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ALLELOPATHIC EFFECT OF *RICINUS COMMUNIS* L. EXTRACTS ON GERMINATION AND SEEDLING GROWTH OF *ZEA MAYS* L.

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Abstract

Allelopathy Aerial parts of *Ricinus communis* extracted in methanol were evaluated for allelopathic effect on growth in maize seedlings (*Zea mays* L.). Maize seeds presoaked in different concentrations of plant extract were grown in petri dishes under laboratory conditions for ten days. Crude extract of the plant at 2.5 and 5 mg/mL concentrations was found to be stimulatory for plant growth with less phytotoxicity, high vigor and high tolerance ability. At concentration of 7.5 mg/mL the extract promoted shoot growth but inhibited root growth while 10 mg/mL concentration caused 100% inhibition of seed germination. The extract also exhibited hydrogen peroxide reducing potential with 40.6% reduction at 1 mg/mL concentration. It was concluded that *R. communis* extract can stimulate growth in maize with different concentrations required for root and shoot growth stimulation.

Keywords
Allelopathy, Hydrogen peroxide scavenging activity, *Ricinus communis*, *Zea mays*

1. Introduction

Allelopathy is the stimulation or inhibition of growth of plants in any area due to the chemical effect of certain compounds released into the environment by neighboring plants (Bhowmik, 2003). The phenomenon of allelopathic plant suppression is not new and the earliest reports of allelopathy have been reported over 2000 years ago (Weston and Duke, 2003). Theophrastus in 300 B.C. reported reduced growth of weeds in surroundings of chickpea while Secundus reported growth inhibition in corn by bitter vetch, barley, and chickpea (Singh *et al*, 2001) during 1 A.D. Allelopathy is different from competition and negative interference and also eco-friendly. Secondary metabolites are released from donor plant and have inhibited effect on the germination, growth and development of other undesirable plants (Mengal *et al*, 2015; Arif *et al.* 2015) reported that metabolites and physiological processes of weeds were affected by allelochemicals because of their phytotoxic effects.

Allelochemicals are the secondary plant metabolites synthesized in plants that help them in execution of their allelopathic effects. They are organ specific and their quantity may vary in various plant parts (Tongma *et al*, 2001) and plant species (Czarnota *et al.*, 2001). In the past, these chemicals in plants were considered as waste materials produced as a byproduct during the biosynthesis of primary metabolites. Allelopathy has been described as the protective role of these allelochemicals in plants to protect them from toxic effects of neighboring plants or other living organisms. These allelochemicals may also serve as agrochemicals agents and have potential to be used as bioherbicides or lead compounds for new herbicides (Inderjit, 2003).

R. communis L. (Euphorbiaceae) is a plant species of tropical areas widely distributed across the world. The plant is of ecological significance being able to grow in waste lands (Roger, 1999) while the oil yielded from the seeds is of economic significance used medicinally as a laxative (Kamal, 2006). The plant also possesses several biological activities including antidiabetic (Shokeen *et al*, 2008), antimicrobial (Panghal *et al*, 2011), antitumor (Lin, 1986) and analgesic activity (Ferraz *et al*, 1999). Research has indicated allelopathic effect of *R. communis* extracts (Nekonam *et al.*, 2013; Seyyedi *et al*, 2013; Nekonam *et al*, 2014). However, no previous data is available regarding allelopathic effect of *R. communis* on maize plant. Therefore the biological effect of crude extract of aerial parts of *R. communis* was determined on physical growth in *Zea mays* (cv. X8F932).

2. Experimental

2.1.Plant Material

Fresh plant material (aerial parts) was collected from natural environment and plant was identified by consulting authentic flora. Authenticated maize grains (cv. X8F932) were provided by Punjab Seed Corporation, Lahore, Pakistan.

2.2.Extraction of plant material

Fresh aerial parts (1785 g) of *R. communis* after washing and drying were grinded to get fine powder (502 g). Extraction in methanol was carried out through maceration by soaking in methanol at ambient temperature (25°C) for five days with occasional stirring. After the desired time period the material was filtered and methanol was recovered in a rotary evaporator at 35°C to get crude methanol extract (31.68 g). Extract was kept in a refrigerator.

2.3.Phytochemical analysis

Phytochemical analysis was performed using standard chemical methods as reported by Harborne (1973).

