

## ***In vitro* allelopathic effect of aqueous extracts of sugarcane on germination parameters of wheat**

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Received January 26, 2017; accepted March 27, 2017.

Delo je prispelo 26. januarja 2017, sprejeto 27. marca 2017.

### **ABSTRACT**

Allelopathy – interactions among plants for resources along with competition – is a composite phenomenon which has spacious potentials of application in agriculture. Understanding of interactions among plants, particularly cultivated crops, may be helpful in modifying crop cultivation pattern with consequent yields increments. In this study, we investigated the allelopathic effects of aqueous extracts of root, stem peels and leaves of sugarcane (*Saccharum officinale* L.) cultivar 51 at concentrations 0, 2.5, 5.0, 7.5 and 10.0 g/l on germination indices and seedling biomass of wheat (*Triticum aestivum* L.) cultivar Pirsabak-2005. Results demonstrated that higher concentration (10.0 g/l) of extracts of root, stem peels and leaves significantly decreased mean germination time (MGT) but increased shoot and seminal root growth and seedling dry biomass; however, germination percentage was affected neither by extract concentration nor by plant parts used in the study. Extract concentrations up to 7.5 g/l had no effect on the studied parameters of wheat. Our result suggests that sugarcane's allelopathy demonstrates healthy effects on wheat growth and that wheat could be cultivated in sequential rotation in field conditions.

**Key words:** allelopathy; sugar cane; common wheat; germination parameters; biomass; crop rotation

### **IZVLEČEK**

#### ***In vitro* ALELOPATSKI UČINKI VODNIH IZVLEČKOV SLADKORNEGA TRSA NA PARAMETRE KALITVE NAVADNE PŠENICE**

Alelopatija – interakcije med rastlinami za vire preko tekmovanja – je kompleksen fenomen, ki ima za uporabo v kmetijstvu velik pomen. Razumevanje teh interakcij med rastlinami, še posebej med gojenimi, lahko pomaga pri spreminjanju načinov pridelave z znatnim povečanjem pridelka. V raziskavi so bili preučevani alelopatski učinki vodnih izvlečkov korenin, stebel in listov sladkornega trsa (*Saccharum officinale* L.), kultivarja 51, v koncentracijah 0, 2.5, 5.0, 7.5 in 10.0 g/l na kalitvene parametre in biomaso kalic krušne pšenice (*Triticum aestivum* L.), sorte Pirsabak-2005. Rezultati so pokazali, da so večje koncentracije (10.0 g/l) izvlečkov korenin, stebela in listov značilno zmanjšale povprečni čas kalitve (MGT), a povečale rast semenskih korenin in poganjkov ter biomaso kalic, na odstotek kalitve pa niti koncentracija izvlečkov niti rastlinski organ nista vplivala. Izvlečki v koncentracijah do 7.5 g/l niso imeli učinka na preučevane parametre pšenice. Rezultati nakazujejo, da ima sladkorni trs pozitivne alelopatske učinke na rast pšenice in da lahko poljščino gojimo v kolobarju neposredno za sladkornim trsom.

**Ključne besede:** alelopatija; sladkorni trs; krušna pšenica; biomasa; parametri kalitve; kolobar

## **1 INTRODUCTION**

Allelopathy is a composite process occurring in natural habitats as well as in cultivated fields and is generally perceived as a mechanism of plants and microbes' capacity to maintain their dominance over others or at least to coexist in a given environment. Through

allelopathy alone or in combination with competition, plants can influence survival capability of in-range plants and other microorganisms in a manner that they are constrained to either migrate to somewhere else or to remain in the habitat in a defensive mode. The

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influential capacity of one plant over the other is because of the metabolic compounds (known as allelochemicals) they release into the surrounding environments either as volatile substances, rain leachates, decomposed products or direct secretion to rhizosphere with potential negative or positive interactions with other plants and microbes (Barkosky et al., 2000; Barto et al., 2010; Rice, 2012). Allelochemicals are generally secondary metabolites present in different concentrations in different plant parts (leaves, stem, barks, flowers, seeds etc.) which upon release into the rhizosphere tend to modify the resource consumption capacity by several mechanisms i.e., alteration of cell membrane permeability, changing enzymatic activity, triggering genetic defects and disturbing photosynthesis of the competitor plants (Gonzalez and Estevez-Braun, 1997; Wu et al., 2000; Barto et al., 2010; Majeed et al., 2012). Interactions among plants for resources through allelopathy lead to physiological and biochemical modifications which may result in the establishment of a successful plant community by eliminating or restricting the susceptible species, although many plants exhibit positive allelopathic effects on the surrounding plants (Elijarrat and Barcelo, 2001; Maharjan et al., 2007; Hussain et al., 2010). Thus allelopathy may be successfully employed in agriculture for enhancing crop productivity and weed management (Fang et al., 2013).

