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Secale cereale L. suppression by aqueous extracts of *Glycine max*

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ABSTRACT

This Study was conducted to evaluate the effect of fresh and dry parts of Soybean (Glycine max) on germination and growth of Secale cereal seedlings in order to determine the inhibitory effect of soybean and effect of drying on the phytotoxic activity of this plant. Results showed that the germination percentage,germination rate, seedling growth and fresh and dry matter production of Secale cereal were retarded by all the four different aqueous extracts applied. The degree of retardatory effects of the aqueous extracts were found to follow this order: dry shoot aqueous extract (DSE) > dry root aqueous extract (DRE) > fresh shoot aqueous extract (FSE)> fresh root aqueous extract (FRE). There was a significantly higher phytotoxic potency at $p<0.05$ in the dry tissue aqueous extract compared with the fresh tissue aqueous extract. Allelopathy appears to be one mechanism for competition among soybeans and weeds. Soybean possessing the ability to chemically inhibit competing weed growth would be a great benefit to the soybean producer.

Key words: Allelopathy, germination, soybean, seedling growth, leaf area.

INTRODUCTION

The phenomenon of allelopathy where one plant exerts a detrimental effect on another through the production of germination and growth inhibiting substances has been widely reported [23,22]. It can play an important role in regulating plant diversity [7]. Chemicals released from plants into the environment are of major significance in adaptation of species and organization of communities [6]. Basic plant processes such as hormonal balance, protein synthesis, respiration, photosynthesis, plant water relations and chlorophyll production may be affected by allelochemicals [29]. Putnam & Duke (1979) introduced the concept of utilizing the allelopathic crop residues for weed control in crops. Cheema *et al*., (1997) found that aqueous extract of sorghum and sunflower has the potential to suppress the weed infestation in wheat crop. Similarly, Mahmood & Cheema (2004) found that sorghum mulch significantly reduced the density and dry biomass of one of the world's worst weed *Cyperus rotundus.* Akhtar *et al*., (2001) reported that aqueous extracts of *Cirsium arvense* and *Ageratum conyzoides* could suppress the germination and early seedling growth of some weeds of wheat. Moradshahi *et al*., (2003) found that aqueous extracts of *Eucalyptus camaldulensis* Schlecht., has the potential to suppress growth of *Echinochloa crus-galli* (L.) Beauv., *Avena fatua* L., and *Rumex acetosella.* Similarly Dahiya & Narwal (2003) found that root exudates of *Helianthus annuus* L., are allelopathic towards *Agropyron repens* (L.) Beauv., *Ambrosia artemsiifolia* L., *Avena fatua* L., *Celosia crustata*, *Chenopodium album* L., *Cynodon dectylon* (L.) Pers. Singh *et al*., (2005) studied the herbicidal effect of volatile oils from leaves of *Eucalyptus citriodora* against the noxious weed *P. hysterophorus* and found that a concentration of 5.0 nL ml^{-1} *Eucalyptus* oil completely inhibited the germination. Uremis *et al*., (2005) have reported significant suppression of *Physalis angulata* L., a problem weed in maize, cotton and soybean fields in Turkey, by aqueous extracts of 6 *Brassica* spp.

Several authors reported that the residues and extracts of soybean might be efficient in weed control due to it's allelopathic properties[11,3,24,27].

The objectives of this study are to determine the susceptibility *of Secale cereal* to phytotoxic activity of *Glycine max*, compare the phytotoxicity of plant parts of *Glycine max* and determine the effects of drying on the phytotoxic activity of *Glycine max.*

MATERIALS AND METHODS

The experiment was carried out at the Department of Biology, Mashhad Branch, Islamic Azad University,Iran(2010). The seeds of *Glycine max* and *Secale cereal* were supplied by the Agricultural Research Center of Khorasan province, Iran. To prepare the extracts, 360 g each of the shoots and roots of six weeks old Soybean were cut into small chips of about 4 cm lengths and finally ground separately with mortar and pestle. Also 360 g each of these parts were oven dried separately in incubator at 60°C for 5 days and ground with a lab mill to pass through a 2 mm screen. The ground plants parts were soaked separately in 5 L of distilled water for 12 h[1]. The filtrates obtained serve as treatments for the seedlings in the different aqueous extract regimes. Experimental pots were randomly allocated to the following regimes control (No application but water) fresh shoot aqueous extract treatment (FSE) regime, dry shoot aqueous extract treatment (DSE) regime, fresh root aqueous extract treatment (FRE) regime, dry root aqueous extract treatment (DRE) regime. The seedlings in the control regime were supplied daily with 400 mL of water while the seedlings in the treatment regime were supplied daily with 400 mL of the appropriate extract.