- **Flavonoids:** Plant extract was treated with conc. H_2SO_4. Yellow/orange color of the solution indicated flavonoids.
- **Glycosides*:*** To 1 ml of KOH (10%) 1 ml of plant sample was added. Appearance of brick red colored precipitates was due to glycosides.
- **Phenolics*:*** 2 drops of $FeCl_3$ (5%) and 1 ml of plant extract were mixed together.

Greenish precipitates, if formed, confirmed phenolics.

- **Saponins:** Formation of persistent froth upon vigorous shaking of the test sample confirmed saponins in the extract.
- **Steroids:** To 1 ml of plant sample 0.5 ml of H_2SO_4 (conc.) was added. A red coloration of solution confirmed steroids.
- **Tannins:** Equal volumes of plant sample and KOH (10%) were mixed. White precipitates formed in the solution confirmed tannins.
- **Triterpenes:** To 1 ml of test extract were added 3-5 drops of H_2SO_4 (conc.). Appearance of blue green color in solution indicated triterpenes.
- **Alkaloids:** Alkaloids were confirmed by adding 0.5 ml of Dragendroff's reagent in 1 mL of crude extract. Orange red coloration confirmed alkaloids.
- **Coumarins:** 1 mL each of plant extract and NaOH (10% solution) were mixed. Yellow color of solution confirmed the presence of coumarins.
- **Anthocyanins and Betacyanins:** 2 ml of plant sample and 1 ml of NaOH (2N) were mixed and heated at 100^0C for 5 minutes. The solution if turned bluish green in color confirmed the presence of anthocyanin and yellow color was indicative of betacyanins.

2.4.Estimation of total phenolic content (TPC)

For estimation of TPC 20 µl of methanol extract, 100 µl of FC reagent and 158 µl of distilled water were added and kept at 25°C. After 10 min 0.3 ml of Na_2CO_3 (25% solution) was added and contents were heated at 40°C. Then absorbance of solution was noted at 765 nm (Cliffe *et al.* 1994). Gallic acid served as control to construct calibration curve for calculation of phenolic content (Figure 1). Phenolic content was reported as mg of Gallic acid equivalents/g dry extract.

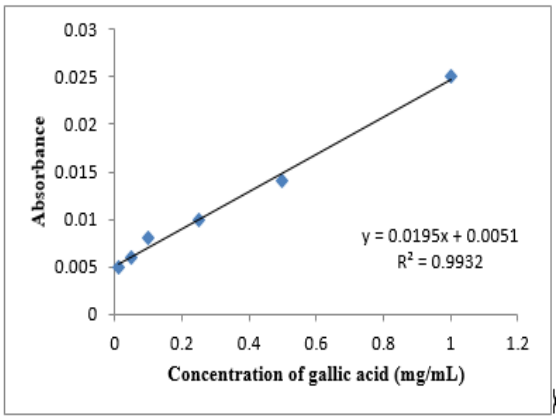

Figure 1: Calibration curve for gallic acid

2.5.Estimation of total flavonoid content (TFC)

For estimation of TFC, method of Dewanto *et al.* (2002) was followed. 250 µL of plant sample, 90 µL of $NaNO_2$ (5%) and 500 µL of deionized water were added in a test tube and left for five min. Then 180 µL of $AlCl_3$ (10%) and 600 µL of NaOH (1 M) were added and final volume was raised to 3 mL by adding distilled water. Finally absorbance of this mixture was noted at 510 nm. Calibration curve of standard quercetin solution was prepared to calculate TFC (Figure 2). Flavonoid contents were reported as mg of quercetin equivalents/g dry extract.

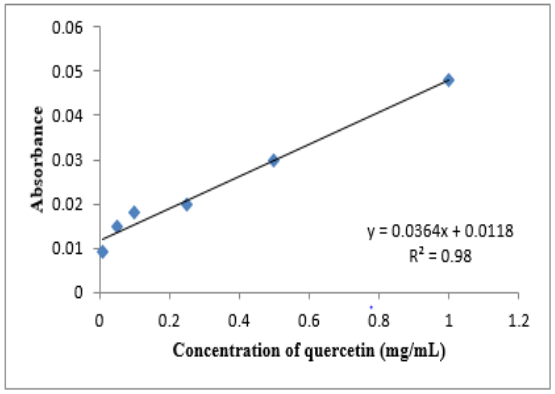

Figure 2: Calibration curve for quercetin

2.6.Estimation of total tannin content (TTC)