Common wheat (*Triticum aestivum* L.) in the family Poaceae is an important agronomic crop widely cultivated for grains which are used in food and several other processed products. After maize and rice, wheat is ranked the third most widely produced grain crops in the world which has significant impact on meeting global food demands and dietary needs (Hou et al., 2014). The crop in Pakistan is generally cultivated in rotation with several other crops such as maize, rice,

tobacco and sugarcane. Knowledge about allelopathic nature of preceding crops may be helpful in modifying cultivation pattern of wheat with other crops. During the last few years, allelopathic activities of several plants and agricultural crops including wheat have been rigorously worked out (Khaliq et al., 2013; Muhammad and Majeed, 2014). Sampietro and Vattuone (2006) observed root elongation of some selected weeds and crops at lower concentrations while declined root growth at higher concentration of aqueous extracts of sugarcane straw. Nikneshan et al. (2011) investigated aqueous extracts of air-dried leaf powder of eight cultivars of sunflower for allelopathic activities on wheat with decrease in germination indices at higher extract concentrations. Majeed et al. (2012) observed drastic effects of higher but stimulatory effects of lower concentrations of aqueous extracts of *Chenopodium album* L. on plant height, tillers, spike length and grain yield of wheat. Variable results were obtained for germination, plant height; fresh and dry mass of shoot and root of wheat under the allelopathic influence of three weeds species viz: *Asphodelus tenuifolius* Cav., *Euphorbia hirta* L., and *Fumaria indica* (Hauskn.) Pugsley (Jabeen et al., 2013). Ullah et al. (2013) recorded suppressive effects on germination of 20 varieties of wheat treated with aqueous leaf extracts of *Eucalyptus camaldulensis* Dehnh., *Acacia nilotica* (L.) Willd. ex Delile, *Helianthus annuus* L. and *Parthenium hysterophorus* L.

Although sugarcane's allelopathy has been worked out against weeds and some crops; however, reports on its allelopathic investigation against wheat are scarce in literature. The aim of this work was to study the allelopathic activity of root, stem peels and leaves of sugarcane on germination and seedling growth of wheat in laboratory conditions.

## 2 MATERIALS AND METHODS

### 2.1 Plant materials

Mature plants of Sugarcane (*Saccharum officinale* L.) cultivar 51 were collected at harvesting stage from cultivated fields in Naguman, Peshawar during 2013. Different plants parts i.e., leaves, stem peels and root were separated and dried under shade conditions. Dried parts were ground to powder with an electric grinder for further use. In order to get aqueous extracts of different concentration, 2.5, 5.0, 7.5 and 10.0 g of dried powder of each part i.e., leaves, stem peels and roots were soaked for 24 h in 1litre distilled water each at room temperature. The soaked materials were filtered through muslin cloth after 24 hours. Filtrate was again filtered through filter paper (Whatman No. 1) in

sterilized flasks to get extracts of different concentrations. Aqueous extracts were stored at 4 °C in a refrigerator.

### 2.2 Bioassay

Seeds of wheat (*Triticum aestivum* L.) cultivar Pirsabak-2005 were obtained from Agricultural Research Institute, Tarnab, Peshawar. Seeds were placed on twice folded filter paper as seed beds in petri-dishes. Each petri-dish was provided with 10 ml of respective concentrated aqueous extracts. Control seeds were provided with the same volume of distilled water. Each Petri dish had 10 seeds; each treatment was further replicated 4 times. The experiment was arranged in a

Completely Randomized Manner at room temperature (20-25 °C) with 12 h photo period. The experiment was performed at Botany Department, Government Degree College Naguman Peshawar during 2013. After 72 hours, data on germination, seminal root and shoot length of seedling was recorded. Germination (%) was calculated as number of germinated seeds in each replicate after 72 hours till final reading. Mean germination time was determined as:  $MGT = \sum (di \times ni) / \sum ni$ ; where *n* represents number of germinated seeds at *i*<sup>th</sup> day and *d* is the number of days counted from the

beginning till the completion of germination (Basra et al., 2005). Dry biomass of seedling was determined as previously described by Muhammad and Hussain (2012).