The seeds of *Secale cereal* L. were soaked in 5% sodium hypochlorate to prevent fungal infection after which they were rinsed for about 5 min in running water. The seeds were washed in distilled water and 20 seeds were placed in clean oven dried Petri dishes which had been lined with a Whatman No. 1 filter paper. The filter paper in each Petri dish allocated to the control was then moistened with 10 mL of distilled water while the filter paper in each of the petri dishes allocated to the other four treatments was moistened with 10 mL of the appropriate aqueous extract. The Petri dishes were incubated at room temperature for 2 weeks. Emergence of 1 mm of the radicle was used as the criterion for germination experiment.

For growth, fresh and dry production, i.e., *Secale cereal* L. seeds were sown in pots (28x15 cm) containing good humus top soil. Seeds *of Secale cereal* L. were watered with 400 mL of tap water every morning. At two weeks, seedlings in each pot were thinned down to 15 seedlings per pot. The pots were then allocated to the control and the four different treatments. Thereafter, the pots in the control regime were supplied with 400 mL of water daily while the pots with the different aqueous extracts were supplied with 400 mL of the appropriate aqueous extract daily. The pots were laid out in a completely randomized design. Plants were harvested just before treatment started. Thereafter, harvesting of the seedlings was on a weekly interval for a period of six weeks. Root length, shoot height, leaf area, fresh weight and dry weight of roots and shoots were determined. For the shoot height the distance between the base of shoot at soil level and the upper point of the terminal bud of the seedling was measured using a metric rule. Leaf area was determined using the formula according to Pearcy *et al.* (1989).

$$LA= 0.5 (LxW)$$

L = Length of leaf
W=Maximum width
Leaf Area Ratio (LAR) was calculated using the formula of West *et al.* (1920).

Total plant dry weight

The root system was carefully excavated. The root was then washed free of soil and the length of the root was measured as distance between the base of plant and root tip. Measurements were carried out on five seedlings and mean values were calculated. Five seedlings were randomly harvested in each regime. Each seedling was separated into shoot and root.The fresh plantparts were then weighed on a Meltler Toledo balance to obtain the freshweight of the plant parts. Five seedlings were randomly harvested in each regime and each seedling was separated into shoot and root. The plants parts were then packaged separately in envelopes and dried to constant weight at 80°C in incubator. The dried plant parts were weighed on a Meltler Toledo balance to obtain the dry weights and then mean weights were calculated. All experiments were conducted in five replicates and the data obtained was subjected to appropriate statistical analysis. Analysis of variance (ANOVA) was carried out for all the data. Treatment means were compared using least significant difference (LSD p<0.05).

RESULTS AND DISCUSSION

The mean percentage germination of the seeds in the DRE regime was comparable to that of the control regime. The germination of seeds treated with root aqueous extracts was slightly higher than that of the seeds in the two regimes treated with shoot extracts (Figure 1). The plumule length of the seedlings in the fresh shoot aqueous extract regime was lower and significantly different from that of the seedlings in the two regimes treated with the root aqueous extracts at $p<0.05$ (Figure 2). The radicle lengths of Seedlings in the dry shoot aqueous extract (DSE), fiesh root aqueous extract (FRE) and dry root aqueous extract (DRE) regimes were found to be slightly variable from that of the seedlings in the control regime and were found not to be statistically different at $p<0.05$. The seedlings treated with fresh shoot aqueous extract (FSE) had a radicle length which was lower and significantly different from that of the seedlings in the control, dry shoot aqueous extract, fresh root aqueous extract and dry root aqueous extract regimes at $p<0.05$ (Figure 2).