For estimation of tannins 0.5 ml FC reagent, 0.1 ml of methanol extract and 1 mL Na_2CO_3 (35%) were combined and diluted with 7.5 ml distilled water. Contents were incubated for 20 min (25°C) and then absorbance was read at 725 nm (Tamilselvi *et al.* 2012). For calculating total amount of tannins standard calibration curve using tannic acid was prepared under the above mentioned conditions (Figure 3). TTC was reported as mg tannic acid equivalents (TAE)/g dry extract.

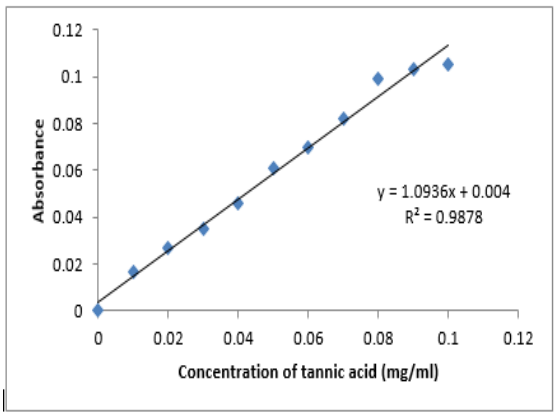

Figure 3: Calibration curve for tannic acid

2.7.Hydrogen peroxide scavenging

To determine potential of extract to scavenge H_2O_2 0.5 ml of methanol extract and 3 mL of H_2O_2 (40 mM in phosphate buffer, pH 7.4) were mixed. Absorbance of H_2O_2 was read at 230 nm after 10 min. Mixture without plant extract served as blank (Ruch *et al.*, 1989). H_2O_2 scavenging potential (%) was determined by the following equation:

% Scavenging = $[(Abs_C - Abs_S)/Abs_C] \times 100$

In this equation Abs_C indicates absorbance of control sample and Abs_S indicates absorbance of plant extract.

2.8.Bioassay for allelopathic effect

Maize seeds were soaked in 1% $HgCl_2$ solution for 5 minutes to ensure surface sterilization followed by several washings with sterilized distilled water. Sterilized seeds were then soaked in different concentrations of *R. communis* extract. Five seeds per petri plate and three replicates of each concentration were used. Maize seeds were placed in petri plates lined with two layers of sterilized filter paper and were kept moist throughout the study period using distilled water.

2.9.Germination and seedling growth

Maize grains with radicle length of approximately 2 mm were considered as germinating. Percentage of germination was calculated by counting the number of germinating maize grains after 24 hrs interval for three consecutive days. Measurement of root and shoot lengths was carried out after every three days for a total period of nine days. Fresh weight of maize seedlings was checked after 9 days.

2.10. Percentage phytotoxicity

Phytotoxicity of various concentrations of extract on physical growth (root and shoot

length) was determined in 9 days old seedlings by the following formula of Chou, 1976:

$$\text{Phytotoxicity (\%)} = \frac{\text{Average length (R/S)in control - Average length (R/S) in treatment}}{\text{Average length in control}} \times 100$$

Where R = root and S = shoot

2.11. Vigor index

Vigor index (VI) of seedlings was estimated by the formula given by Iqbal, 1992:

Vigor Index = (average root length + average shoot length) × % seed germination

2.12. Tolerance index

The ability of maize seedlings to tolerate each tested concentration of *R. communis* extract was estimated by the following formula (Koornneef *et al*, 1997).

$$I_t = (I_{me}/I_c) \times 100$$

In the equation I_{me} indicates increase in root length in seeds incubated in plant sample and I_c indicates increase in root length in water.

2.13. Statistical analysis

Treatments were repeated in triplicates and values recorded as mean ± SD (standard deviation). Statistical analysis was carried out by using Microsoft Excel 2007. For control and experimental samples means were compared using Student's t-test ($p < 0.05$).

