to that of control (dH_2O). The shoot length of wheat was significantly increased by the application of F3 formulation, whereas shoot length of weed was significantly reduced by treatments of F7 and F9 formulations. The root and shoot growth inhibitory effect on weed may be attributed to the permeability of allelochemicals into the tissues that may inhibit cell division at meristimatic tissue of the growing tip of root and shoot (Rawat *et al.,* 2017). Sunflower contains allelochemicals that exerts negative effect on germination, protein contents, and root and shoot lengths of the weed crops (Jabran, 2017). It has been found that plants stimulate the production of secondary metabolites with induction of stress which suggested that theses metabolite may be used not only for medicinal purpose but also as herbicides (Ologundudu, 2016).

Among the biochemical attributes of plant responses, proline gathered the key role in maintaining the integrity of cells. It has been an essential component of protein in plant cells and found to be over produced during stress conditions; most often to protect plants form the adverse environment (Lam *et al.,* 2012). Therefore, proline accumulation is a regular physiological response to various stresses where it acts as osmolyte and store nitrogen and carbon (Tshewang *et al.,* 2017). Besides acting as an excellent osmolyte, proline is also considered as a multifunctional amino acid **(**Szabados & Savoure, 2010). Although the way proline metabolism may stabilize cellular homeostasis during stress conditions is poorly understood, it has exhibited its role in proteins and protein complexes stabilization in the chloroplast and cytosol. Proline plays the major roles in cell protection during stress conditions as molecular chaperone, an antioxidative defense molecule and a signaling molecule (Hayat *et al.,* 2012). Herbicides also induce stressful environment in vicinity of plants. For example synthetic herbicides induce oxidative stress in plants which causes cell death of plant cells. The biological response of wheat plants to the chlorotoluron-induced oxidative stress was studied by Song *et al*., (2007) and found that application of herbicide (0–25 mg/kg) had induced the accumulation of O^{2-} and H_2O_2 in plant leaves and resulted in the lipid peroxidation of plasma membrane. In the chlorotoluron-exposed roots and leaves cells of the wheat plants the significant levels of accumulated endogenous proline were also noted affirming its role in alleviation of stress responses of wheat. In the present study it was found that the proline contents were increased in wheat plants while decreased in canary grass, after application of F9 and F1 formulations. We also noted the increased antioxidant potential of the wheat plants as compared to that of the canary grass plants. Therefore we propose that the accumulated proline also exhibited its antioxidant role and stabilized the wheat plant cells under bioherbicide application, the condition that was not developed in the canary grass plants due to decreased proline contents. Dar *et al.*, (2016) also described proline accumulation in plant tissues for plant stabilization as it acts as osmoregulator in plant cells (Dar *et al.*, 2016). We also suggest from our findings that the better growth of root and shoot length of wheat plants under bioherbicide application is also due to the presence of higher levels of proline contents. Similarly, phenolic compounds have shown promising role in crop production and development. The phenolic compounds in plants exhibit numerous biological activities including the antioxidant; free radical scavenging activities, and the anti-inflammatory activities (Miled *et al.*, 2017). It is reported that natural antioxidant are mainly extracted from plants consists of phenolic compounds like phenolic acids, flavonoid, tocopherols etc. Earlier, Akhtar & Arshad, (2013) compared the phytotoxic ability of aqueous extracts of new and old mango leaves against canary grass and wheat. All the extracts showed inhibition of germination and growth of canary grass (Akhtar & Arshad, 2013). The inhibitory action was attributed to the presence of high concentration of phenolic compounds. They suggested phenolic contents as effective constituents to be included in the preparation of herbicides to suppress canary grass growth in the wheat field (Gomez-Macpherson, 2016). However, in the present study we found that the application of most of the formulation treatments has increased the amount of phenolics in wheat as compared to the weed plants suggesting that the presence of higher amount of phenolic contents may affect growth of plants. Among the test formulations, F13 showed the better activity in increasing the phenolic contents of wheat plants, while F10 and F11 showed the decreased amount of phenolic contents in canary grass. Thus, findings of present study suggest that the application of formulations; F10 and F11 have significantly reduced the phenolic contents of the weed seedlings which may has resulted in decrease in the root length of weed plants (Table 2). Therefore, it may be inferred that phenolic compounds may helped in tissue stabilization and maintained the integrity of the cell membrane to promote growth of wheat plants. Among all the formulations, F1 formulation showed the most significant positive effect on antioxidant potential of wheat, while F6 formulation showed significant reduction in antioxidant potential of weed plants. Application of F6 formulation also showed the reduction in root length of the weed plants (Table 2) which suggested that the lowering of antioxidant potential of weed plants also influenced the root lengths of the plants and weed root tissues might not scavenge the stress induced generated free radicals so efficiently, causing tissue deterioration.