### 2.3 Statistical analysis

Results were statistically analyzed by the analysis of variance (ANOVA). Least significant difference (LSD) was used to determine significant mean values of the studied parameters at  $p \leq 0.05$ .

## 3 RESULTS

### 3.1 Germination (%)

In this experiment, the allelopathic activity of root, stem peels and leaves of sugarcane which were prepared as aqueous extracts in different concentrations were studied on germination parameters and dry biomass of wheat. Results revealed that germination percentage was neither affected by different plant parts nor by extract concentrations. Interaction between plant parts and concentrations were also non-significant.

Germination (%) was maximum (91.8) in control conditions. Slighter decrease in germination was observed in petri-dishes treated with different extract concentrations of different plant parts; however, the differences among means of concentrations and plant parts for germination percentage were insignificant and they ranged between 89.7 - 90.9 % which did not differ significantly from 91.8 % in control (Table 1).

**Table 1:** Effect of different concentrations of aqueous extracts of plant parts (root, stem peel and leaves) of sugarcane on germination (%) of wheat

Plant parts	Concentration (g/l)					Plant parts means
	Control 0	2.5	5.0	7.5	10.0	
Roots	91.8a	90.9a	90.7a	90.9a	91.4a	90.98a
Stem peel	91.8a	90.7a	91.1a	90.9a	91.0a	91.10a
Leaves	91.8a	90.8a	90.9a	90.7a	89.7a	90.64a
Concentration means	91.8a	90.8a	90.9a	90.83a	90.7a	

### 3.2 Mean Germination Time

MGT was calculated to assess the average number of days taken from the beginning of germination of seeds till completion of germination. MGT was significantly affected by plant parts as well as concentration of extracts. Interaction between plant parts and concentration was also recorded as significant. It was observed that lower extract concentrations up to 7.5 g/l of each plant part had no effect on this parameter; however, 10.0 g/l extract significantly minimized germination time particularly when roots and peel extracts were applied (Table 2). Leaf extract at the highest dose (10 g/l) significantly prolonged meant time of germination which was recorded as 5.8 days. In each plant part at highest extract concentration, MGT was lowest than control where it was 4.7 days except in leaf extract which revealed maximum value for this parameter.

### 3.3 Shoot growth

Analysis of variance (ANOVA) determined significant differences for values of shoot length of wheat seedlings at 10g/l concentrated extracts but insignificant effect at lower concentrations. Interactions were also significant. Shoot length was 15.9 mm in control plates which were treated with distilled water. There were no significant differences between values in extract concentrations 2.5, 5 and 7.5 g/l of respective plant parts which revealed results almost consistent with those of control; however, 10 g/l extract of root and stem peels significantly increased shoot length (16.8 and 16.7 mm respectively). Unexpectedly, the same concentrated extract of leaves had adverse effect on shoot growth which was significantly reduced (14.2 mm) as compared to control and other plant part extracts (Table 3).

**Table 2:** Effect of different concentrations of aqueous extracts of plant parts (root, stem peel and leaves) of sugarcane on mean germination time (MGT) (days) of wheat

Plant parts	Concentration (g/l)					Plant parts means
	<i>Control 0</i>	2.5	5.0	7.5	10.0	
Roots	4.7a	4.3a	4.6a	4.8a	3.4c	4.36a
Stem peel	4.7a	4.4a	4.7a	4.9a	3.7b	4.48a
Leaves	4.7a	4.8a	4.8a	4.9a	5.8bc	4.4a
Concentration means	4.7a	4.5a	4.7a	4.86a	3.3c	

LSD ( $p \leq 0.05$ ) for plant parts = 3.245; concentration = 2.769; interaction 3.120

Values in columns and rows with different alphabets are significantly different

**Table 3:** Effect of different concentrations of aqueous extracts of plant parts (root, stem peel and leaves) of sugarcane on shoot length (mm) of wheat

Plant parts	Concentration (g/l)					Plant parts means
	<i>Control 0</i>	2.5	5.0	7.5	10.0	
Roots	15.9a	16.0a	15.9a	15.3a	16.8b	90.98a
Stem peel	15.9a	15.8a	15.7a	15.5a	16.7b	91.1a
Leaves	15.9a	15.4	15.6a	15.7a	14.2bc	90.64a
Concentration means	15.9a	15.8a	15.7a	15.5a	15.9a	

LSD ( $p \leq 0.05$ ) for plant parts = 1.971; concentration = 3.963; interaction 4.7612