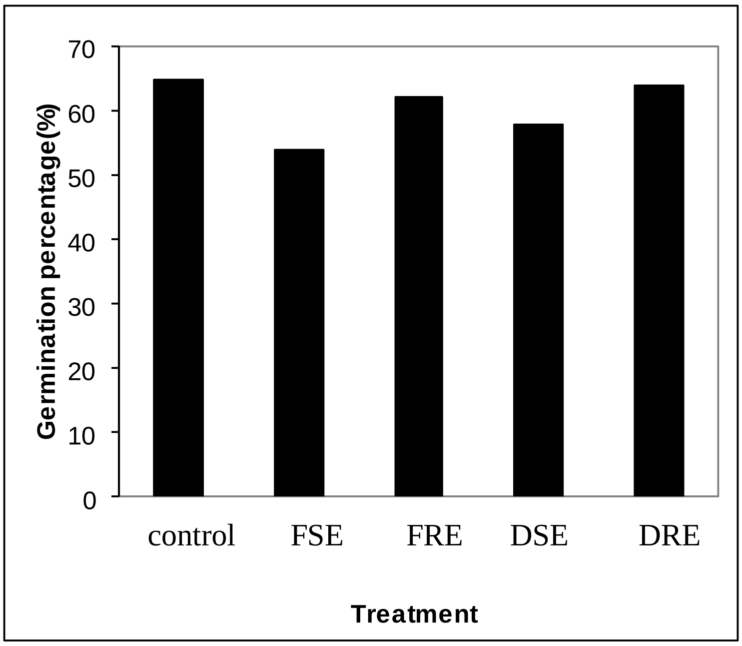

Figure 1: Effect of aqueous extract of *Glycine max* on seed germination *of Secale cereal*

The fresh weight of the shoot of the seedlings in the control and dry root aqueous extract (DRE) regimes increased gradually from the start of the experiment to week four and then increased sharply until the end of the experiment (Figure 3). The shoot fresh weight of the seedlings in the control regime remained highest throughout the duration of the experiment when compared with that of the seedlings in all the aqueous extract treatment regimes. There was a significant difference between the fresh weight of the shoot of the seedlings in the control regime and that of the seedlings in all the aqueous extract treatment regimes at $p<0.05$. The root fresh weight of the seedlings in the control regime was significantly different and higher than of the seedlings treated with the aqueous extracts. Significant differences were observed between the fresh weights of the root of the seedlings in the FSE and FRE regimes which were significantly different from that of the root of the seedlings in the DSE and DRE regimes at $p<0.05$ (Figure 4). The dry weight of the shoot of the seedlings in the control regime was slightly higher than that of seedlings in all the extract treatment regimes from week two until the end of the experiment (Figure 5). Significant difference was observed between the shoot dry weight of the seedlings in the control regime and that of the seedlings in the aqueous extract treatment regimes at $p<0.05$. Significant differences were observed between the shoot dry weights of the seedlings in the FSE and FRE regimes and between those of the seedlings in the DSE and DRE regimes. Also the shoot dry weights of the seedlings in the FSE and FRE regimes were significantly different from those of the DSE and DRE respectively. The effect of different aqueous extracts of soybean on the dry weight of the root *of* Secale cereal L. is presented in Figure 6. The dry weight of the root of the control seedlings and that of the root of the seedlings belonging to all the other regimes showed the same pattern with that of control being slightly highest.

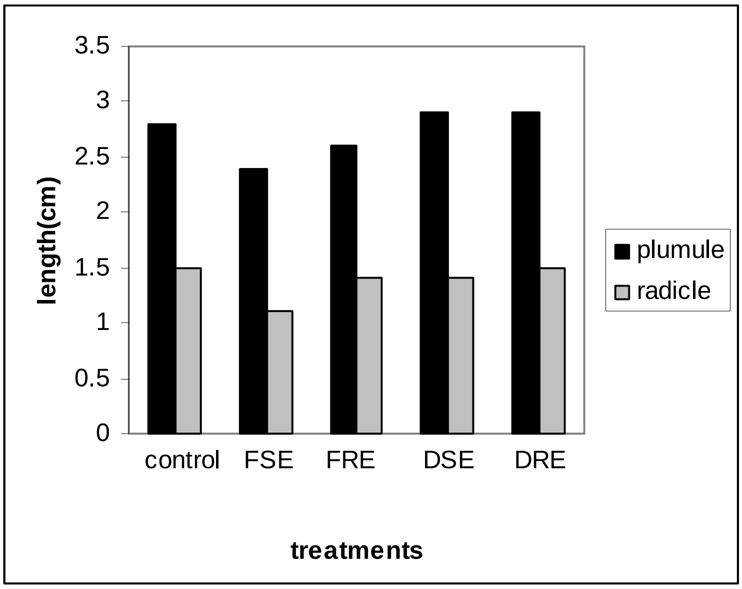

Figure 2: Effect of the application of fresh shoot, dry shoot, fresh root and dry root aqueous extract treatments on the plumule length and radicle length of the germinating seedlings of *Secale cereal*