Based on observations of present research investigation, we found that among the 16 formulations, 7 formulations; F1, F3, F6, F7, F9, F10 and F11, affected profoundly at least two growth parameters of plants (Table 2). Therefore, we screened out the most active formulations exhibiting the growth stimulatory effects on the wheat and the growth inhibitory effects on canary grass plants, as shown in Table 4.

Further analysis of each selected active formulations showed that the formulations; F1 [S (10%) + N (10%)], F3 [S (10%) + N (30%)] and F6 [S (20%) + N (20%)] exhibited both the growth stimulatory effects on wheat and the growth inhibitory effects on weed plants (Table 4). Among these, only the F3 formulation; constituted by 1-part of sunflower and 3-parts of neem aqueous extracts, stimulated the shoot length of wheat plants and also inhibited the root length of canary grass (Table 4), so we propose this formulation as the most effective one and suggest it as best allelopathic alternative to synthetic herbicides.

Table 4A. Screening of active formulations with significant biological activity.

Plant Attributes	Growth stimulatory effects on wheat	Growth inhibitory effects on weed
Root length	F6	F3, F7, F10, F11, F12
Shoot length	F3	F7, F9
Proline contents	F9	F1
Total phenolic content	F13	F10, F11
Antioxidant potential	F1	F6

Table 4B. Screening of effective formulations exhibiting the growth stimulatory effects on wheat while the inhibitory effects on weed plants.

Formulations	Stimulatory effect on wheat	Inhibitory effect on weed
F1	+	-
F3	+	-
F6	+	-

In the present study, remarkable allelopathic potential was exhibited by 16 different formulations prepared by combining the aqueous extracts of sunflower and neem leaves to suggest the active formulation to control growth of lesser canary grass in wheat field as alternative to synthetic herbicides. Among all, the formulations F1 [S (10%) + N (10%)], F3 [S (10%) + N (30%)] and F6 [S (20%) + N (20%)] were screened out with strong allelopathic potential as exhibited by the growth stimulation of wheat, and inhibition of the weed plants. The F3 was the most effective allelopathic formulation which stimulated the shoot length of wheat plants and also inhibited the root length of canary grass plants. Further isolation and characterization of the active allelochemicals from this formulation will help to develop some effective bioherbicide that will not only reduce the growth of canary grass, but also stimulate the growth of wheat plants. Thus, we proposed F3 formulation as the effective bioherbicide for farmers to lessen the use of synthetic herbicides in wheat field, and also to reduce the health risks.

References

Akhtar, K.P. and H.M.I. Arshad. 2013. Identification of phenolics in mango leaves extract and their allelopathic effect on canary grass and wheat. *Pak. J. Bot.*, 45: 1527-1535.

Bates, L.S., R.P. Waldren and I.D. Teare. 1973. Rapid determination of free proline for water-stress studies. *Plant Soil*, 39: 205-207.

Blois, M.S. 2002. Antioxidants determinations by the use of stable free radical. *Nature*, 26: 1199-1200.

Cheema, Z.A., A. Zahid, M. Farooq and A. Khaliq. 2013. Application of allelopathy in crop production: success story from Pakistan. In*: Allelopathy current trends and future applications.* (Eds.): Cheema, Z.A., A. Zahid, M. Farooq and A. Khaliq. Springer, Berlin, Heidelberg, 113-143.