Values in columns and rows with different alphabets are significantly different

### 3.4 Seminal root length

Like other indices, seminal root length was significantly influenced by concentrations of the extracts as well as different plant parts. Lower concentration had no effect on RL whose values were similar to control with slight variations. Interaction between plant parts and concentrations at highest dose were significant. Control condition and extract concentrations up to 7.5 g/l recorded almost similar values of seminal root length which ranged between 27.3-27.9 mm. Maximum length (30.1 mm) of seminal root was observed in root extract at 10g/l concentration followed by the same extract concentration of stem peels and leaves which yielded consistent values 29.4 and 29.3 mm respectively (Table 4).

### 3.5 Seedling dry biomass

Dry biomass of seedling was calculated in each replicate of each treatment and then averaged to determine individual seedling's biomass. Results demonstrated significantly different values for different plant parts, 10g/l extract concentration and interactions but insignificant differences were observed for lower concentrations (2.5 - 7.5 g/l). Values of dry biomass slightly varied under lower extract concentrations of each plant part; however, they were statistically similar to control values. Root and stem peels extract at 10 g/l resulted in highest dry biomass of seedlings which were recorded as 40.1 and 39.2 mg respectively when compared to control (36.1 mg). The lowest value for dry biomass was observed in 10 g/l leaves extract which was 34.2 mg, significantly different from control as well as root and peel extracts at the same concentration (Table 5).

**Table 4:** Effect of different concentrations of aqueous extracts of plant parts (root, stem peel and leaves) of sugarcane on seminal root length (mm) of wheat

Plant parts	Concentration (g/l)					Plant parts means
	Control 0	2.5	5.0	7.5	10.0	
Roots	27.8a	27.7a	27.6a	28.0a	30.1b	28.4a
Stem peel	27.8a	27.5a	27.7a	27.9a	29.4b	28.06a
Leaves	27.8a	27.3a	27.4a	27.8a	29.3b	27.9a
Concentration means	27.8a	27.5a	27.5a	27.9a	29.6b	

LSD ( $p \leq 0.05$ ) for plant parts = 11.09; concentration = 7.939; interaction 1.890

Values in columns and rows with different alphabets are significantly different

**Table 5:** Effect of different concentrations of aqueous extracts of plant parts (root, stem peel and leaves) of sugarcane on dry biomass seedling<sup>-1</sup> (g) of wheat

Plant parts	Concentration (g/l)					Plant parts means
	Control 0	2.5	5.0	7.5	10.0	
Roots	36.1a	35.8a	36.9a	36.5a	40.1b	37.08c
Stem peel	36.1a	36.4a	36.3a	36.9a	39.2b	36.98a
Leaves	36.1a	36.7a	36.4a	36.7a	34.2c	36.02a
Concentration means	36.1a	36.3a	36.5a	36.7a	37.8ab	

LSD ( $p \leq 0.05$ ) for plant parts = 5.213; concentration = 1.7821; interaction 6.379

Values in columns and rows with different alphabets are significantly different

#### 4 DISCUSSION

Germination is an important indicator which depicts the plants' response to changes in the environment, resources or any allelopathic stress induced as a result of allelochemicals released from donor plants (Hussain et al., 2010). Germination indices are generally used to detect potential stimulatory or inhibitory allelopathic activity of the test plant (Hussain and Reigosa, 2014). In our study, germination percentage was not affected by aqueous extract of different plant parts of *S. officinale*. Moreover, concentrations of the extracts were also unable to stimulate or inhibit the germination percentage of wheat. This is in contradiction with previous studies on the allelopathy of *Hypericum myrianthum* Cham. & Schltld. (Fritz et al., 2007), *Eucalyptus camaldulensis* (Ahmed et al., 2008), *Prosopis juliflora* (Sw.) DC. (Siddiqui et al., 2009), *Dodonaea viscosa* Jacq. (Barkatullah et al., 2010) and *Halianthus annus* L. (Muhammad and Majeed, 2014) on germination of wheat and other target crops which revealed significant retardation of germination under the allelopathy of the respective plants at different extract concentrations. We used the highest concentration as 10 g l<sup>-1</sup> which is relatively low as compared to extract

concentrations used in other studies. Thus, non-responsiveness of seed germination to allelopathic stress in this study might be due to relatively low concentration or possible resistance exhibited by wheat seeds to potential allelopathic activity of sugarcane.