3. Results And Discussion

3.1.Phytochemical analysis

Analysis of crude plant extract confirmed the flavonoids, coumarins, alkaloids, glycosides, triterpenes, phenolics and betacyanins in the extract. Anthocyanins, saponins, tannins and steroids were absent. Phenolics, alkaloids, flavonoids and terpenoids are the major plant components identified as allelochemicals in allelopathic effects of several plant extracts on crops (Kanchan, 1980; Aziz, 2014). These phytochemicals present in *R. communis* extract might be controlling the allelopathic effect of the plant observed.

3.2.Total contents of phytochemicals

Total contents of phenols, flavonoids and tannins in methanol extract of *R. communis* are presented in Figure 4. Phenolic compounds including flavonoids impose inhibitory effect on seed germination and physical growth in plants at high concentrations (Einhelling, 1995; Yukiko *et al*, 2001). The stimulatory effect of extract at lower concentrations might be related to the low contents of phenolics in the extract as compared to the amount of phenolics present in high extract concentrations.

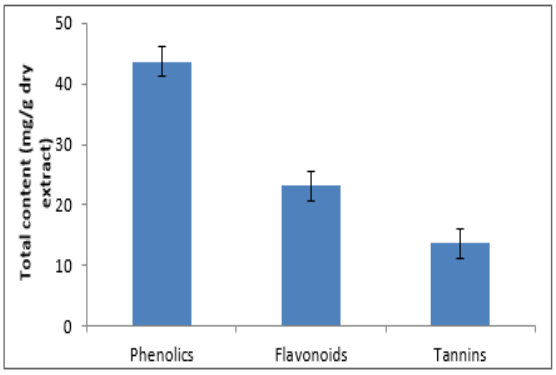

Figure 4: Total phenolic, flavonoid and tannin content in *R. communis* extract

3.3.Hydrogen peroxide scavenging activity

R. communis extract showed 26.55, 37.91 and 40.63% H_2O_2 scavenging at 0.25, 0.5 and 1 mg/mL concentrations respectively (Figure 5). The activity was significantly lower than ascorbic acid and quercetin used as standard antioxidants with H_2O_2 scavenging activity

ranging between 76 to 82% for quercetin and between 80.5 to 86% for ascorbic acid. H_2O_2 scavenging by extracts is usually attributed to their phenolic contents, which neutralize H_2O_2 to water by donating electrons (Nabavi *et al.*, 2008; Ebrahimzadeh *et al*, 2009).

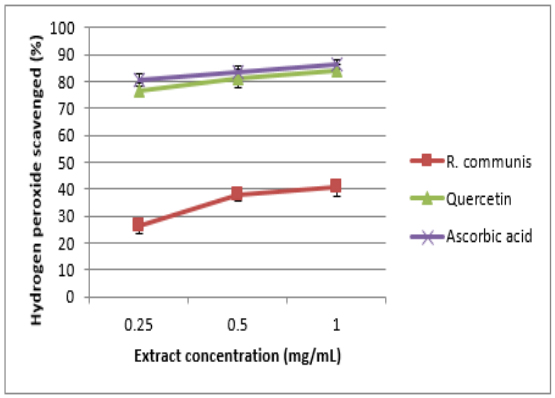

Figure 5: Hydrogen peroxide scavenging activity of crude methanol extract of *R. communis*

3.4.Effect on seed germination

R. communis extract stimulated seed germination with maximum germination in seeds incubated with 5 mg/mL concentration of plant extract (70%) comparable to control group (70%). The extract also enhanced the germination time with 70% germination on day 1 as compared to only 40% in control group. At higher concentrations the extract was highly toxic with only 10% germination at 7.5 mg/mL while no germination was observed even after day 4 at 10 mg/mL (100% inhibition) (Figure 6). Allelochemicals present in plant extracts inhibit seed germination by affecting several physiological reactions in target plant by blocking hydrolysis of nutrient reserves and cell division (Weir *et al.*, 2003; Irshad, 2004). In a study by Nekonam *et al*, 2013 dried powder and aqueous extracts of *R. communis* inhibited germination of seeds and growth of seedlings in convolvulus with maximum reduction at highest concentration (10 g/L). Seyyedi *et al*, 2013 and Nekonam *et al.* 2014 also observed strong inhibition of seed germination in *Cuscuta compestris* and *Sorghum vulgare* by *R. communis* extracts.