Cheng, F., Z. Cheng, H. Meng and X. Tang. 2016. The garlic allelochemical diallyl disulfide affects tomato root growth by influencing cell division, phytohormone balance and expansin gene expression. *Front. Plant Sci.*, 7.

Chhokar, R.S., S. Singh and R.K. Sharma. 2008. Herbicides for control of isoproturon-resistant Little seed Canary grass (*Phalaris minor*) in wheat. *Crop Protec.*, 27: 719-726.

Choudhary, S. 2016. Biochemical tests of different food products used frequently by the human population (Doctoral dissertation, Mahatma Jyoti Rao Phoole University).

Dar, M.I., M.I. Naikoo, F. Rehman, F. Naushin and F.A. Khan. 2016. Proline accumulation in plants: roles in stress tolerance and plant development. In: *Osmolytes and Plants Acclimation to Changing Environment: Emerging Omics Technologies.* Springer India. pp. 155-166.

Folin, O. and V. Ciocalteu. 1927. On tyrosine and tryptophane determinations in proteins. *J. Biol. Chem*., 73: 649-672.

Gomes, M.P., Q.S. Garcia, L.C. Barreto, L.P.S. Pimenta, M.T. Matheus and C.C. Figueredo. 2017. Allelopathy: An overview from micro-to macroscopic organisms, from cells to environments, and the perspectives in a climate-changing world. *Biologia*, 72: 113-129.

Gomez-Macpherson, H., J.A. Gomez, F. Orgaz, F.J. Villalobos and E. Fereres. 2016. Soil Conservation. In: *Principles of Agronomy for Sustainable Agriculture.* Springer International Publishing, pp. 241-254.

Hamad, S.W. 2017. Bioherbicidal properties of sunflower (*Helianthus annuus* L.) and its activities in weed management. Ph.D. Thesis, Newcastle University, UK.

Hayat, S., Q. Hayat, M.N. Alyemeni, A.S. Wani, J. Pichtel and A. Ahmad. 2012. Role of proline under changing environments: a review. *Plant Signal Behav.,* 7: 1456-1466.

Ho, C.H., H.S. Lur, M.H. Yao, F.C. Liao, Y.T. Lin, N. Yagi and H.J. Lu. 2017. The impact on food security and future adaptation under climate variation: a case study of Taiwan's agriculture and fisheries. *Mitig. ADAPT. Strat. Gl.*, 1-37.

Huang, M. T., Y.C. Lu, S. Zhang, F. Luo and H. Yang. 2016. Rice (*Oryza sativa*) laccases involved in modification and detoxification of herbicides atrazine and isoproturon residues in plants. *J. Agri. Food Chem.,* 64: 6397-6406.

Jabran, K. 2017. Allelopathy: Introduction and Concepts. In: Eds. *Manipulation of Allelopathic Crops for Weed Control.* Springer International Publishing, pp. 1-12.

Jamil, M., Z.A. Cheema, M.N. Mushtaq, M. Farooq and M.A. Cheema. 2009. Alternative control of wild oat and canary grass in wheat fields by allelopathic plant water extracts. *Agron. Sustain. Dev.*, 29: 475-482.

Javaid, A. 2010. Herbicidal potential of allelopathic plants and fungi against Parthenium hysterophorus–a review. *Allelopath. J.*, 25: 331-334.

Kasarkar, A.R. and A.N. Barge. 2016. Effect of aqueous extract of neem (*Azadirachta indica* A. Juss) leaves on germination and growth of some agricultural crops. *J. Med. Plant Res.*, 4*:*11-13.

Khaliq, A., A. Matloob, A. Tanweer and M.B. Khan. 2012. Naturally occurring phytotoxins in allelopathic plants help reduce herbicide dose in wheat. *Nat. Prod. Res.,* 26: 1156-1160.