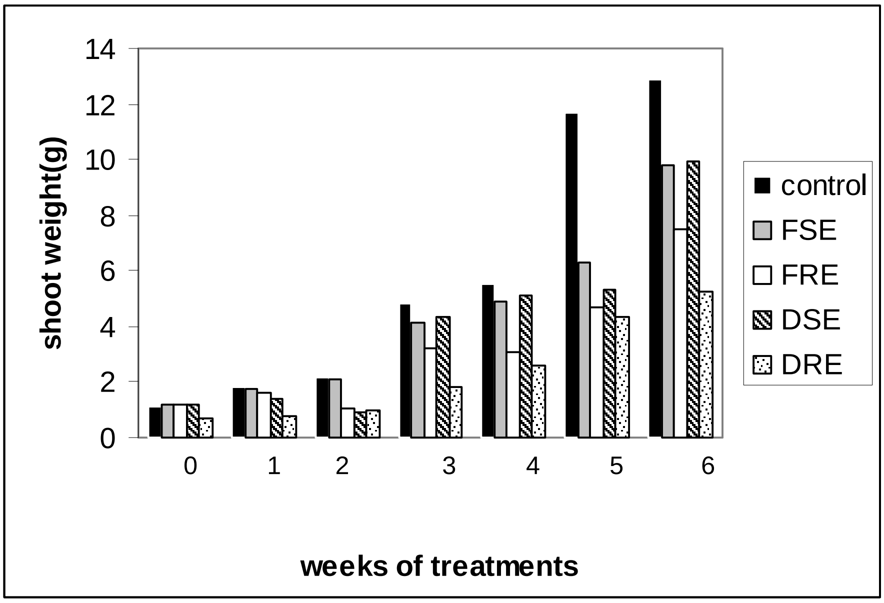

Figure 3: Effect of aqueous extract of *Glycine max* on shoot weight (g) of *Secale cereal*

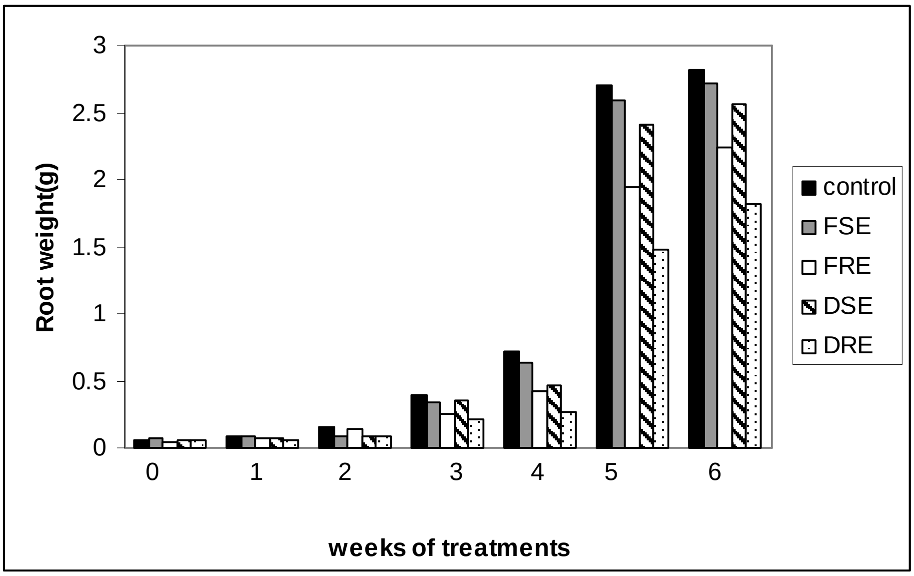

Figure 4: Effect of aqueous extract of *Glycine max* on Root Fresh weight (g) of *Secale cereal*