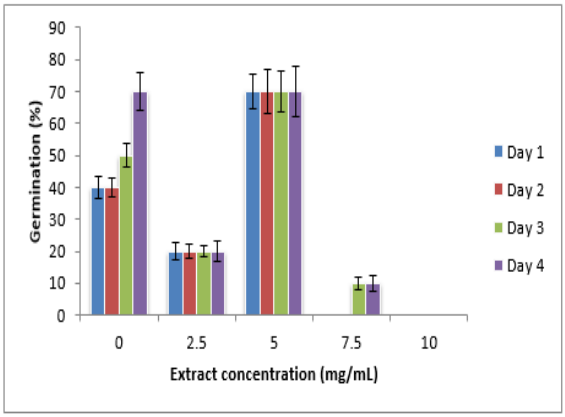

Figure 6: Effect of methanol extract of *R. communis* on seed germination of maize

3.5.Effect on seedlings growth

R. communis extract at 2.5 mg/mL caused considerable increase in root growth ($P < 0.05$) with 62% increase in root length. At a dose of 7.5 mg/mL the extract inhibited root growth causing 40% reduction in root length while 10 mg/mL concentration of the extract exerted 100% inhibitory effect on growth (Figure 7).

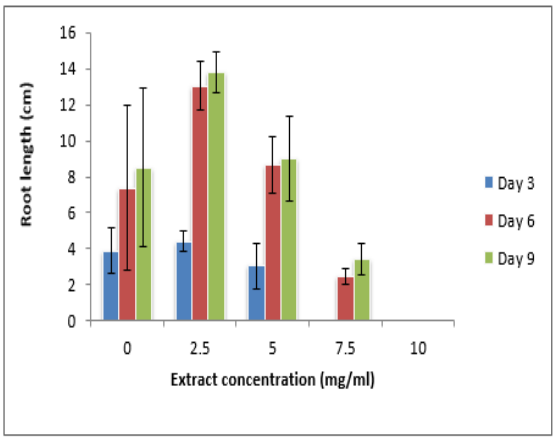

Figure 7: Effect of *R. communis* extract on root length of *Z. mays* L. seedlings

A strong increase in shoot length ($P < 0.05$) as compared to control was recorded at 2.5, 5 and 7.5 mg/mL with 165, 80 and 58% increase respectively while there was 100% inhibition of shoot growth at 10 mg/mL (Figure 8). Plant extracts are reported to cause growth inhibition in seedlings of many plant species (Mucciarelli *et al*, 2000; Reigosa *et al*, 2007) that might be due to anti-mitotic potential of specific allelochemicals present in these extracts (Hernandeza *et al*, 2008; Ninfali *et al*, 2007). The allelochemicals which inhibit plant growth at certain concentration may be stimulatory for plant growth at lower concentrations (Zhung *et al*, 2005). This is indicated by stimulation of growth in root and shoot at low extract concentration and inhibition at high concentration.

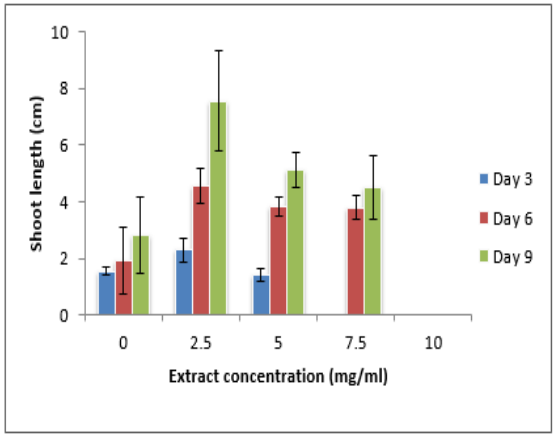

Figure 8: Effect of *R. communis* extract on shoot length of *Z. mays* L. seedlings

3.6.Effect on biomass

A slight increase ($P > 0.05$) in fresh biomass over control group was recorded in maize seedlings incubated in 2.5 and 5 mg/mL extract concentrations. In case of dry weights of *Z. mays* seedlings no significant differences were observed as compared to control by treating with *R. communis* extract at all concentrations (Figure 9).