Khan, E.A., A.A. Khakwani, M. Munir and Ghazanfarullah. 2015. Effects of allelopathic chemicals extracted from various plant leaves on weed control and wheat crop productivity. *Pak. J. Bot.*, 47: 735-740.

Khan, F., S.K. Khalil, A. Rab, I. Khan and H. Nawaz. 2017. Allelopathic potential of sunflower extract on weeds density and wheat yield. *Pak. J. Weed Sci. Res.,* 23.

Khan, S., S. Irshad, F. Mehmood and M. Nawaz. 2018. Combined application of sorghum and mulberry water extracts is effective and economical way for weed management in wheat. *Asian J. Agri. & Biol*., 6: 221-227.

Lam, Y., C.W. Sze, Y. Tong, T.B. Ng, S.C.W. Tang, J.C.M. Ho and Y. Zhang. 2012. Research on the allelopathic potential of wheat. *Agr. Sci.*, 3*:* 979.

Liu, X., F. Tian, Y. Tian, Y. Wu, F. Dong, J. Xu and Y. Zheng. 2016. Isolation and identification of potential allelochemicals from aerial parts of *Avena fatua* L. and their allelopathic effect on wheat. *J. Agri. Food Chem.*, 64: 3492-3500.

Miled, H. B., M. Saada, I. Jallali, Z.B. Barka, M. Tlili, H. Alimi and O. Tebourbi. 2017. Variability of antioxidant and biological activities of *Rhus tripartitum* related to phenolic compounds. *EXCLI J.*, 16:439.

Ologundudu, A.F. 2016. Allelopathic potential of neem leaf aqueous extract and rice husk residue on the growth and yield of tomato (*Solanum lycopersicum* L.). *Biosci. Methods*, 7.

Palma-Tenango, M., M. Soto-Hernández and E. Aguirre-Hernández. 2017. Flavonoids in Agriculture. In: *Flavonoids-From Biosynthesis to Human Health.* In Tech.

Peerzada, A.M., H.H. Ali, Z. Hanif, A.A. Bajwa, L. Kebaso, D. Frimpong and S. Manalil. 2017. Eco-biology, impact, and management of *Sorghum halepense* (L.) Pers. *Biol. Invasions*, 1-19.

Rawat, L.S., R.K. Maikhuri, Y.M. Bahuguna, N.K. Jha and P.C. Phondani. 2017. Sunflower allelopathy for weed control in agriculture systems. *J. Crop Sci. Biotechnol.,* 20: 45-60.

Rice, E.L., 2012. *Allelopathy*, Academic press.

Sithole, N.J., L.S. Magwaza and P.L. Mafongoya. 2016. Conservation agriculture and its impact on soil quality and maize yield: A South African perspective. *Soil Tillage Res.,* 162: 55-67.

Song, N.H., X. Le Yin, G.F. Chen and H. Yang. 2007. Biological responses of wheat (*Triticum aestivum*) plants to the herbicide chlorotoluron in soils. *Chemosphere*, 68:1779-1787.

Su, Z., S. Jin, Y. Lu, G. Zhang, S. Chao and G. Bai. 2016. Single nucleotide polymorphism tightly linked to a major QTL on chromosome 7A for both kernel length and kernel weight in wheat. *Mol. Breed.,* 36: 15.

Szabados, L. and A. Savoure. 2010. Proline: a multifunctional amino acid. *Trends Plant Sci.*, 15: 89-97.

Tauro, P. and S.S. Narwal. 1992. Proceedings First National Symposium. In: *Allelopathy in Agroecosystems (Agriculture And Forestry).* Indian Society of Allelopathy and Icar; Hisar.

Tshewang, S., R.F. Park, B.S. Chauhan and A.K. Joshi. 2017. Challenges and prospects of wheat production in Bhutan: A review. *Exp. Agr.*, 1-15.

Willis, R.J. and G.J. Rosinska. 2016. *Allelopathy.* In: *The Life of Plants and Their Communities.* Scientific Publishers.

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