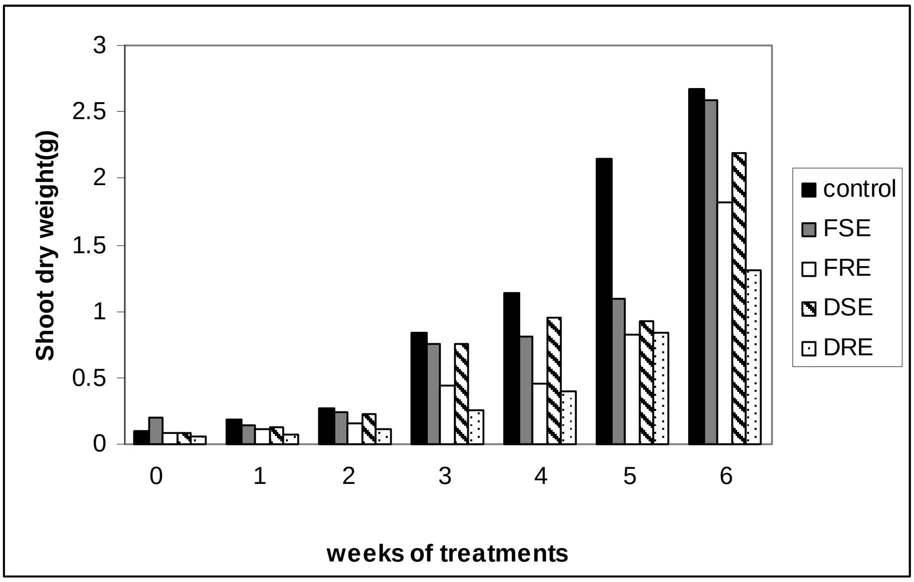

Figure 5: Effect of aqueous extract of *Glycine max* on Shoot dry weight (g) of *Secale cereal*

The shoot height of the seedlings in the control regime and all the four treatment regimes followed essentially the same trend. The height of the shoot of the control seedlings remained slightly higher than that of the treated seedlings throughout the duration of the experiment. The same applied to the shoot height of the seedlings treated with root aqueous extracts (FRE and DRE) which remained slightly higher than that of the seedlings treated with shoot aqueous extracts (FSE and DSE) from the second week until the end of the experiment (Figure 7). The shoot height of the seedlings treated with dry shoot and dry root aqueous extracts remained higher than that of the seedlings treated with fresh shoot and fresh root aqueous extracts respectively throughout the experiment. The shoot height of the seedlings in the control regime was statistically significantly different from the shoot height of the seedlings in all the treatment regimes at $p<0.05$.

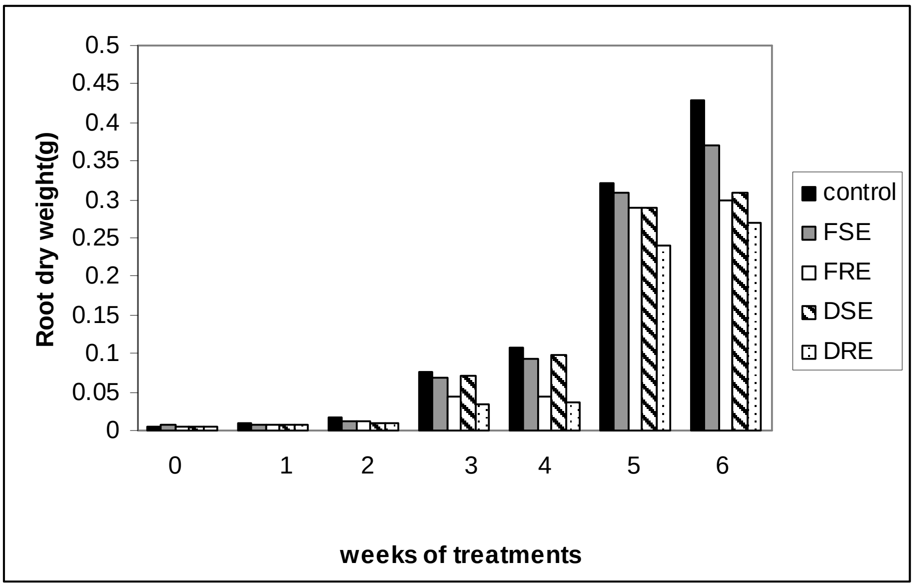

Figure 6: Effect of aqueous extract of *Glycine max* on Root dry weight (g) of *Secale cereal*