Mean germination time (MGT) is another important parameter which determines the energy of germination capacity of seeds in a stressed environment (Bonciarelli, 1995). MGT of target plants in a particular allelopathic assay may either be increased or decreased, depending on the concentration and type of allelopathic compounds. Phenolic compounds, in general, have been sought to prolong germination time as they may possibly interfere with seed dormancy and enzymatic activity necessary for rapid germinability. In this study, MGT was significantly reduced by root and peel extracts at 10 g/l concentration but prolonged under leaves extract. This suggest that root and peels extracts of sugarcane have some potent allelochemicals with stimulatory effect on germination time. On the other hand, leaves extract may possibly possess phenolics which had detrimental effect on this parameter.

Previously, Sampietro & Vattuone (2006) isolated phenolic compounds from straw of sugarcane which had deteriorating effects on germination and general growth of different weeds and crops.

Shoot and seminal root growth were significantly increased by root and stem peels extract at higher concentration which demonstrated stimulatory allelopathy of sugarcane. We assume that root and peel extracts of sugarcane may contain carbohydrates complexes and unknown diffusible allelochemicals which induced stimulatory response in wheat seedling with consequent increase in shoot and seminal root length. However, leaf extract showed detrimental effects on the lengths of shoot and seminal root. Previously stimulatory effect of rice hull extracts (Seyyednejad et al., 2010) but inhibitory effect of *Jatropha curcas* L. (Aburge and Sam, 2010), *Dodonaea viscosa* (Barkatullah et al., 2010) and oleander and walnut leaf extracts (Unal, 2013) on shoot length of different plants including wheat have been reported. Similarly, our results regarding reduced shoot and seminal root length under leaf extracts are in line with findings of Batlang and Shusho (2007), Singh et al. (2009), Sadehgi et al. (2010) and Unal (2013) who reported shoot and seminal root suppression of wheat in response to aqueous extracts of different allelopathic plants. Primary effect of allelochemicals may probably be alteration in cell membrane permeability of the target plant which can cause secondary effects such as changes in water and mineral absorptions potentials, changes in pH, enzymatic alterations etc. thus causing either stimulatory or inhibitory effects (Barkosky et al. 2000; Gatti et al., 2010; Majeed et al., 2012). Allelopathic stress may either elevate the level or induce the inhibition of carbohydrates and protein contents of target plant which build up more proline content as stress indicator; consequently plant growth is either

increased or reduced (Batish et al., 2007; Al-Johani et al., 2012).

Increase in dry biomass was observed under allelopathic effects of 10 g/l root and peel extract which are supported by the findings of Mubeen et al. (2012) on barley and Han et al. (2013) on lettuce who documented increase in dry biomass in response to allelopathic aqueous extracts of diverse plants. Decrease in biomass of seedlings treated with leaf extracts in this study are in agreement with Singh et al. (2005), Jamil et al. (2009), Ullah et al. (2013) and Jabeen et al. (2013) who reported suppression in fresh and dry biomass of seedling in *Brassica* sp., wild oat and wheat under the influence of allelopathy of some weeds and crops. Differences in results of different studies may be due to different experimental conditions, extract concentrations and test plants they used. The increase in dry biomass of wheat seedlings treated with root and stem peel extract in the current study may probably be due to increase in photosynthetic rate and greater accumulation of photo-assimilate in the shoot, increase in thickness and length of seminal root, decrease in proline content and greater potassium ion uptake induced by allelopathic extracts of *S. officinale*; as in earlier studies some authors argued that botanical extracts could have positive influence on these phenomena thus stimulating biomass of the receptor plant (Rizvi and Rizvi, 1992; Gatti et al., 2010; Ibrahim et al., 2013). Similarly, reduced biomass, seminal root and shoot length under leaf extract suggests the presence of phenolic and growth inhibitory compounds in leaves of sugarcane and these reductions may be regarded as a result of induced oxidative damage and activation of cellular anti-oxidant systems which in turn changed permeability patterns of cell membrane, irregularities in mineral and ion uptake and the activation of stress enzymes (Oracz et al., 2007; Khaliq et al., 2012).

## 5 CONCLUSION

The present study suggests that different plant parts of sugarcane exhibit both stimulatory and inhibitory allelopathy against wheat in germination bioassay. Roots and stem peel extracts at higher concentration

promoted germination time, shoot and seminal root growth and dry biomass of seedling while leaf extract had negative effects on these parameters. Pot culture study is underway to confirm our lab bioassay findings.

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