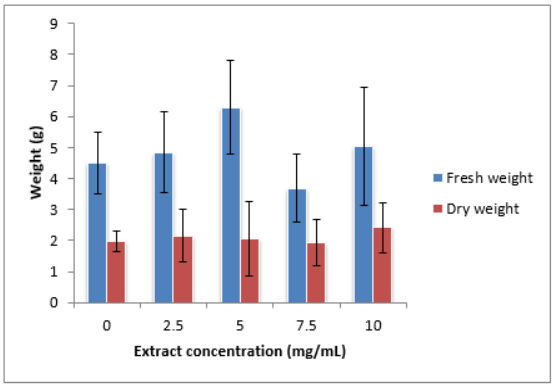

Figure 9: Effect of *R. communis* extracts on fresh and dry weight of *Z. mays* seedlings

3.7.Phytotoxicity

In maize grains soaked in 2.5 and 5 mg/mL extract concentration no toxic effect was observed in seedlings rather there was a stimulatory effect on root development (% phytotoxicity -62.35 and -6% respectively). However, 7.5 mg/mL extract concentration had a toxic effect on growth of root with 60% phytotoxicity while concentration of 10 mg/mL was 100% phytotoxic. The extract was not phytotoxic to shoot growth at the tested concentrations except 10 mg/mL and had stimulated shoot growth with values of phytotoxicity-58, -80 and -165%, for 7.5, 5 and 2.5 mg/mL (Figure 10).

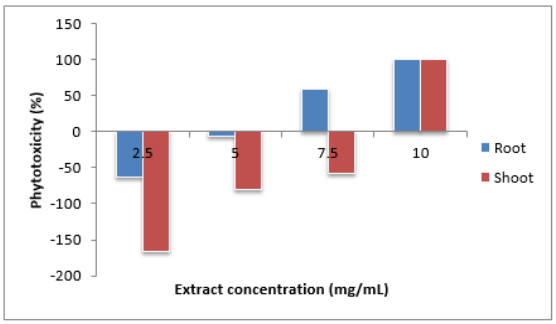

Figure 10: Effect of *R. communis* extracts on % phytotoxicity of root and shoot of *Z. mays* seedlings

3.8.Effect on vigor index

Vigor index of *Z. mays* seedlings treated with *R. communis* extracts was higher at 5 mg/mL (989.1) than control group (567) while all the other concentrations decreased the seedling vigor (Figure 11).

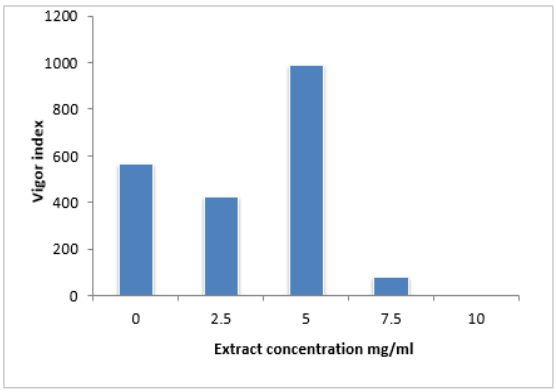

Figure 11: Effect of *R. communis* extract on seedling vigor index of *Z. mays*

3.9.Effect of plant extract on tolerance index

The tolerance indices of maize seedlings for *R. communis* extract decreased at higher extract concentrations with highest value of tolerance index (162.35) determined for 2.5 mg/mL and lowest for 10 mg/mL (0) (Figure 12).

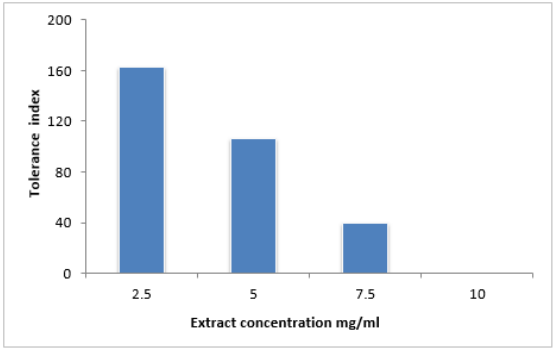

Figure 12: Tolerance indices of *Z. mays* against *R. communis* extract

4. Conclusion

It was concluded from the resutls that the methanol extract of aerial parts of *R. communis* can stimulate growth of maize plants at lower concentration. However, at high concentrations the extract had inhibitory effect on maize growth. Moreover, the extract stimulated shoot growth more as comaprd to root. Thus it might be concluded that the introduction of this plant in crop fields may help to enhance the physical growth of these plants through production of allelochemicals.

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