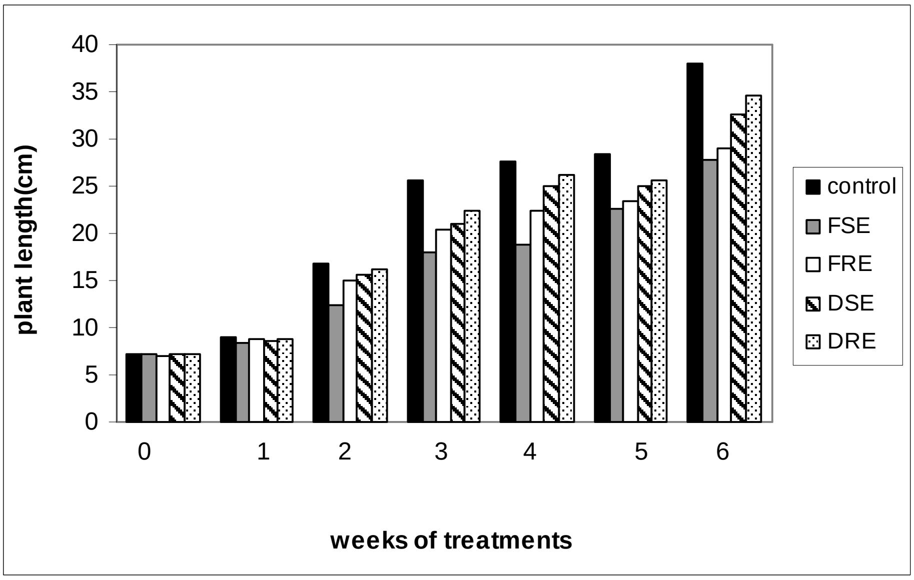

Figure 7: Effect of aqueous extract of *Glycine max* on plant length(cm) of *Secale cereal*

The root length of the seedling in the control regime and all the four treatment regimes were similar in the first week of growth after which the root length of the seedling increased steadily until the end of the experiment. The root length of the seedlings in the control regime was slightly longer than that of the seedlings in all the treatment regimes. Seedlings in the two root aqueous extract treatment regimes (FRE and DRE regimes) had a root length that was longer than that of the seedlings in the two shoot aqueous extracts (FSE and DSE) treatment regimes (Figure 7). The root length of the seedlings in the control regime was significantly different when compared with that of the seedlings treated with the FSE, DSE and FRE at $p<0.05$. The root length of the seedlings treated with fresh shoot aqueous extract was significantly different from that of the seedlings treated with dry shoot extract at $p<0.05$. Significant difference was also observed between the root length of the seedlings treated with fresh root aqueous extract and that of the seedlings treated with dry root aqueous extract at $p<0.05$ (Figure 8). The leaf area of the seedlings in the FSE regime remained lowest throughout the duration of the experiment while the leaf area of the seedlings in the control regime was continuously higher than that of the seedlings in the other regimes throughout the duration of the experiment (Figure 9). The leaf area ratio of the seedlings treated with dry root aqueous extract was higher than that of the seedlings in the other treatment regimes while that of the seedlings is the fresh shoot aqueous extract was lowest (Figure 10).

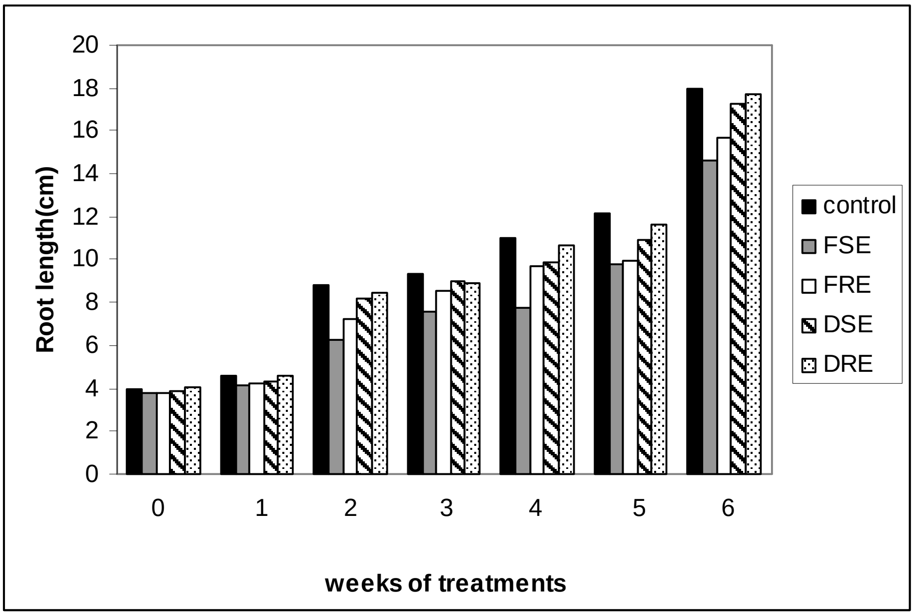

Figure 8: Effect of aqueous extract of *Glycine max* on Root length(cm) of *Secale cereal*

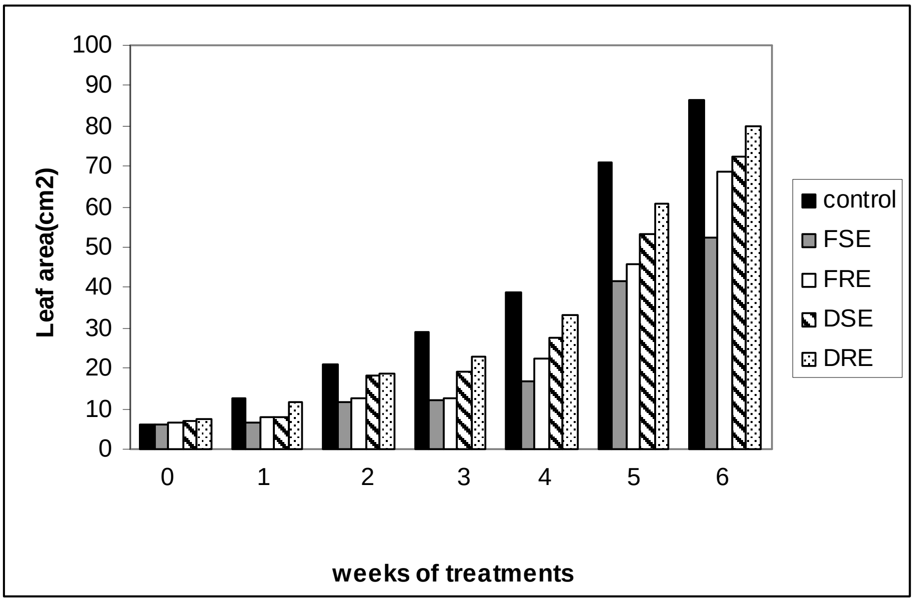

Figure 9: Effect of aqueous extract of *Glycine max* on Leaf area(cm^2) of *Secale cereal*

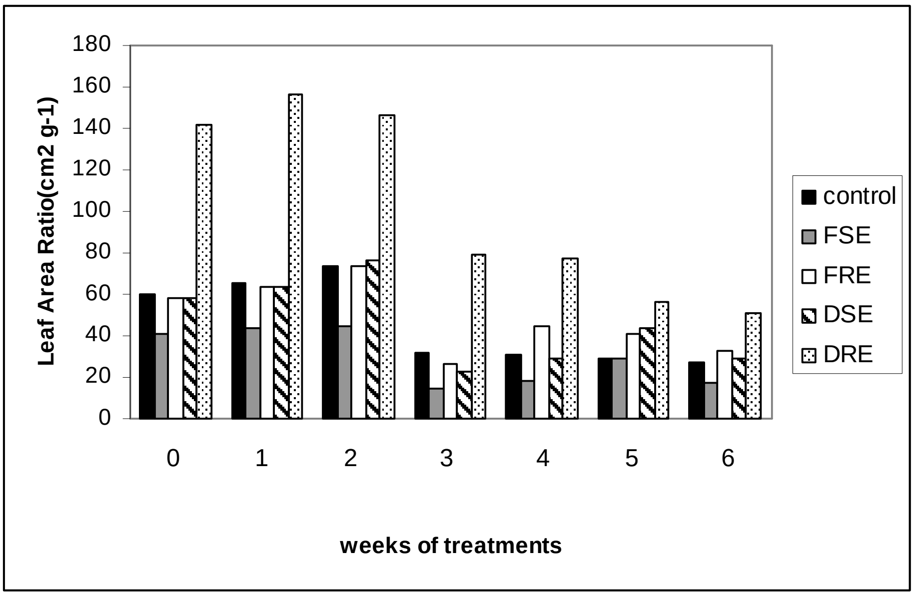

Figure10:Effect of aqueous extract of *Glycine max* on Leaf area ratio(cm^2g^{-1}) of *Secale cereal*

DISCUSSION

Extensive studies have been carried out on the colonizing plants of the Fabaceae family and it has been suggested that these plants could compete effectively and suppress other plants in the same habitat as a result of their allelopathic activity. From this result, the extracts from the fresh shoot, fresh root and dry shoot tissues of *Glycine max* had slight inhibitory effect on the germination of seeds of *Secale cereal* L. This observation, however was contrary to that of Shunjie *et al.* (2008) who found that soybean root exudates did not significantly reduce the germination of seeds *of* wheat seeds However, it was found to be consistent with that of Iman et al. (2006) who observed that allelochemicals from shoot aqueous extracts of soybean inhibited germination in corn and soybean varieties. Huber and Abney(1986) also found that the soybean residues inhibited the germination of *Triticum aestivum*. A significant difference was observed between germination of seeds treated with the fresh tissue aqueous extracts and those treated with the dry tissue aqueous extracts. In fact, the percentage germination of seeds of *Secale cereal* in the dry root regime was actually almost equivalent with that of the control regime. This indicated that the amount or potency of allelochemicals present in the dry tissue aqueous extracts were considerably lower compared to that of the fresh tissues aqueous extracts.

The radicle growth of germinating *Secale cereal* L. seedlings treated with the aqueous extract prepared from fresh and dried shoot of *Glycine max* was observed to be inhibited. A similar result was obtained by Iman et al.(2006) on the effect of aqueous extract derived from stem and leaves of soybean on the growth of radicle and plumule of corn. However, in this study, the aqueous extracts prepared from the fresh and dried root of *Glycine max* did not affect the radicle growth of germinating seeds of *Secale cereal* . This probably could be attributed to low concentration of allelochemicals in the two root aqueous extracts. In support of this was the finding of Miller (1996) who stated that water extract of top growth of *Medicago sativa* L. produced more allelopathic effect on seedlings than extracts from the roots.

The fresh weight and dry weight of the shoot of the control seedlings of *Secale cereal* remained highest in most parts of the experiment and was significantly different from that of the shoot of the seedlings in the different aqueous extract treatment regimes. This result agreed with that of Ahn and Chung (2000) who found that aqueous extract of rice hull inhibited the shoot fresh weight of Barnyard grass *(Echmochloa crusgalli).* The root fresh weight of aqueous extract treated seedlings of *Secale cereal* were observed to be significantly reduced when compared to that of the control seedlings. Huber *et al.* (2002) had earlier observed that exogenously applied phenolic acids reduced root fresh weight and dry weight of soybean. Although the aqueous extracts prepared from the shoot and root of *Glycine max* were observed to retard the shoot height of *Secale cereal* it was however evident that the shoot extracts were more phytotoxic and had more inhibitory effect on the shoot height of the treated seedlings than the root aqueous extracts. The shoot height of *Secale cereal* seedlings treated with the dry shoot aqueous extract and dry root aqueous extract were higher than those treated with the fresh shoot and fresh root aqueous extracts respectively. The drying process could have reduced the amount of volatile allelochemical in these plant tissues hence the low inhibitory effect of the extract prepared from the dried tissue. It has been fairly well established that root length was more sensitive to phytotoxic compounds than either seed germination or shoot elongation in many crops [10, 12, 16]. Huber *et al.* (2002) showed that exogenously applied phenolic acids reduced root length of soybeans. In this work, the root length of the treated seedlings of *Secale cereal* was reduced by aqueous extract treatments applied. This indicated that the extracts applied contain some growth inhibitory substances in amount sufficient to suppress the growth of the root of these seedlings. Variation in the root length of the control and treated seedlings followed the same pattern as observed for the shoot height. The shoot aqueous extract regimes had seedlings with shortest root length during most part of the experiment. This observation was supported by the findings of Eze and Gill (1992) who stated that *Chromolaena odorata* L. had a high concentration of allelochemicals especially in its leaves.

Canston and Venus (1981) were of the opinion that leaves are the most important photosynthetic producers of the plant. According to these workers, light interception and photosynthetic rate depend to a large extent upon the available leaf area. Therefore, the amount of light intercepted is assumed to be directly proportional to the leaf area. In this study, the leaf area of seedlings in the control regime was significantly higher than that of seedlings in all the aqueous extract treatment regimes. That is, the application of the different aqueous extract was observed to have reduced the leaf area of these seedlings. This observation was consistent with the findings of Patterson (1981) who detected that the application of some synthetic allelochemicals reduced the leaf area of soybean.

CONCLUSION

According to our results, the growth of *Glycine max* in association with weeds may lead to reduction in growth of them. There is possibility of using this allelopathic plant directly or as structural leads for the discovery and development of environment friendly herbicides to control of the world's worst weeds.

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